JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

ELUCIDATION OF THE PLANT GROWTH –PROMOTING EFFECT OF *Hartmannibacter diazotrophicus* ON TOLERANCE OF BARLEY TO SALT STRESS

Cumulative Dissertation
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The present work was carried out at the Institute for Applied Microbiology, Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management, Justus-Liebig-University, Gießen during the period from January 2017 to January 2021 under the guidance of **Prof. Dr. Sylvia Schnell**.

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Declaration

I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation.
I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the "Statutes of the Justus Liebig University Gießen for the Safeguarding of Good Scientific Practice".

Date Julian Rojas

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List of peer-reviewed publications

Rojas, J., Ambika Manirajan, B., Ratering, S., Suarez, C., Geissler-Plaum, R., & Schnell, S. (2021). *Spirosoma endbachense* sp. nov., isolated from a natural salt meadow. International Journal of Systematic and Evolutionary Microbiology, 71(1), 1–7. https://doi.org/10.1099/ijsem.0.004601

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List of Abbreviations

16S rRNA	16S ribosomal RNA
ABA	Abscisic acid
ACC	1-aminociclopropane
ACC-deaminase	Enzyme 1-aminociclopropane-1-carboxylase
aRuMP	Assimilatory ribulose monophosphate pathway
Asp	Aspartic acid
ASV	Amplicon sequence variations
BS	Bifidobacterium shunt
C1	One-carbon metabolism
CDS	Coding sequence
DDH	DNA-DNA hybridization
DAP	Dihydroxyacetone phosphate
DPG	2,3-diphosphoglycerate
E19^T	*Hartmanibacter diazotrophicus*
EDD	Entner-Doudoroff pathway
EMP	Embden-Meyerhof-Parnas pathway
E4P	Erythrose-4-phosphate
FAO	Food and Agriculture Organization
Fdh	Formate dehydrogenase
FDH	Formate dehydrogenase
F6P	Fructose 6-phosphate
F1,6P	Fructose 1,6-bisphosphate
G3P	Glyceraldehyde 3-phosphate
G6P	Glucose 6-phosphate

He6P	3-hexulose 6-phosphate
HKT	High-affinity potassium transporter
HPi	3-hexulose 6-phosphate isomerase
HPR	Hydroxypyruvate reductase
HPS	3-hexulose 6-phosphate synthase
H_4FP	Folate-linked C1 transfer
H_4MTP	Methanopterin-linked C1 transfer
IAA	Indole acetic acid
KDPG	2-keto-3-deoxy 6-phosphogluconate
MCL	Malyl coenzyme A lyase
MD	Malate dehydrogenase
MDH	Methanol dehydrogenase
MMO	Methane monooxygenase
MtdA	Methylene tetrahydromethanopterin dehydrogenase
MTK	Malate thiokinase
mxaF	Methanol dehydrogenase-gene
NDH_2	NADH:quinone oxidoreductase
6PG	6-phosphogluconate
PEP	phosphoenolpyruvate
PGA	3-phosphoglycerate
PGPR	Plant Growth-Promoting Rhizobacteria
Pi	Inorganic phosphate
PPi	Diphosphate
PQQ	Pyrrolo-quinoline quinone
pMMO	Particulate form methane monooxygenase
REEs	Rare earth elements

ROS	Reactive oxygen species
RuMP	Ribulose monophosphate pathway
Ru5P	Ribulose 5-phosphate
R5P	Ribose 5-phosphate
SC	Serine cycle
S7P	Sedoheptulose 7-phosphate
sMMO	Soluble form Methane monooxygenase
STHM	Serine hydroxymethyl transferase
VOC	Volatile organic compounds
xoxF	Methanol dehydrogenase-gene
X5P	Xylulose 5-phosphate

Summary

A novel type of methanol dehydrogenase was recently identified, this dehydrogenase is encoded by the gene *xoxF* and for its activity requires lanthanum (La^{3+}) as a cofactor. The *xoxF*-gene was detected in the bacterium *Hartmannibacter diazotrophicus* which stands out by its activity as a plant growth promoter under saline stress by mechanisms that include production of ACC-deaminase, nitrogen fixation, phosphorus solubilization, among other. Methanol dehydrogenase *xoxF* activity of *H. diazotrophicus* was evaluated by its growth on methanol in a mineral medium supplemented with lanthanum and the suitable concentration of lanthanum of optimal growth was determined. Moreover, a greenhouse experiment with barley plants growing in saline soil (NaCl 3%) was reassessed and six experimental treatments were set up namely: Two treatments of barley seeds inoculated with *H. diazotrophicus* with/without lanthanum amendment, two treatments of seeds with addition of *H. diazotrophicus* death biomass with/without lanthanum amendment, one treatment of seeds with lanthanum amendment and the controls without amendments respectively. Variations in shoots and roots dry weights, changes in microbial diversity and colonization of *H. diazotrophicus* in roots and rhizosphere were evaluated. Inoculated plants with/without lanthanum showed significant increases of leave and root dry weights in comparison to controls. Plants growing with only La^{3+} also exhibited higher leave and root dry weights in contrast to treatments with death biomass addition and the controls. Plants inoculated with dead biomass of *H. diazotrophicus* with and without lanthanum had no significant effect in plant growth under salt stress compared to non-inoculated plants. Changes in alpha and beta bacterial diversity in communities of rhizosphere and roots were evidenced among the treatments; alpha diversity indicators showed an increase in the number of ASVs (Amplicon sequence variations) for treatments with *H. diazotrophicus,* whereas beta diversity analysis revealed variation in microbial communities throughout the treatments. Furthermore, for *H. diazotrophicus* a high colonization capacity of roots and rhizosphere was demonstrated in presence/absence of lanthanum.

Furthermore, samples from natural saline environments of soil and waters were collected for bacterial enrichments in liquid mineral medium with methanol and lanthanum in order to isolate potential halotolerant bacteria with methylotrophic

activity. Thirty-one bacteria were isolated and among them, one new species of *Spirosoma sp.* genus was identified and after polyphasic approach was proposed and accepted as *Spirosoma endbachense* I-24^T. The draft genome of *S. endbachense* I-24^T confirmed the position as new species and 4 clusters involved in biosynthesis of ladderane, terpene, polyketide synthese type I and III and non-ribosomal peptide synthese were identified. For *Spirosoma agri* KCTC 52727^T and *Spirosoma terrae* KCTC 52035^T which are the next relatives to *S. endbachense* I-24^T, draft genome sequences were also assembled. The annotation revealed carbohydrate-active enzymes and secondary metabolite biosynthesis gene clusters as well as alkaline phosphatase, cellulose and amylase activity. The genomes contribute to the genomic knowledge of the members of the genus *Spirosoma*.

Zusammenfassung

Vor kurzem wurde ein neuartiger Typ einer Methanol-Dehydrogenase identifiziert. Diese Dehydrogenase wird durch das Gen *xoxF* kodiert und benötigt für ihre Aktivität Lanthan (La^{3+}) als Cofaktor. Das *xoxF*-Gen wurde im Bakterium *Hartmannibacter diazotrophicus* nachgewiesen, das sich durch seine Aktivität als Pflanzenwachstumsförderer unter Salzstress auszeichnet, und zwar durch Mechanismen, die u.a. die Aktivitäten von ACC-Deaminase, Stickstofffixierung und Phosphorsolubilisierung umfassen. Die Methanol-Dehydrogenase *xoxF*-Aktivität von *H. diazotrophicus* wurde durch sein Wachstum auf Methanol in einem mit Lanthan supplementierten Mineralmedium untersucht und die geeignete Lanthankonzentration für optimales Wachstum wurde bestimmt. Außerdem wurde ein Gewächshausexperiment mit Gerstenpflanzen, die in salzhaltigem Boden (NaCl 3%) wachsen, durchgeführt und dafür sechs Versuchsgruppen eingerichtet, nämlich: Zwei Versuchsgruppen von Gerstensamen mit *H. diazotrophicus* beimpften mit/ohne Lanthan-Zusatz, zwei Versuchsansätze von Samen mit Zusatz von abgetöteter Biomasse von *H. diazotrophicus* mit/ohne Lanthan-Zusatz, ein Versuchsansatz von Samen mit Lanthan-Zusatz und Kontrollen ohne Zusatz. Die Trockengewichte von Blättern und Wurzeln, die Veränderungen der mikrobiellen Diversität und die Kolonisierung von *H. diazotrophicus* an Wurzeln und in der Rhizosphäre wurden ausgewertet. Inokulierte Pflanzen mit/ohne Lanthan zeigten einen signifikanten Anstieg des Blatt- und Wurzeltrockengewichts im Vergleich zu

den Kontrollen. Pflanzen, die nur mit La^{3+} wuchsen, zeigten ebenfalls ein höheres Blatt- und Wurzeltrockengewicht im Vergleich zu den Versuchsansätzen mit Zusatz von toter Biomasse und den Kontrollen. Pflanzen, die mit toter Biomasse von *H. diazotrophicus* mit oder ohne Lanthan inokuliert wurden, zeigten keine signifikante Wirkung auf das Pflanzenwachtum unter Salzstress im Vergleich zu nicht-beimpften Pflanzen. Veränderungen der Alpha- und Beta-Diversität der bakteriellen Rhizosphäre- und Wurzelgemeinschaften wurden analysiert; Alpha-Diversitätsindikatoren zeigten eine Zunahme der ASVs (Amplicon sequence variations) für die Versuchsgruppen mit *H. diazotrophicus*, während die Analyse der Beta-Diversität Unterschiede der mikrobiellen Gemeinschaften entsprechend der Versuchsansätzen zeigte. Für *H. diazotrophicus* wurde eine hohe Kolonisierungskapazität der Wurzeln und in der Rhizosphäre in Anwesenheit/Abwesenheit von Lanthan gezeigt.

Um potenziell halotolerante Bakterien mit methylotropher Aktivität neu zu isolieren wurden Proben aus natürlichen salzhaltigen Umgebungen von Böden und Gewässern gesammelt und für Bakterienanreicherungen in flüssigem Mineralmedium mit Methanol und Lanthan verwendet. Einunddreißig Bakterienstämme wurden isoliert und darunter wurde eine neue Art der Gattung *Spirosoma* identifiziert und nach einem polyphasischen Ansatz als *Spirosoma endbachense* I-24^T vorgeschlagen und akzeptiert. Das (draft) Genom von *S. endbachense* I-24^T bestätigte die Position der neuen Art und 4 Gengruppen für die Biosynthese von Ladderan, Terpen, Polyketid Synthese Typ I und III sowie eine nicht-ribosomale Peptidsynthetase wurden identifiziert. Für *Spirosoma agri* KCTC 52727^T and *Spirosoma terrae* KCTC 52035^T als nächst verwandte Arten von *Spirosoma endbachense* wurden ebenfalls die Genome sequenziert und assembiert (draft genomes). Die Annotation ergab Gene für kohlenhydrataktive Enzyme und Gencluster für die Biosynthese von Sekundärmetaboliten sowie für alkaline Phosphatase, Zellulase und Amylase Die Genomsequenzen ergänzen das genomischen Wissen über Vertreter der Gattung *Spirosoma*.

CHAPTER 1.
Introduction and Background

1. Introduction and background

Climate change plays a role in increasing environmental stresses such as drought, salinization, floods and extreme temperatures that lead to reduction in crop productivity (Mahajan and Tuteja, 2005). Among them, soil salinity is one of the major threats to crops quality and productivity (Apse et al., 1999). Salinization has affected more than 20% of cultivated lands according to the Food and Agriculture organization (FAO) (Munns and Tester, 2008) and may increase with the accumulation of salt due to ongoing climate change (Corwin, 2020; Shrivastava and Kumar, 2015). The exponentially growing world population requires increasing food supply and hence soil remediation and salinity mitigation strategies are required to increase crop yields.

1.1 Salinity

Salt stress is the excessive concentration of soluble salts such as NaCl (most common), Na_2SO_4, $MgSO_4$, $CaSO_4$, $MgCl_2$, KCl, and Na_2CO_3 (Ilangumaran and Smith, 2017; Mukhopadhyay et al., 2020). A soil with an electrical conductivity (of the saturated paste extract) of 4 dS m^{-1} or more is considered a saline soil (Ilangumaran and Smith, 2017), equivalent to 40 mM NaCl and 0.2 MPa osmotic pressure (Munns and Tester, 2008). These conditions cause a significant reduction in the productivity of most crops.

The source of dissolved salts in soils are physical or chemical weathering of primary rocks, geological depositions that releases salts, evapotranspiration phenomena from salt-affected groundwater that originates and deposits salts near surface, inflow of seawater or seawater flooding in coastal areas; these types of occurrences are also referred to as primary salinization (Daliakopoulos et al., 2016; Mustafa et al., 2019). Salts can also be introduced as a result of human activities such as land clearing, irrigation with saline water, cultivation activities, addition of chemical fertilizers, inadequate drainage; practices that are associated with secondary salinization (Daliakopoulos et al., 2016; Mustafa et al., 2019).

Increasing climate change is negatively affecting the hydrological cycle, leading to a decrease in rainfall followed by prolonged droughts (Corwin, 2020). Thus, when rainfall levels are low to leach salt ions from the topsoil, salts accumulate, which is one of the main reasons for salinization (Akhtar, 2019).

1.2 Effects of soil salinity on plant growth

Plants are able to detect salt stress and trigger a series of changes at hormonal and physiological levels in response (Munns, 1992). This set of responses develops in two phases; an immediate phase known as the osmotic phase and an ionic phase that occurs after prolonged exposure to salt stress (several days or weeks) (Munns et al., 1995); both phases result in most vital processes in the plant being negatively affected, including, mineral transport, reproduction, photosynthesis and transpiration. The main consequence of osmotic phase is the loss of intracellular water, plant cells under normal conditions have higher osmotic potential than soil, which allows the uptake of water and nutrients from the soil solution, but under salt stress, the osmotic potential in the soil is higher, causing an osmotic imbalance, which impedes the plant from carrying out the normal uptake process from the soil (Passioura and Munns, 2000; Yeo et al., 1991).

As an effect of the disturbed water balance, the synthesis of abscisic acid (ABA) is stimulated, whereupon the stomata are closed to prevent water loss. In addition, the closure of the stomata leads to the generation of reactive oxygen species (ROS) such as hydroxyl radicals, superoxides, disrupt cellular components and processes (Claeys et al., 2014; Pel et al., 2000; Wise and Naylor, 1987).
Another consequence of osmotic shock is the reduction of photosynthesis rate, which leads to plant growth limitation as energy is expended to maintain ionic balance and water conservation (Allakhverdiev et al., 1999, 2000).

On the other hand, the ionic phase is manifested by a constant accumulation of salt in the leaves, the ions of Na^+ are transported to the leaves through the xylem, most of the ions remain in the leaves, but a small portion is returned through the phloem, causing Na^+ to accumulate in the leaves, resulting in a mineral imbalance that leads to an interruption of leaf growth, a decrease in the photosynthetic process and senescence (Flowers and Yeo, 1986; Munns and Passioura, 1984).

1.3 Plant growth-promoting rhizobacteria (PGPR)

Soil bacteria are the most abundant group of microorganisms in soil with about 10^8-10^9 bacteria per gram of soil (Schoenborn et al., 2004). Most of them are found with a high population density in the rhizosphere zone of plant roots (DeAngelis et al., 2009), attracted by the presence of various metabolites such as amino acids, sugars and organic acids exuded by the plant (Zhalnina et al., 2018). A group of bacteria known as plant growth-promoting rhizobacteria (PGPR) are found in the rhizosphere and are of particular interest because of their beneficial relationship with plants; these bacteria have several mechanisms that promote plant growth, such as nutrient supply through biological nitrogen fixation (Marroquí et al., 2001)(Christiansen-Weniger and van Veen, 1991), phosphorus solubilization (Kumar et al., 2001; Rodriguez et al., 2004) siderophore production (Radzki et al., 2013), inhibition of phytopathogens agents (Mazurier et al., 2009), likewise, PGPR are able to produce or modulate phytohormones for plant growth, including indoleacetic acid (IAA), cytokinins, gibberellins and ethylene (Nieto and Frankenberger, 1989; Tien et al., 1979); they participate in the biodegradation of toxic organic compounds which promotes phytoremediation processes (Muratova et al., 2005; Weyens et al., 2015)

1.3.1 Alleviation of salt stress by PGPR

PGPR have been reported to increase the tolerance of plants to environmental stress (Kumar et al., 2007; Ortiz et al., 2015; Staudinger et al., 2016) and especially in alleviating stress caused by salt in plants (Hmaeid et al., 2014; Porras-Soriano et al., 2009; Sukweenadhi et al., 2015) hence the application of halotolerant bacteria with PGPR activity on salt-affected soils is suggested to reactivate crop yields of plants.

The mechanisms of PGPR conferring salt tolerance include the synthesis of cytokinins, indoleacetic acid, ACC deaminase, volatile organic compounds (VOC), exopolysaccharides, restoration of osmotic balance, regulation of oxidative stress, and modulation of gene expression (Forni et al., 2017; Ilangumaran and Smith, 2017).

Plant growth-promoting rhizobacteria have been shown to be able to restore osmotic balance by modulating gene expression of proteins such as plasma

membrane aquaporin protein (ZmPIP), which is involved in stomata opening. Studies by Marulanda et al., 2010 showed that maize plants exposed to salt stress and inoculated with *Bacillus megaterium* increased the expression of ZmPIP, leading to a reduction in leaf's damage and improvement of water conductance.

The accumulation of osmolytes with osmoprotective properties maintains cell turgor and allows water to move from the soil into the roots; examples of osmolytes include proline, trehalose, and glycine betaines produced or induced by PGPR. Bacteria belonging to the genera *Bacillus* and *Arthrobacter* enhanced the accumulation of proline in maize plants under salinity conditions, thereby improving plant growth (Ullah and Bano, 2015), another study using soybean plants inoculated with *Bacillus* and *Pseudomonas* demonstrated an increase in proline concentration in roots, which allowed water uptake and improved shoot growth under salinity conditions (Kumari et al., 2015).
Due to ion imbalance, plants have different mechanisms for Na^+ efflux and K^+ influx to diminish ion toxicity, one of which is the high-affinity potassium transporter (HKT), which increases the uptake of K^+ over Na^+ ions, PGPR can upregulate the expression of HKT genes in the roots and thus restrict the entry of Na^+ into roots (Safdarian et al., 2019). Microbes also minimize the uptake of salt ions by producing exopolysaccharides that act as a physical barrier around the roots, trapping cations in their matrix to prevent uptake by plants (Akhtar, 2019).

Another mechanism to restore osmotic balance regulated by PGPR is the release of volatile organic compounds (VOCs), these low molecular weight compounds are able to induce the production of ROS scavenger enzymes such as catalase, superoxide dismutase, glutathione reductase which protect the plant from cellular damage (Timmusk et al., 2014). Moreover, VOCs are found to upregulate the production of HKT proteins and subsequently promote growth and increase plant leaf biomass. HKT1 gene expression in roots and shoots is up-regulated in *Arabidopsis thaliana* in association with *Bacillus subtilis* under salt stress by exposure to VOCs, which induces a lower Na^+ accretion (Zhang et al., 2008).

Rhizobacteria favour the production of phytohormones, leading to the activation of various response mechanisms that help the plant cope with biotic and abiotic stress effects.

One of the most studied phytohormones produced by PGPR is indole-3-acetic acid (IAA) which is taken up by the plant and is involved in the stimulation of cell division (Khalid et al., 2004). The synthesis of IAA is given by the utilization of tryptophan, which is secreted by plants as a metabolite in the rhizosphere, which in turn is transformed to IAA by rhizobacteria (Dodd et al., 2010). During salt stress in tomato plants, IAA was shown to accumulate in the roots at a concentration of 100 mM NaCl (Albacete et al., 2008). However, once NaCl concentration is increased to 300 mM, IAA levels decrease significantly (Dunlap and Binzel, 1996). PGPR can alleviate salt stress in plants by producing IAA, shoot and root growth were stimulated by inoculating wheat with IAA-producing bacteria *Pseudomonas aurantiaca* and *Pseudomonas extremorientalis* at 100 mM NaCl (Egamberdieva, 2009).

As described above, abscisic acid (ABA) is also induced under salt stress, ABA is induced in roots and then transported via xylem to leaves where it is involved in closing stomata to minimize transpiration activity and regulate water potential (Borel et al., 2001), PGPR are capable of producing ABA but their role in the plant-microbe interaction has not been elucidated, however, these microorganisms could enhance plant survival by regulating ABA pathways. The concentration of ABA in cucumber plants under salt stress inoculated with PGPR was reduced compared to plant controls, which improved the plant growth (Kang et al., 2014); in another report, ABA accumulation was reduced in cotton plants associated with *Pseudomonas putida* RS-198, which induced salt tolerance and increased plant biomass in salinized soil (Yao et al., 2010).

Another important plant hormone associated with biotic and abiotic stress is ethylene, which is critical for seed dormancy breaking, rhizobial nodule formation, fruit ripening and leaf abscission, among others, but is also induced in high concentrations in response to stress phenomena that cause senescence (Glick, 2014). As a means to reduce stress and promote plant growth, PGPR have been shown to be able to inhibit ethylene biosynthesis by cleaving the precursor 1-aminocyclopropane-1-carboxylase (ACC) to α-ketobutyrate and ammonia with the release of the enzyme ACC-deaminase, thus reducing ethylene levels in plants (Glick, 2014); a study indicated the decrease in ethylene synthesis in red pepper plants grown at 150 mM NaCl and inoculated with ACC-deaminase-producing halotolerant bacterial strains *Bacillus licheniformis* RS656, *Zihhengliuela alba* RS111" and *Brevibacterium iodinum* RS16, resulting in an increase in nutrient

assimilation and increased tolerance to salt stress compared to non-inoculated control plants (Siddikee et al., 2011). Strains of *Pseudomonas fluorescens* and *Enterobacter* spp. containing ACC deaminase decreased induced ethylene levels and increased maize yield in salt-affected soils compared to the non-inoculated control (Nadeem et al., 2009).

In conclusion, PGPR under salt stress induce systemic tolerance that promotes plant growth and development. Moreover, it is important to point out the enormous potential of microorganisms associated with the rhizosphere of halophytic plants or in the vicinity of saline soils to alleviate salt stress and promote crop plant growth.

1.4 Methylotrophs

Methane and methanol are among the C1 compounds (one carbon-molecule) that are part of the carbon cycle and are widely distributed in nature (Iguchi et al., 2015). The production of methane occurs in anoxic environments by anaerobic archaea, also known as methanogenic archaea, which reduce CO_2 with H_2 (hydrogenotrophic) to CH_4 or convert acetate to methane and CO_2 (acetoclastic) and mostly depend of the activity of H_2- and acetate-producing fermenting bacteria co-occurring in the same habitat. This group of microorganism can be found as free-living archaea in lakes, paddy fields (Maksimavičius and Roslev, 2020; Whiticar, 2020), as well as in specialized compartments such as the rumen of ruminants like cows (Vanwonterghem et al., 2016). Methane is also produced in abiotic processes such as coal mining, oil and gas operations, anaerobic waste treatment plants, landfills etc. (Iguchi et al., 2015; Whiticar, 2020). These processes have made methane the second most important gas produced by anthropogenic activities, and it is in turn part of the atmospheric gases that promote the greenhouse effect (Whiticar, 2020).

On the other hand, methanol is a volatile carbon compound and the main contributors to its emissions are plants through the processes of repair and demethylation of pectin polymers in the cell wall as well as lignin breakdown (Fall and Benson, 1996; Galbally and Kirstine, 2002); however, other sources of methanol have been mentioned, including the chemical industry, where it is commonly used as a solvent (Fall and Benson, 1996), and other anthropogenic

activities (Singh et al., 2000) and it is one of the most abundant volatile organic compounds in the atmosphere (Galbally and Kirstine, 2002).

Methane and methanol, as well as other C1 compounds, are used by methylotrophic organisms as the sole source of energy; furthermore, facultative methylotrophic organisms capable of utilizing multicarbon compounds have been identified (Chistoserdova et al., 2009; Nazaries et al., 2013); methylotrophs are widely distributed in ecosystems such as geothermal reservoirs, soils, peat bogs or aquatic environments and sediments, where most of them grow under oxic conditions at neutral pH (6.0–8.0) and temperatures between 10 and 40 °C (Nazaries et al., 2013), but acidic, alkaliphilic, thermophilic and psychrophilic species have also been described (Op den Camp et al., 2009); they are also representative members of *Proteobacteria*, *Verrucomicrobia*, *Flavobacteria* and *Actinobacteria* (Kolb, 2009; Stacheter et al., 2013).

As mentioned above, plants can produce methanol as result of their biochemical activities, which can be exuded from the leaves or the roots, whereby phyllosphere (Stacheter et al., 2013) and the rhizosphere (Macey et al., 2020) have been described as zones with high densities of methylotrophs. Moreover, the presence of proteins involved in methylotrophic metabolism has been demonstrated in the phyllosphere and roots of rice plants (Knief et al., 2012) and of taxonomic groups with methylotrophic activity in wheat roots (Turner et al., 2013). Activities to promote plant growth under abiotic stress have been reported for methylotrophic bacteria relieving the adverse effects through activities such as increased uptake of essential elements through nitrogen fixation, P, K and Zn solubilization, production of chelating compounds, increase in tolerance through production of phytohormones (Kumar et al., 2019).

1.4.1 Aerobic oxidation of methane to formaldehyde

Methanotrophic bacteria oxidize methane under oxic conditions via methane monooxygenase and dehydrogenases, producing intermediates such as methanol, formaldehyde, and formate, which are further converted to CO_2 (Jiang et al., 2010; Macey et al., 2020). To catalyze the oxidation of methane to methanol, methanotrophic bacteria can produce two forms of methane monooxygenases (MMO) enzymes with the same metabolic function; particulate (pMMO) or soluble

methane monooxygenases (sMMO). The pMMO is a membrane-bound enzyme with copper in its active site, requiring a copper-sufficient environment for its functionality, the pMMO is the most common enzyme among methylotrophic bacteria; the sMMO, on the other hand, is a cytoplasmic enzyme with a di-iron active site that is released at low copper concentrations and is found in selected groups of methylotrophs (Jiang et al., 2010; Park and Lee, 2013).

The methanol produced is subsequently oxidized to formaldehyde by means of a periplasmic pyrroloquinolinequinone (PQQ)-dependent methanol dehydrogenase (MDH), encoded by the calcium dependent *mxaF*-gene or the *xoxF*-gene, the latter recently discovered to have lanthanide dependent activity; then the formaldehyde is converted to formate via tetrahydrofolate or tetrahydromethanopterin intermediaries, the pathway ends with the final conversion of formate to CO_2 by NAD-dependent formate dehydrogenase. Alternatively, another part of formaldehyde can be assimilated into the cell biomass via the assimilatory ribulose monophosphate (aRuMP) pathway or via the serine cycle pathway (Figure. 1) (Kalyuzhnaya, 2016; Nazaries et al., 2013)

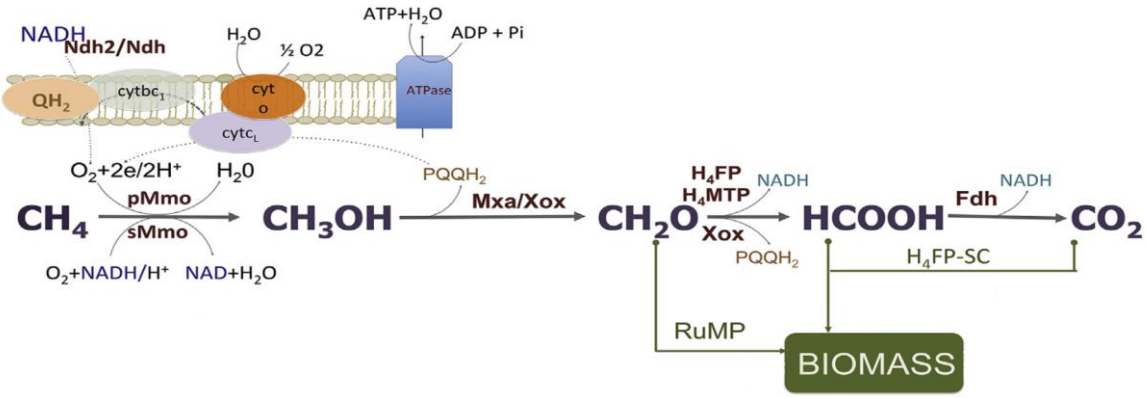

Figure 1. Aerobic methane metabolism; membrane-bound methane monooxygenase (**pMMO**); soluble methane monooxygenase (**sMMO**); PQQ-linked methanol dehydrogenases (**Mxa**); PQQ-linked methanol and formaldehyde dehydrogenases (**Xox**); methanopterin-linked C1 transfer (**H_4MTP**); folate-linked C1 transfer (**H_4FP**); formate dehydrogenase (**Fdh**); NADH:quinone oxidoreductase (**NDH_2**); assimilatory ribulose monophosphate pathway (**RuMP**); serine cycle (**SC**) (Modified from Kalyuzhnaya, 2016)

The ribulose monophosphate pathway (RuMP pathway) occurs with the condensation of formaldehyde on ribulose 5-phosphate by 3-hexulose 6-phosphate synthase (Hps) to form hexulose 6-phosphate and its isomerization to fructose 6-phosphate by 3-hexulose 6-phosphate isomerase (Hpi). The fate of this six-carbon compound could occur via three predicted RuMP pathways, including the Embden-

Meyerhof-Parnas pathway (EMP) (glycolytic); the Entner-Doudoroff (EDD) pathway (KDPG variant); and the Bifidobacterium shunt (BS) variant (phosphoketolase, Xfp variant)(Figure. 2) (Kalyuzhnaya, 2016). Via all pathways important intermediates for biomass synthesis (sugars, acetyl-CoA, glycerin-phosphate) are provided.

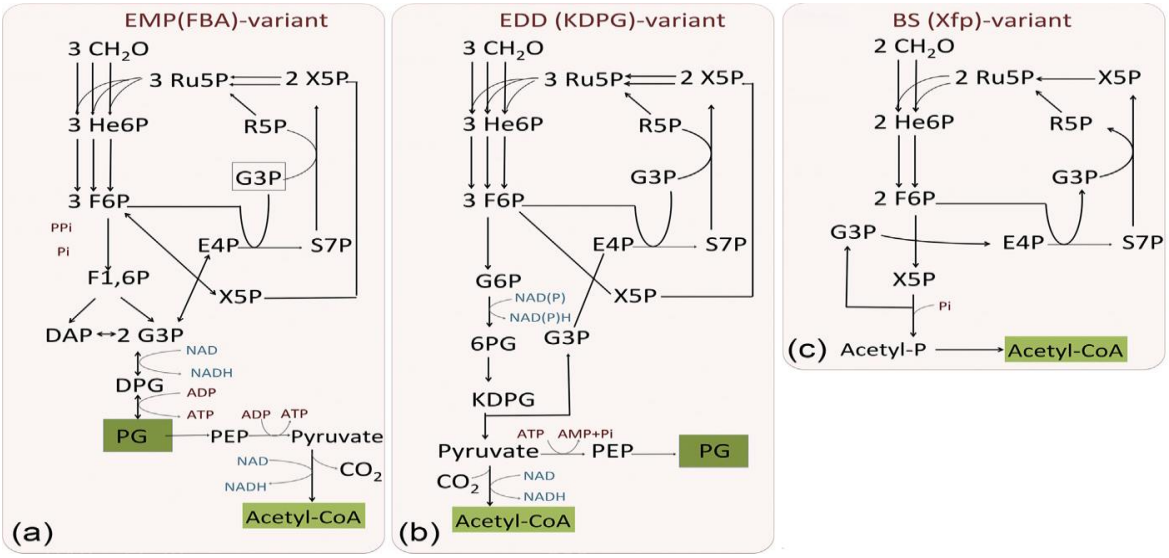

Figure 2. The ribulose monophosphate (RuMP) represented with the three predicted routes for cleavage for formaldehyde assimilation **(a)** EMP (FBA) variant; **(b)** EDD (KDPG) variant; and **(c)** BS (Xfp) variant. Dihydroxyacetone phosphate (**DAP**); 2,3-diphosphoglycerate (DPG); erythrose-4-phosphate (**E4P**); fructose 6-phosphate (**F6P**); fructose 1,6-bisphosphate (**F1,6P**); glyceraldehyde 3-phosphate (**G3P**); glucose 6-phosphate (**G6P**); 3-hexulose 6-phosphate (**He6P**); 2-keto-3-deoxy 6-phosphogluconate (**KDPG**); 6-phosphogluconate (**6PG**); phosphoenolpyruvate (**PEP**); 3-phosphoglycerate (**PGA**); ribulose 5-phosphate (**Ru5P**); ribose 5-phosphate (**R5P**); sedoheptulose 7-phosphate (S7P); (**X5P**) xylulose 5-phosphate; diphosphate (**PPi**); inorganic phosphate (**Pi**). (Modified from Kalyuzhnaya, 2016)

The serine cycle, on the other hand, begins with the condensation of methylene-H4F and glycine to serine, which in turn, after several transformations, is converted to phosphoenolpyruvate, which is carboxylated to malate, followed by CoA-addition to generate malyl-CoA, which is cleaved to glyoxylate and acetyl-CoA, glyoxylate is finally converted back to glycine (Figure. 3) (Chistoserdova and Lidstrom, 2013).

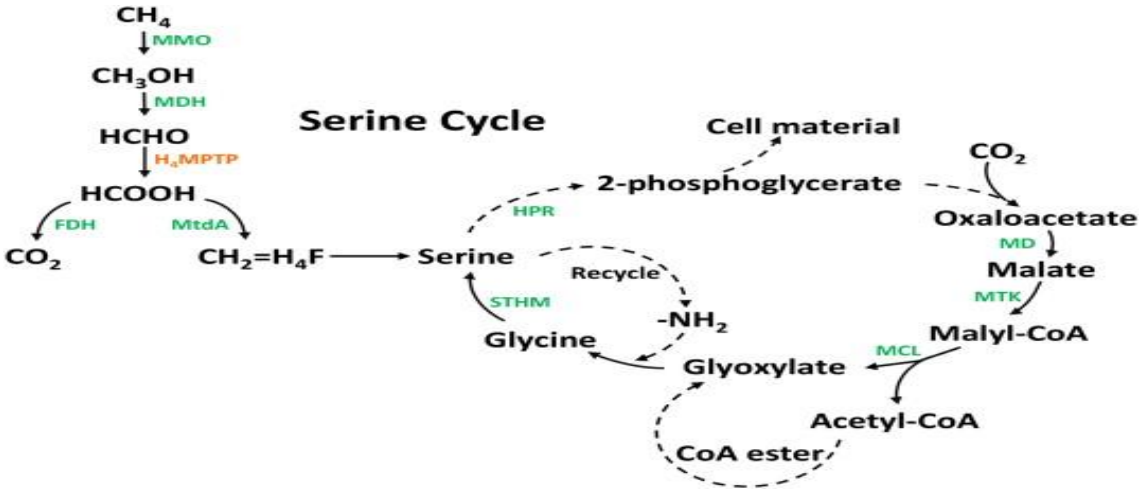

Figure 3. The serine cycle in in methanotrophic bacteria. Methane monooxygenase (**MMO**); methanol dehydrogenase (**MDH**); methylene tetrahydromethanopterin pathway (**H4MPTP**); methylene tetrahydromethanopterin dehydrogenase (**MtdA**); formate dehydrogenase (**FDH**); serine hydroxymethyl transferase (**STHM**); hydroxypyruvate reductase (**HPR**); malate dehydrogenase (**MD**); malate thiokinase (**MTK**); malyl coenzyme A lyase (**MCL**). (Adapted from Fei et al., 2014)

1.4.2 Methanol dehydrogenase (MDH)

The MDH is a protein found in the periplasm of most model methylotrophs; this protein has a prosthetic group (PQQ) placed in the center of a large α_2 subunit folded in a β-propeller structure and a β_2-subunit surrounding the α_2- subunit (Figure 4). The mechanism of enzyme action begins when PQQ is reduced with a proton that is withdrawn from the alcohol group present in the methanol molecule by an active site dominated by Asp^{303} and a cofactor that acts as a Lewis acid (Anthony, 2004); this reduced PQQ is reoxidized transferring two electrons to the heme group of cytochrome C_L, which in turn is oxidized by cytochrome C_H. This latter compound transfers the electrons to a membrane-bound C-type, which could be cytochrome aa3 or CO (oxidase); the electron chain ends with a final step where the electrons reach the final acceptor oxygen (Anthony, 1992; Read et al., 1999), this process of electron transfer generates a proton motive force that can generate one molecule of ATP per molecule of oxidized methanol (Williams et al., 2005).

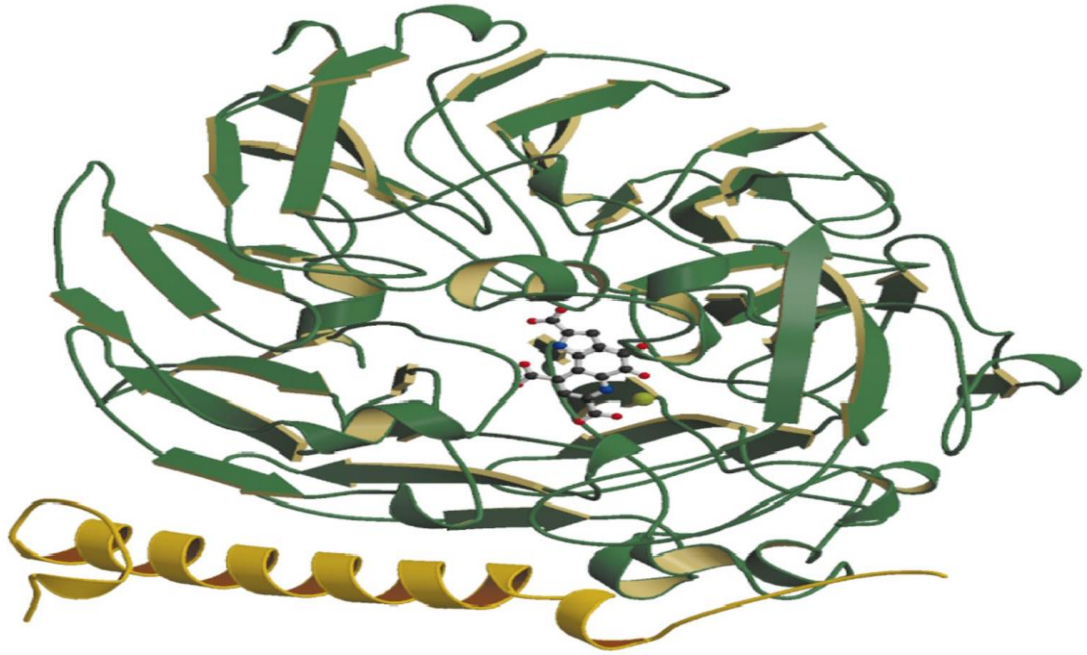

Figure 4. Structure of *Methylobacterium extorquens* MDH. The α-subunit folded in propeller-structure (green) coupled with a PQQ and a calcium ion (green sphere) in the active center, the small β-subunit (yellow) (Adapted from Williams *et al.*, 2005).

The first MDH was discovered in *Methylobacterium extorquens*, which is encoded by the *mxaF*-gene that requires calcium (Ca^{+2}) as a cofactor for its activation; the *mxa* cluster in *M. extorquens* consists of the *mxaF* and *mxaI* genes, which express the large and small subunits of MDH, *mxaG*, which encodes cytochrome C_L, mxaACKL which encodes proteins for calcium insertion, and other genes required for MDH activity (Chistoserdova, 2011). This MDH-Ca^{2+} dependence was considered the most traditional and widely used enzyme model for methanol oxidation in methylotrophic bacteria such as *Methylobacterium sp.*, *P. denitrificans*, *Hyphomicrobium methylovorum*, and *Methylophilus methylotrophus* (Hibi et al., 2011a). Recently, however, Hibi et al., 2011 found an alternative MDH in *Methylobacterium radiotolerans* encoded by a *xoxF-gene*, a homologous gene of *mxaF* that is dependent on lanthanum (Ln^{3+}) as a cofactor and widely predominant in methylotrophic bacteria and co-occurs with the *mxaF* gene or alone (Lv et al., 2018). The operon of *XoxF* includes the *xoxF*-gene, encoding Ln-dependent MDH, the *xoxG*-gene, encoding cytochrome C_L and the *xoxJ*-gene, encoding a protein for La^{3+} transport (Featherston and Cotruvo, 2021; Keltjens et al., 2014). MDH encoded by *xoxF* shows optimal activity at neutral pH and is not dependent on ammonium, in contrast to *mxaF,* which requires pH around 9-10 and is dependent

on ammonium for its activity (Pol et al., 2014). Some of methylotrophic bacteria with *xoxF* activity include, *Methylobacterium radiotolerans*, *Bradyrhizobium* sp., "*Candidatus Methylacidiphilum fumariolicum* SolV", *M. extorquens* AM1, and others (Hibi et al., 2011a; Pol et al., 2014).

The rare earths elements (REEs) comprise a group of 17 chemical elements from lanthanum to lutetium with atomic numbers 57 to 71 in the periodic table and also include yttrium and scandium; rare earths elements are widely distributed as mixtures in the Earth's crust and are classified into two groups according to electron configuration, mineralogy and chemical behavior: Light rare earths (LREEs), which include from La to Eu, and heavy rare earths (HREEs), which include Gd-Lu and Y (Hoshino et al., 2016). The rare earth elements (REEs) are used as components of electronic devices such as mobile-phones, solar cells, and computers (Pol et al., 2014) and are often found in insoluble forms such as oxides, silicates, carbonates, and phosphates (Habashi, 2013). Due to their low solubility, unestablished association with biological functions and mechanisms, these REEs were usually considered nonessential until their role as cofactors of MDH for methanol oxidation in methylotrophic species was discovered (Pol et al., 2014). The association of REEs coupled to PQQ exhibit a stronger Lewis character, which plays an important purpose in polarizing atoms and enhances the redox activity of MDH compared to Ca^{2+} (Bogart et al., 2015; Pol et al., 2014). It is evident that REEs are assimilated as described above, but, the mechanisms have not been elucidated, however, despite their low solubility, some evidence for the assimilation of these elements has been suggested. The presence of binding groups for REEs in the bacterial cell surface such as carboxylates and phosphates has been pointed out (Takahashi et al., 2005), another study on REEs uptake mechanism described the involvement of molecules with similar activity to chalkophores or siderophores involved in copper or iron acquisition, these compounds are known as lanthanophores which solubilize La^{3+} from mineral complexes, the lanthanophores are transferred to the periplasm by a TonB-dependent receptor in the outer membrane, once in the periplasm the La^{3+} is released from the lanthanophore and the La^{3+} is recognized by apo-*xoxF* or a periplasm binding protein that induces the expression of the *xoxF* cluster; another pathway involves the transfer of La^{3+} from the periplasm to the cytoplasm through

a ABC transport system coupled to the internal membrane; in the cytoplasm, La^{3+} also activates transcription of the *xoxF* cluster (Ochsner et al., 2019).

1.4.3 Expression Regulation of *xoxF*

The gene *XoxF* is involved in carbon metabolism and is expressed when lanthanide species such as La^{3+}, Ce^{3+}, $Pr^{3+}$and Nd^{3+} are available, but it prefers La^{3+}, as shown by an experiment in which *Methylorubrum extorquens* strain AM1, growing in a mineral medium containing a mixture of Ln-species, used La^{3+} as a preferred cofactor (Xu et al., 2007). As mentioned above, methylotrophic bacteria can harbor both MDH-genes (*mxaF-xoxF*) and under growth conditions in an environment with methanol and free of lanthanides, normally express *mxaF* but preferentially induce *xoxF* at low concentrations of lanthanide elements, even when the Ca^{2+} concentration is higher. This mechanism is known as the "lanthan-switch", in which transcription of the *mxaF* gene is repressed and expression of the *xoxF* gene is up-regulated in the presence of lanthanides (Chu and Lidstrom, 2016; Vu et al., 2016). A periplasmic binding protein has been identified in *M. extorquens*, that may be associated with the "lanthan-switch". This is the La^{3+}-selective chelator called Lanmodulin (LanM), which occurs in the periplasm and contains four carboxylate-rich metal coordination motifs known as EF hands, allowing it to selectively recognize La^{3+} ions.

Depending on the organism, expression regulation may vary. Three-component systems, *MxbDM*, *MxcQE*, *MxaB* are involved in the regulatory mechanism of *M. extorquens*, but only the *MxbM* component is required for repression of the *xoxF* cluster in the absence of lanthanum, however, *XoxF* is required for expression of the *mxa* cluster and the *MxbDM* component system leads to repression of itself (Featherston and Cotruvo, 2021; Vu et al., 2016); to fulfill the requirement of *xoxF* for expression of the *mxa* cluster, Skovran et al., 2019 propose the involvement of apo-*xoxF* (metal not incorporated into *xoxF*) as a lanthanide sensor in periplasm that interacts with either *MxcQ* or *MxbD*; once apo-*xoxF* reacts with La^{3+}, it triggers transcription of the *xoxF* operon and represses *mxa* expression. In another methylotrophic bacteria *Methylomicrobium buryatense*, the component system of *MxbDM* and *MxcQE* is not present, but a sensor kinase known as *MxaY*, which can recognize Ln^{3+}, regulates the expression of *mxa* and *xoxF* clusters (Chu et al., 2016).

1.5 *Hartmannibacter diazotrophicus* E19^T

Hartmannibacter diazotrophicus E19^T is a gram-negative bacterium isolated from the rhizosphere of the plant *Plantago winteri Wirtg*., as part of bacterial screening project with PGPR potential associated with halotolerant plants growing in a natural salt meadow. Taxonomically, the bacterium belongs to the Domain *Bacteria*, Phylum *Proteobacteria*, Class *Alphaproteobacteria* (Suarez et al., 2014) and was recently assigned to the Order *Hyphomicrobiales*, novel family *Pleomorphomonadaceae* along with other genera such as *Chthonobacter*, *Methylobrevis*, *Mongoliimonas*, *Oharaeibacter*, and *Pleomorphomonas* (Hördt et al., 2020).

H. diazotrophicus is described as motile, strictly aerobic, oxidase-positive and catalase-negative, grows in 1–3% (w/v) NaCl, the major respiratory quinone is Q-10, DNA G+C content is 59.9% ± 0.7 mol%. The activities described to promote plant growth of *H. diazotrophicus* include: Phosphate solubilization, nitrogen fixation, ACC and VOC production, iron uptake. The ability of phosphate solubilisation by *H. diazotrophicus* was tested in liquid medium with inorganic phosphorus complex sources [$Ca_3(PO_4)_2$, $AlPO_4$ and $FePO_4$] and organic ones such as inositol hexaphosphate (IHP), obtaining values ranging from 0.15 to 0.37 $mg \cdot l^{-1}$ of soluble phosphate; it was also observed that gluconic acid is the most produced organic acid for solubilization activity (Suarez et al., 2019). The gluconic acid is synthetized in the oxidation pathway of glucose by means of enzyme glucose dehydrogenase and a cofactor pyrroloquinoline quinone (PQQ) (Suleman et al., 2018). The gene *pqqBCDE*, encoding the biosynthesis of PQQ and a substitute of the enzyme glucose dehydrogenase encoded by *yliI* are present in the *H. diazotrophicus* genome, but genes for acidic phosphatase, alkaline phosphatase and phosphatidic acid production are also present (Suarez et al., 2019). Nitrogen fixation by *H. diazotrophicus* was confirmed by its growth on nitrogen-free medium and nitrogenase activity by acetylene reduction to ethylene; genes involved in the biological nitrogen cycle were annotated in its genome. The genes described for nitrogen fixation activity include *nifH*, *nifD* and *nifK*, which encode the structural subunits of nitrogenase, genes involved in the synthesis and incorporation of *FeMoco* into nitrogenase, such as *nifE*, *nifN*, *nifX*, *nifB*, *nifQ* and *nifV*, as well as other genes involved in the protection, stabilization and regulation of nitrogenase

(Suarez et al., 2019). Mechanisms for the acquisition and metabolism of sulfur and iron in *H. diazotrophicus* are possible through specific protein complexes in the outer membrane; mixed sulfur compounds, *e.g.* alkane sulfonates, are taken up by an aliphatic sulphonate ABC transport encoded by *ssuABC*; once inside the cell, sulfur compounds are mineralized to sulfite by an alkane sulfonate monooxygenase and an NADPH-dependent FMN reductase encoded by the genes *ssuD* and *ssuE* genes respectively. Second, the iron is chelated by a siderophore, which in turn is transported by the protein complex of *TonB*, *ExbB* and *ExbD* into the periplasmic space, where the siderophore releases the ferrous ion and the iron is taken up by a Fe-binding protein and transported to the cytoplasm via an ATP transporter. It has been mentioned that ACC-deaminase can degrade the precursor 1-aminocyclopropane-1-carboxylase (ACC), thereby lowering the ethylene levels in plants while reducing stress from salt (Glick, 2014), *H. diazotrophicus* was able to grow in a ACC-supplemented medium (Suarez et al., 2014), moreover, genes with enzymatic potential for ACC-degradation are present in its genome (Suarez et al., 2019). A metabolic pathway for the production of VOCs is also evidenced with the gene *ilv*, which encodes the enzyme acetolactate synthase for acetolactate production, and the gene *budC*, which encodes diacetyl reductase that subsequently converts acetolactate to acetoin by decarboxylation. *H. diazotrophicus* possesses genes for proline and glutamate biosynthesis, the genes *otsA* and *otsB*, which express the production of trehalose synthase and trehalose phosphatase, involved in the synthesis of trehalose, and the genes *betA*, *betB*, which encode for choline dehydrogenase and betaine aldehyde dehydrogenase, essential enzymes for betaine synthesis (Suarez et al., 2019). Trehalose, proline, glutamate, betaine belong to the group of compatible solutes that plants accumulate as osmoprotectants during salt stress (Ullah and Bano, 2015).

Other gene-activities identified in *H. diazotrophicus* that may help the plant to cope with salt stress are peroxidases, superoxidase and glutathione S-transferase, which are encoded in coding sequences (CDS) and are involved in the protection of the cell against oxidative stress, also the *KDP* operon, which is involved in the expression of high-affinity K^+ and favors the uptake of K^+ over Na^+, and the gene cluster *mrpABCDEFG*, which stimulates Na^+ efflux and regulates cell volume and pH homeostasis (Suarez et al., 2019).

In methylotrophic bacteria, two genes have been associated with methanol dehydrogenase expression, *mxaF* and *xoxF* (Daumann, 2019). In *H.*

diazotrophicus, the presence of the *xoxF4*-lanthanide-dependent gene was detected and its activity was confirmed by growth of the bacteria in mineral medium supplemented with methanol and lanthanum (Lv et al., 2017). The genome of *H. diazotrophicus* harbors genes for other methylotrophy activities associated such as PQQ synthesis, H4MPT and H4F pathways, formate oxidation, serine cycle and methylamine dehydrogenase (Lv and Tani, 2018; Suarez et al., 2019).

The plant growth-promoting activity of *H. diazotrophicus in planta* was demonstrated when barley plants growing under salt stress were inoculated, which increased shoot and root dry weights compared to non-inoculated plants; moreover, ethylene levels were reduced as a possible consequence of ACC deaminase activity (Suarez et al., 2015); another study showed that inoculation of alfalfa plants with *H. diazotrophicus* under salinity enhanced K^+ uptake and carotenoids production and increased relative water content (Ansari et al., 2019).

1.6 Barley

Barley (*Hordeum vulgare, vulgare L*.) is a cereal crop which grows in a variety of climates from sub-Arctic to subtropical and is listed on position five of crops with the highest dry weight production in the world. Barley is composed primarily of soluble and insoluble dietary fiber, with β-glucans predominating, but is also a source of protein, carbohydrates, vitamin E, B-complex vitamins, minerals, and phenolic compounds. Barley is incorporated in breakfast cereals, soups, baking flour and is an important food source that is also used in the production of alcoholic beverages such as beer (Gupta et al., 2010).

Barley belongs to the grass family *Poaceae*, subfamily *Festucoideae*, tribe *Triticeae and* genus *Hordeum*. The tribe *Triticeae* includes the best known cereals, namely: wheat (*Triticum* , several species), rye (*Secale cereale*), barley *(H. vulgare*) and others (Von Bothmer and Komatsuda, 2011). Of genus *Hordeum*, thirty-one species have been described (Von Bothmer and Komatsuda, 2011) but the cultivated barley varieties belongs to the species: *vulgare* and the wild form which belongs to the species *spontaneum*. Morphologically, barley is similar to other grasses and has spikes consisting of a series of spikelets which in turn contain

florets, the organization of the spikelets can be two-rowed or six-rowed, resulting in differences in composition and reproduction (MacGregor, 2003).

1.7 Taxonomical description of a new *Spirosoma* species

The study of the biodiversity and relationships among living organisms is facilitated by taxonomical classification (Gevers et al., 2005); for bacteria, the taxonomy enables their reliable identification from clinical and environmental samples (Moore et al., 2010). Currently, a polyphasic approach is the standard method for bacterial novel taxa classification (Tindall et al., 2010), which consists of a series of tests for the evaluation of phenotypic, genotypic, and chemotaxonomic traits (Stackebrandt et al., 2002). Phenotypic tests comprises morphological, physiological, and biochemical characteristics (Wayne et al., 1987); genotypic methods are referred to DNA-based analysis such as %G+C content, DNA-DNA hybridization (DDH) and 16S rRNA gene sequence analysis (Stackebrandt and Ebers, 2006; Tindall et al., 2010), and chemotaxonomic characterization on the other hand, include tests to evaluate differences in the structural components such as the cell wall, the cell membrane or cytoplasm (Tindall et al., 2010).

In bacterial taxonomy, the species is the basic unit of bacterial taxonomy (Wayne et al., 1987) and can cluster similar strains which share 70% or more DNA-DNA relatedness. For the delineation of new species multiple rules of thumb have been proposed such as, DDH value which calculate genetic similarities at genome level between two species, value that should be less 70% to be designated as different species (Wayne et al., 1987); comparative sequence analysis of the 16S rRNA gene with identity values < 98,7% (Stackebrandt and Ebers, 2006) in combination with tests for evaluation of chemotaxonomy, physiological and cultural characteristics.

The genus *Spirosoma* was described first time by Migula, 1894 and is assigned to the proposed family *Spirosomaceae*, phylum *Bacteroidetes*, the family description was emended by García-López et al., 2019. The genus comprises 42 members validly published (Euzéby, 2018) that were isolated from natural environments such as water, soil, dust and soil (Lee, et al., 2018; Ten, et al., 2018). Members of the genus are described as Gram-negative, aerobic or facultatively anaerobic, non-spore-forming, variable motility, rods with various morphologies such as

rings, coils, filaments, colonies pink to yellow pigmented, the major menaquinone is MK-7 (Ahn et al., 2014), phosphatidylethanolamine as major polar lipid and G+C content from 47.2 to 57.0 mol% (Chang et al., 2014).

1.8 Comparative genomics

Genome sequencing is a tool of valuable importance, as it can aid in the taxonomic classification, the study of dynamics, evolution, and function of an unknown organism in an ecosystem. (Andersson and Goodman, 2012; Loman and Pallen, 2015) The use of NGS (Next generation sequencing) technologies in combination with informatic tools enable to sequence any bacterial genome project. A downstream analysis for a genome sequence involves the assembly of individual sequence reads into larger contigs; gene annotation and functional prediction based on referenced sequence databases to check genetic and metabolic capabilities of the bacteria; and comparative sequence analysis of single genes or whole genomes to genotypic characterization (Del Chierico et al., 2015; Roumpeka et al., 2017), core and even pan-genome analysis for multiples strains of species (Bentley, 2009).

1.9 Aim of the study

Soil salinization negatively affects plant growth and all their metabolic processes and is also one of the major challenges for agriculture due to increasing climate change (Mukhopadhyay et al., 2020). PGPRs have been shown to alleviate the negative effects caused by salt stress in plants through ACC deaminase, phytohormones and VOCs production (Etesami and Maheshwari, 2018). Among PGPRs, salt halotolerant bacteria were found to have activities as plant growth promoters *eg. H. diazotrophicus* which showed improved growth of barley and alfalfa under saline conditions (Ansari et al., 2019; Suarez et al., 2015), however, the gene *xoxF*-lanthanide-dependent was amplified in *H. diazotrophicus* which confers the ability to methylotrophy through methanol uptake (Lv et al., 2017). The study of lanthanide dependent methylotrophy has attracted particular interest in recent years and most studies on methylotrophic bacteria with *xoxF* activity associated with plants have been conducted in the phyllosphere, but there is little

information on *xoxF*-methylotrophic bacteria with plant-promoting growth activity associated with roots, apart from a few studies on halotolerant bacteria dependent on lanthanides.
Since methanol is a by-product of cell wall metabolism in plants, it can be released from either leaves and shoots (Macey et al., 2020; Stacheter et al., 2013), which could allow methylotrophic metabolism to influence plant-associated microbes and benefit the plant as PGPRs.

In this study a greenhouse experiment with barley growing in saline soil was carried out, several treatments were established where plant seeds grow with/ without bacteria inoculation, with/without lanthanum supplementation, non-inoculated seeds grown in presence/absence of lanthanum and the controls of seeds only growing in saline soil without further amendments. The aims of this study were as follow: (I) Reassess the growth of *H. diazotrophicus* with methanol supported by lanthanum and to find the optimal concentration required as a metal cofactor for methanol dehydrogenase. (II) Evaluate, if performance of *H. diazotrophicus* as plant growth-promoting rhizobacterium on barley plants under salt stress is enhanced in presence of lanthanum. (III) Evaluate, if the presence of lanthanum without seed inoculation with *H. diazotrophicus* does not affect plant growth. (IV) Availability of lanthanum improves rhizosphere abundance of *H. diazotrophicus*. (V) Bacterial communities are affected by E19^{T} inoculation and lanthanum application in rhizosphere and roots.
Furthermore, methylotrophic lanthanum-dependent bacteria from saline natural environments samples have been enriched in a methanol lanthanum containing liquid medium. The isolated strains were characterized for their ability to grow with lanthanum and for taxonomic identification.

1.10 References

Ahn, J. H., Weon, H. Y., Kim, S. J., Hong, S. B., Seok, S. J., & Kwon, S. W. (2014). *Spirosoma oryzae* sp. nov., isolated from rice soil and emended description of the genus *Spirosoma*. International Journal of Systematic and Evolutionary Microbiology, 64(Pt_9), 3230–3234. https://doi.org/10.1099/ijs.0.062901-0

Akhtar, M. S. (2019). Salt stress, microbes, and plant interactions: Causes and solution. In Salt Stress, Microbes, and Plant Interactions: Causes and Solution: Volume 1 (Vol. 1). https://doi.org/10.1007/978-981-13-8801-9

Albacete, A., Ghanem, M. E., Martínez-Andújar, C., Acosta, M., Sánchez-Bravo, J., Martínez, V., Lutts, S., Dodd, I. C., & Pérez-Alfocea, F. (2008). Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum L.*) plants. Journal of Experimental Botany, 59(15), 4119–4131. https://doi.org/10.1093/jxb/ern251

Allakhverdiev, S. I., Nishiyama, Y., Suzuki, I., Tasaka, Y., & Murata, N. (1999). Genetic engineering of the unsaturation of fatty acids in membrane lipids alters the tolerance of *Synechocystis* to salt stress. Proceedings of the National Academy of Sciences of the United States of America, 96(10), 5862–5867. https://doi.org/10.1073/pnas.96.10.5862

Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., & Murata, N. (2000). Inactivation of photosystems I and II in response to osmotic stress in *Synechococcus*. Contribution of water channels. Plant Physiology, 122(4), 1201–1208. https://doi.org/10.1104/pp.122.4.1201

Andersson, S. G. E., & Goodman, A. L. (2012). Bacterial genomes: Next generation sequencing technologies for studies of bacterial ecosystems. In Current Opinion in Microbiology (Vol. 15, Issue 5, pp. 603–604). Elsevier Current Trends. https://doi.org/10.1016/j.mib.2012.10.001

Ansari, M., Shekari, F., Mohammadi, M. H., Juhos, K., Végvári, G., & Biró, B. (2019). Salt-tolerant plant growth-promoting bacteria enhanced salinity tolerance of salt-tolerant alfalfa (*Medicago sativa L.*) cultivars at high salinity. Acta Physiologiae Plantarum, 41(12), 1–13. https://doi.org/10.1007/s11738-019-2988-5

Anthony, C. (1992). The c-type cytochromes of methylotrophic bacteria. BBA - Bioenergetics, 1099(1), 1–15. https://doi.org/10.1016/0005-2728(92)90181-Z

Anthony, C. (2004). The quinoprotein dehydrogenases for methanol and glucose. Archives of Biochemistry and Biophysics, 428(1), 2–9. https://doi.org/10.1016/j.abb.2004.03.038

Apse, M. P., Aharon, G. S., Snedden, W. A., & Blumwald, E. (1999). Salt Tolerance Conferred by Overexpression of a Vacuolar Na^+/H^+ Antiport in *Arabidopsis*. Science, 285(5431), 1256–1258.

https://doi.org/10.1126/SCIENCE.285.5431.1256

Bentley, S. (2009). Sequencing the species pan-genome. Nature Reviews Microbiology, 7(4), 258–259. https://doi.org/10.1038/nrmicro2123

Bogart, J. A., Lewis, A. J., & Schelter, E. J. (2015). DFT Study of the Active Site of the XoxF-Type Natural, Cerium-Dependent Methanol Dehydrogenase Enzyme. Chemistry - A European Journal, 21(4), 1743–1748. https://doi.org/10.1002/chem.201405159

Borel, C., Frey, A., Marion-Poll, A., Tardieu, F., & Simonneau, T. (2001). Does engineering abscisic acid biosynthesis in *Nicotiana plumbaginifolia* modify stomatal response to drought? Plant, Cell and Environment, 24(5), 477–489. https://doi.org/10.1046/j.1365-3040.2001.00698.x

Chang, X., Jiang, F., Wang, T., Kan, W., Qu, Z., Ren, L., Fang, C., & Peng, F. (2014). *Spirosoma arcticum* sp. nov., isolated from high Arctic glacial till. International Journal of Systematic and Evolutionary Microbiology, 64(Pt_7), 2233–2237. https://doi.org/10.1099/ijs.0.061853-0

Chistoserdova, L. (2011). Modularity of methylotrophy, revisited. Environmental Microbiology, 13(10), 2603–2622. https://doi.org/10.1111/j.1462-2920.2011.02464.x

Chistoserdova, L., Kalyuzhnaya, M. G., & Lidstrom, M. E. (2009). The expanding world of methylotrophic metabolism. Annual Review of Microbiology, 63, 477–499. https://doi.org/10.1146/annurev.micro.091208.073600

Chistoserdova, L., & Lidstrom, M. E. (2013). Aerobic methylotrophic prokaryotes. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), The Prokaryotes: Prokaryotic Physiology and Biochemistry (4th ed., pp. 267–285). Springer-Verlag Berlin Heidelberg. https://doi.org/10.1007/978-3-642-30141-4

Christiansen-Weniger, C., & van Veen, J. A. (1991). Nitrogen fixation by *Azospirillium brasilense* in soil and the rhizosphere under controlled environmental conditions. Biology and Fertility of Soils, 12(2), 100–106. https://doi.org/10.1007/BF00341483

Chu, F., Beck, D. A. C., & Lidstrom, M. E. (2016). MxaY regulates the lanthanide-mediated methanol dehydrogenase switch in *Methylomicrobium buryatense*. PeerJ, 1–16. https://doi.org/10.7717/peerj.2435

Chu, F., & Lidstrom, M. E. (2016). XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*.

Journal of Bacteriology, 198(8), 1317–1325. https://doi.org/10.1128/JB.00959-15

Claeys, H., Van Landeghem, S., Dubois, M., Maleux, K., & Inzé, D. (2014). What Is Stress? Dose-response effects in commonly used in vitro stress assays. In Plant Physiology (Vol. 165, Issue 2, pp. 519–527). American Society of Plant Biologists. https://doi.org/10.1104/pp.113.234641

Corwin, D. L. (2020). Climate change impacts on soil salinity in agricultural areas. European Journal of Soil Science, June, 1–21. https://doi.org/10.1111/ejss.13010

Daliakopoulos, I. N., Tsanis, I. K., Koutroulis, A., Kourgialas, N. N., Varouchakis, A. E., Karatzas, G. P., & Ritsema, C. J. (2016). The threat of soil salinity: A European scale review. Science of the Total Environment, 573, 727–739. https://doi.org/10.1016/j.scitotenv.2016.08.177

Daumann, L. J. (2019). Essential and Ubiquitous: The Emergence of Lanthanide Metallobiochemistry. Angewandte Chemie - International Edition, 58(37), 12795–12802. https://doi.org/10.1002/anie.201904090

DeAngelis, K. M., Brodie, E. L., DeSantis, T. Z., Andersen, G. L., Lindow, S. E., & Firestone, M. K. (2009). Selective progressive response of soil microbial community to wild oat roots. ISME Journal, 3(2), 168–178. https://doi.org/10.1038/ismej.2008.103

Del Chierico, F., Ancora, M., Marcacci, M., Cammà, C., Putignani, L., & Conti, Salvatoref Next-Generation Sequencing Pipelines. In: Mengoni A., Galardini M., F. M. (2015). Choice of next-generation sequencing pipelines. In A. Mengoni, M. Galardini, & M. Fondi (Eds.), Bacterial Pangenomics (1st ed., Vol. 1231, pp. 31–47). Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-1720-4

Dodd, I. C., Zinovkina, N. Y., Safronova, V. I., & Belimov, A. A. (2010). Rhizobacterial mediation of plant hormone status. Annals of Applied Biology, 157(3), 361–379. https://doi.org/10.1111/j.1744-7348.2010.00439.x

Dunlap, J. R., & Binzel, M. L. (1996). NaCl Reduces Indole-3-Acetic Acid Levels in the Roots of Tomato Plants lndependent of Stress-lnduced Abscisic Acid'. Plant Physiol, 112(1), 379–384. https://doi.org/https://doi.org/10.1104/pp.112.1.379

Egamberdieva, D. (2009). Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiologiae Plantarum, 31(4), 861–864. https://doi.org/10.1007/s11738-009-0297-0

Etesami, H., & Maheshwari, D. K. (2018). Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. Ecotoxicology and Environmental Safety, 156 (October 2017), 225–246. https://doi.org/10.1016/j.ecoenv.2018.03.013

Euzéby, J. (2018). List of new names and new combinations previously effectively, but not validly, published. International Journal of Systematic and Evolutionary Microbiology, 59(5), 923–925. https://doi.org/10.1099/ijs.0.013961-0

Fall, R., & Benson, A. A. (1996). Leaf methanol - The simplest natural product from plants. Trends in Plant Science, 1(9), 296–301. https://doi.org/10.1016/1360-1385(96)88175-1

Featherston, E. R., & Cotruvo, J. A. (2021). The biochemistry of lanthanide acquisition, trafficking, and utilization. Biochimica et Biophysica Acta - Molecular Cell Research, 1868(1), 118864. https://doi.org/10.1016/j.bbamcr.2020.118864

Fei, Q., Guarnieri, M. T., Tao, L., Laurens, L. M. L., Dowe, N., & Pienkos, P. T. (2014). Bioconversion of natural gas to liquid fuel: Opportunities and challenges. Biotechnology Advances, 32(3), 596–614. https://doi.org/10.1016/j.biotechadv.2014.03.011

Flowers, T. J., & Yeo, A. R. (1986). Ion Relations of Plants under Drought and Salinity. In Aust. J. Plant Physiol (Vol. 13).

Forni, C., Duca, D., & Glick, B. R. (2017). Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant and Soil, 410(1–2), 335–356. https://doi.org/10.1007/s11104-016-3007-x

Galbally, I. E., & Kirstine, W. (2002). The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry, 43, 195–229. https://doi.org/10.1023/A:1020684815474

García-López, M., Meier-Kolthoff, J. P., Tindall, B. J., Gronow, S., Woyke, T., Kyrpides, N. C., Hahnke, R. L., & Göker, M. (2019). Analysis of 1,000 Type-Strain Genomes Improves Taxonomic Classification of *Bacteroidetes*.

Frontiers in Microbiology, 10, 2083. https://doi.org/10.3389/fmicb.2019.02083

Gevers, D., Cohan, F. M., Lawrence, J. G., Spratt, B. G., Coenye, T., Feil, E. J., Stackebrandt, E., Van de Peer, Y., Vandamme, P., Thompson, F. L., & Swings, J. (2005). Re-evaluating prokaryotic species. In Nature Reviews Microbiology (Vol. 3, Issue 9, pp. 733–739). Nature Publishing Group. https://doi.org/10.1038/nrmicro1236

Glick, B. R. (2014). Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiological Research, 169(1), 30–39. https://doi.org/10.1016/j.micres.2013.09.009

Gupta, M., Abu-Ghannam, N., & Gallaghar, E. (2010). Barley for brewing: Characteristic changes during malting, brewing and applications of its by-products. Comprehensive Reviews in Food Science and Food Safety, 9(3), 318–328. https://doi.org/10.1111/j.1541-4337.2010.00112.x

Habashi, F. (2013). Extractive metallurgy of rare earths. In Canadian Metallurgical Quarterly (Vol. 52, Issue 3, pp. 224–233). https://doi.org/10.1179/1879139513Y.0000000081

Hibi, Y., Asai, K., Arafuka, H., Hamajima, M., Iwama, T., & Kawai, K. (2011). Molecular structure of La^{3+}-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. Journal of Bioscience and Bioengineering, 111(5), 547–549. https://doi.org/10.1016/j.jbiosc.2010.12.017

Hmaeid, N., Metoui, O., Wali, M., Zorrig, W., & Abdelly, C. (2014). Comparative effects of Rhizobacteria in promoting growth of *Hordeum maritimum L*. plants under salt stress. Journal of Plant Biology Research, 3(1), 37–50. http://www.inast.org/jpbr.html

Hördt, A., López, M. G., Meier-Kolthoff, J. P., Schleuning, M., Weinhold, L.-M., Tindall, B. J., Gronow, S., Kyrpides, N. C., Woyke, T., & Göker, M. (2020). Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. Frontiers in Microbiology, 11, 468. https://www.frontiersin.org/article/10.3389/fmicb.2020.00468

Hoshino, M., Sanematsu, K., & Watanabe, Y. (2016). REE Mineralogy and Resources. In Handbook on the Physics and Chemistry of Rare Earths (1st ed., Vol. 49, pp. 129–291). Elsevier B.V. https://doi.org/10.1016/bs.hpcre.2016.03.006

Iguchi, H., Yurimoto, H., & Sakai, Y. (2015). Interactions of Methylotrophs with Plants and Other Heterotrophic Bacteria. Microorganisms, 3(2), 137–151. https://doi.org/10.3390/microorganisms3020137

Ilangumaran, G., & Smith, D. L. (2017). Plant Growth Promoting Rhizobacteria in Amelioration of Salinity Stress: A Systems Biology Perspective. Frontiers in Plant Science, 8, 1768. https://doi.org/10.3389/fpls.2017.01768

Jiang, H., Chen, Y., Jiang, P., Zhang, C., Smith, T. J., Murrell, J. C., & Xing, X. H. (2010). Methanotrophs: Multifunctional bacteria with promising applications in environmental bioengineering. Biochemical Engineering Journal, 49(3), 277–288. https://doi.org/10.1016/j.bej.2010.01.003

Kalyuzhnaya, M. G. (2016). Methane Biocatalysis: Selecting the Right Microbe. In Biotechnology for Biofuel Production and Optimization (pp. 353–383). Elsevier B.V. https://doi.org/10.1016/B978-0-444-63475-7.00013-3

Kang, S. M., Khan, A. L., Waqas, M., You, Y. H., Kim, J. H., Kim, J. G., Hamayun, M., & Lee, I. J. (2014). Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. Journal of Plant Interactions, 9(1), 673–682. https://doi.org/10.1080/17429145.2014.894587

Keltjens, J. T., Pol, A., Reimann, J., & Op Den Camp, H. J. M. (2014). PQQ-dependent methanol dehydrogenases: Rare-earth elements make a difference. Applied Microbiology and Biotechnology, 98(14), 6163–6183. https://doi.org/10.1007/s00253-014-5766-8

Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology, 96(3), 473–480. https://doi.org/10.1046/j.1365-2672.2003.02161.x

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., Von Mering, C., & Vorholt, J. A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME Journal, 6(7), 1378–1390. https://doi.org/10.1038/ismej.2011.192

Kolb, S. (2009). Aerobic methanol-oxidizing Bacteria in soil. FEMS Microbiology Letters, 300(1), 1–10. https://doi.org/10.1111/j.1574-6968.2009.01681.x

Kumar, B., Trivedi, P., & Pandey, A. (2007). *Pseudomonas corrugata*: A suitable bacterial inoculant for maize grown under rainfed conditions of Himalayan

region. Soil Biology and Biochemistry, 39(12), 3093–3100. https://doi.org/10.1016/j.soilbio.2007.07.003

Kumar, M., Kour, D., Yadav, A. N., Saxena, R., Rai, P. K., Jyoti, A., & Tomar, R. S. (2019). Biodiversity of methylotrophic microbial communities and their potential role in mitigation of abiotic stresses in plants. Biologia, 74(3), 287–308. https://doi.org/10.2478/s11756-019-00190-6

Kumar, V., Behl, R. K., & Narula, N. (2001). Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under green house conditions. Microbiological Research, 156(1), 87–93. https://doi.org/10.1078/0944-5013-00081

Kumari, S., Vaishnav, A., Jain, S., Varma, A., & Choudhary, D. K. (2015). Bacterial-Mediated Induction of Systemic Tolerance to Salinity with Expression of Stress Alleviating Enzymes in Soybean (*Glycine max L. Merrill*). Journal of Plant Growth Regulation, 34(3), 558–573. https://doi.org/10.1007/s00344-015-9490-0

Li, W., Lee, S. Y., Kang, I. K., Ten, L. N., & Jung, H. Y. (2018). *Spirosoma agri* sp. nov., isolated from apple orchard soil. Journal of Microbiology, 56(2), 90–96. https://doi.org/10.1007/s12275-018-7430-y

Li, W., Ten, L. N., Lee, S. Y., Lee, D. H., & Jung, H. Y. (2018). *Spirosoma jeollabukense* sp. nov., isolated from soil. Archives of Microbiology, 200(3), 431–438. https://doi.org/10.1007/s00203-017-1453-3

Loman, N. J., & Pallen, M. J. (2015). Twenty years of bacterial genome sequencing. Nature Reviews Microbiology, 13(12), 787–794. https://doi.org/10.1038/nrmicro3565

Lv, H., Masuda, S., Fujitani, Y., Sahin, N., & Tani, A. (2017). *Oharaeibacter diazotrophicus* gen. nov., sp. nov., a diazotrophic and facultatively methylotrophic bacterium, isolated from rice rhizosphere. International Journal of Systematic and Evolutionary Microbiology, 67(3), 576–582. https://doi.org/10.1099/ijsem.0.001660

Lv, H., Sahin, N., & Tani, A. (2018). Isolation and genomic characterization of *Novimethylophilus kurashikiensis* gen. nov. sp. nov., a new lanthanide-dependent methylotrophic species of *Methylophilaceae*. Environmental Microbiology, 20(3), 1204–1223. https://doi.org/10.1111/1462-2920.14062

Lv, H., & Tani, A. (2018). Genomic characterization of methylotrophy of *Oharaeibacter diazotrophicus* strain SM30T. Journal of Bioscience and

Bioengineering, 126(6), 667–675. https://doi.org/10.1016/j.jbiosc.2018.05.023

Macey, M. C., Pratscher, J., Crombie, A. T., & Murrell, J. C. (2020). Impact of plants on the diversity and activity of methylotrophs in soil. Microbiome, 8(1), 1–17. https://doi.org/10.1186/s40168-020-00801-4

MacGregor, A. W. (2003). Barley - Origin Adaptation, and Production. In L. Trugo & P. Finglas. (Eds.), Encyclopedia of Food Sciences and Nutrition (Second, pp. 379–382). Academic Press. https://www.sciencedirect.com/science/article/pii/B012227055X00081X

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. Archives of Biochemistry and Biophysics, 444(2), 139–158. https://doi.org/10.1016/j.abb.2005.10.018

Maksimavičius, E., & Roslev, P. (2020). Methane emission and methanotrophic activity in groundwater-fed drinking water treatment plants. Water Science and Technology: Water Supply, 20(3), 819–827. https://doi.org/10.2166/ws.2020.009

Marroquí, S., Zorreguieta, A., Santamaría, C., Temprano, F., Soberón, M., Megías, M., & Downie, J. A. (2001). Enhanced symbiotic performance by *Rhizobium tropici* glycogen synthase mutants. Journal of Bacteriology, 183(3), 854–864. https://doi.org/10.1128/JB.183.3.854-864.2001

Marulanda, A., Azcón, R., Chaumont, F., Ruiz-Lozano, J. M., & Aroca, R. (2010). Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays L.*) plants under unstressed and salt-stressed conditions. Planta, 232(2), 533–543. https://doi.org/10.1007/s00425-010-1196-8

Mazurier, S., Corberand, T., Lemanceau, P., & Raaijmakers, J. M. (2009). Phenazine antibiotics produced by fluorescent *pseudomonads* contribute to natural soil suppressiveness to Fusarium wilt. ISME Journal, 3(8), 977–991. https://doi.org/10.1038/ismej.2009.33

Migula, W. (1894). Über ein neues System der Bakterien, Arbeiten aus de, Bakteriologischen Institut der Technischen Hochschule zu Karlsruhe.

Moore, E. R. B., Mihaylova, S. A., Vandamme, P., Krichevsky, M. I., & Dijkshoorn, L. (2010). Microbial systematics and taxonomy: Relevance for a microbial commons. Research in Microbiology, 161(6), 430–438. https://doi.org/10.1016/j.resmic.2010.05.007

Mukhopadhyay, R., Sarkar, B., Jat, H. S., Sharma, P. C., & Bolan, N. S. (2020). Soil salinity under climate change: Challenges for sustainable agriculture and food security. Journal of Environmental Management, June, 111736. https://doi.org/10.1016/j.jenvman.2020.111736

Munns, R., & Passioura, J. B. (1984). Effect of prolonged exposure to NaCl on the osmotic pressure of leaf xylem sap from intact, transpiring barley plants. Australian Journal of Plant Physiology, 11(6), 497–507. https://doi.org/10.1071/PP9840497

Munns, R., Schachtman, D. P., & Condon, A. G. (1995). The significance of a two-phase growth response to salinity in wheat and barley. Australian Journal of Plant Physiology, 22(4), 561–569. https://doi.org/10.1071/PP9950561

Munns, R. (1992). A Leaf Elongation Assay Detects an Unknown Growth Inhibitor in Xylem Sap From Wheat and Barley. Functional Plant Biology, 19(2), 127. https://doi.org/10.1071/pp9920127

Munns, Rana, & Tester, M. (2008). Mechanisms of salinity tolerance. Annual Review of Plant Biology, 59, 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Muratova, A. Y., Turkovskaya, O. V., Antonyuk, L. P., Makarov, O. E., Pozdnyakova, L. I., & Ignatov, V. V. (2005). Oil-oxidizing potential of associative rhizobacteria of the genus *Azospirillum*. Microbiology, 74(2), 210–215. https://doi.org/10.1007/s11021-005-0053-4

Mustafa, G., Akhtar, M. S., & Abdullah, R. (2019). Global concern for salinity on various agro-ecosystems. In Salt Stress, Microbes, and Plant Interactions: Causes and Solution: Volume 1 (pp. 1–19). Springer Singapore. https://doi.org/10.1007/978-981-13-8801-9_1

Nadeem, S. M., Zahir, Z. A., Naveed, M., & Arshad, M. (2009). Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Canadian Journal of Microbiology, 55(11), 1302–1309. https://doi.org/10.1139/W09-092

Nazaries, L., Murrell, J. C., Millard, P., Baggs, L., & Singh, B. K. (2013). Methane, microbes and models: Fundamental understanding of the soil methane cycle for future predictions. Environmental Microbiology, 15(9), 2395–2417. https://doi.org/10.1111/1462-2920.12149

Nieto, K. F., & Frankenberger, W. T. (1989). Biosynthesis of cytokinins by *Azotobacter chroococcum*. Soil Biology and Biochemistry, 21(7), 967–972. https://doi.org/10.1016/0038-0717(89)90089-8

Ochsner, A. M., Hemmerle, L., Vonderach, T., Nüssli, R., Bortfeld-Miller, M., Hattendorf, B., & Vorholt, J. A. (2019). Use of rare-earth elements in the phyllosphere colonizer *Methylobacterium extorquens* PA1. Molecular Microbiology, 111(5), 1152–1166. https://doi.org/10.1111/mmi.14208

Op den Camp, H. J. M., Islam, T., Stott, M. B., Harhangi, H. R., Hynes, A., Schouten, S., Jetten, M. S. M., Birkeland, N. K., Pol, A., & Dunfield, P. F. (2009). Environmental, genomic and taxonomic perspectives on methanotrophic Verrucomicrobia. Environmental Microbiology Reports, 1(5), 293–306. https://doi.org/10.1111/j.1758-2229.2009.00022.x

Ortiz, N., Armada, E., Duque, E., Roldán, A., & Azcón, R. (2015). Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. Journal of Plant Physiology, 174, 87–96. https://doi.org/10.1016/j.jplph.2014.08.019

Park, D., & Lee, J. (2013). Biological conversion of methane to methanol. Korean Journal of Chemical Engineering, 30(5), 977–987. https://doi.org/10.1007/s11814-013-0060-5

Passioura, J. B., & Munns, R. (2000). Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate. Australian Journal of Plant Physiology, 27(10), 941–948. https://doi.org/10.1071/pp99207

Pel, Z. M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G. J., Grill, E., & Schroeder, J. I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature, 406(6797), 731–734. https://doi.org/10.1038/35021067

Pol, A., Barends, T. R. M., Dietl, A., Khadem, A. F., Eygensteyn, J., Jetten, M. S. M., & Op den Camp, H. J. M. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environmental Microbiology, 16(1), 255–264. https://doi.org/10.1111/1462-2920.12249

Porras-Soriano, A., Soriano-Martín, M. L., Porras-Piedra, A., & Azcón, R. (2009). Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance

to salinity in olive trees under nursery conditions. Journal of Plant Physiology, 166(13), 1350–1359. https://doi.org/10.1016/j.jplph.2009.02.010

Radzki, W., Gutierrez Mañero, F. J., Algar, E., Lucas García, J. A., García-Villaraco, A., & Ramos Solano, B. (2013). Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 104(3), 321–330. https://doi.org/10.1007/s10482-013-9954-9

Read, J., Gill, R., Dales, S. L., Cooper, J. B., Wood, S. P., & Anthony, C. (1999). The molecular structure of an unusual cytochrome c 2 determined at 2.0 å; the cytochrome c h from *Methylobacterium extorquens*. Protein Science, 8(6), 1232–1240. https://doi.org/10.1110/ps.8.6.1232

Rodriguez, H., Gonzalez, T., Goire, I., & Bashan, Y. (2004). Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften, 91(11), 552–555. https://doi.org/10.1007/s00114-004-0566-0

Roumpeka, D. D., Wallace, R. J., Escalettes, F., Fotheringham, I., & Watson, M. (2017). A review of bioinformatics tools for bio-prospecting from metagenomic sequence data. Frontiers in Genetics, 8(MAR), 1–10. https://doi.org/10.3389/fgene.2017.00023

Safdarian, M., Askari, H., Shariati J, V., & Nematzadeh, G. (2019). Transcriptional responses of wheat roots inoculated with *Arthrobacter nitroguajacolicus* to salt stress. Scientific Reports, 9(1), 1–12. https://doi.org/10.1038/s41598-018-38398-2

Schoenborn, L., Yates, P. S., Grinton, B. E., Hugenholtz, P., & Janssen, P. H. (2004). Liquid serial dilution is inferior to solid media for isolation of cultures representative of the phylum-level diversity of soil bacteria. Applied and Environmental Microbiology, 70(7), 4363–4366. https://doi.org/10.1128/AEM.70.7.4363-4366.2004

Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences, 22(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Siddikee, M. A., Glick, B. R., Chauhan, P. S., Yim, W. jong, & Sa, T. (2011). Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum L.*) by regulating stress ethylene synthesis with halotolerant bacteria

containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiology and Biochemistry, 49(4), 427–434. https://doi.org/10.1016/j.plaphy.2011.01.015

Singh, H., Chen, Y., Tabazadeh, A., Fukui, Y., Bey, I., Yantosca, R., Jacob, D., Arnold, F., Wohlfrom, K., Atlas, E., Flocke, F., Blake, D., Blake, N., Heikes, B., Snow, J., Talbot, R., Gregory, G., Sachse, G., Vay, S., & Kondo, Y. (2000). Distribution and fate of selected oxygenated organic species in the troposphere and lower stratosphere over the Atlantic. Journal of Geophysical Research: Atmospheres, 105(D3), 3795–3805. https://doi.org/10.1029/1999JD900779

Skovran, E., Raghuraman, C., & Martinez-gomez, N. C. (2019). Lanthanides in Methylotrophy. In L. Chistoserdova (Ed.), Methylotrophs and Methylotroph Communities (2019th ed., Vol. 33, pp. 101–115). Caister Academic Press. https://doi.org/10.21775/cimb.033.101

Stacheter, A., Noll, M., Lee, C. K., Selzer, M., Glowik, B., Ebertsch, L., Mertel, R., Schulz, D., Lampert, N., Drake, H. L., & Kolb, S. (2013). Methanol oxidation by temperate soils and environmental determinants of associated methylotrophs. ISME Journal, 7(5), 1051–1064. https://doi.org/10.1038/ismej.2012.167

Stackebrandt, E., & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. Microbiology Today, 33, 152–155.

Stackebrandt, E., Frederiksen, W., Garrity, G. M., Grimont, P. A. D., Kämpfer, P., Maiden, M. C. J., Nesme, X., Rosselló-Mora, R., Swings, J., Trüper, H. G., Vauterin, L., Ward, A. C., & Whitman, W. B. (2002). Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. International Journal of Systematic and Evolutionary Microbiology, 52(3), 1043–1047. https://doi.org/10.1099/00207713-52-3-1043

Staudinger, C., Mehmeti-Tershani, V., Gil-Quintana, E., Gonzalez, E. M., Hofhansl, F., Bachmann, G., & Wienkoop, S. (2016). Evidence for a rhizobia-induced drought stress response strategy in *Medicago truncatula*. Journal of Proteomics, 136, 202–213. https://doi.org/10.1016/j.jprot.2016.01.006

Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A. M. Z., Geissler-Plaum, R., & Schnell, S. (2015). Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare L.*)

under salt stress. Applied Soil Ecology, 95, 23–30. https://doi.org/10.1016/j.apsoil.2015.04.017

Suarez, C., Ratering, S., Geissler-Plaum, R., & Schnell, S. (2014). *Hartmannibacter diazotrophicus* gen. nov., Sp. nov., A phosphate-solubilizing and nitrogen-fixing alphaproteobacterium isolated from the rhizosphere of a natural salt-meadow plant. International Journal of Systematic and Evolutionary Microbiology, 64, 3160–3167. https://doi.org/10.1099/ijs.0.064154-0

Suarez, C., Ratering, S., Hain, T., Fritzenwanker, M., Goesmann, A., Blom, J., Chakraborty, T., Bunk, B., Spröer, C., Overmann, J., & Schnell, S. (2019). Complete Genome Sequence of the Plant Growth-Promoting Bacterium *Hartmannibacter diazotrophicus* Strain E19^{T}. International Journal of Genomics, 2019, 1–12.

Sukweenadhi, J., Kim, Y. J., Choi, E. S., Koh, S. C., Lee, S. W., Kim, Y. J., & Yang, D. C. (2015). *Paenibacillus yonginensis* DCY84T induces changes in *Arabidopsis thaliana* gene expression against aluminum, drought, and salt stress. Microbiological Research, 172, 7–15. https://doi.org/10.1016/j.micres.2015.01.007

Suleman, M., Yasmin, S., Rasul, M., Yahya, M., Atta, B. M., & Mirza, M. S. (2018). Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. PLoS ONE, 13(9), 1–28. https://doi.org/10.1371/journal.pone.0204408

Takahashi, Y., Châtellier, X., Hattori, K. H., Kato, K., & Fortin, D. (2005). Adsorption of rare earth elements onto bacterial cell walls and its implication for REE sorption onto natural microbial mats. Chemical Geology, 219(1–4), 53–67. https://doi.org/10.1016/j.chemgeo.2005.02.009

Tien, T. M., Gaskins, M. H., & Hubbell, D. H. (1979). Plant Growth Substances Produced by *Azospirillum brasilense* and Their Effect on the Growth of Pearl Millet (*Pennisetum americanum L.*) . Applied and Environmental Microbiology, 37(5), 1016–1024. https://doi.org/10.1128/aem.37.5.1016-1024.1979

Timmusk, S., Abd El-Daim, I. A., Copolovici, L., Tanilas, T., Kännaste, A., Behers, L., Nevo, E., Seisenbaeva, G., Stenström, E., & Niinemets, Ü. (2014). Drought-Tolerance of Wheat Improved by Rhizosphere Bacteria from Harsh Environments: Enhanced Biomass Production and Reduced Emissions of

Stress Volatiles. PLoS ONE, 9(5), e96086. https://doi.org/10.1371/journal.pone.0096086

Tindall, B. J., Rosselló-Móra, R., Busse, H. J., Ludwig, W., & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. International Journal of Systematic and Evolutionary Microbiology, 60(1), 249–266. https://doi.org/10.1099/ijs.0.016949-0

Turner, T. R., Ramakrishnan, K., Walshaw, J., Heavens, D., Alston, M., Swarbreck, D., Osbourn, A., Grant, A., & Poole, P. S. (2013). Comparative metatranscriptomics reveals kingdom level changes in the rhizosphere microbiome of plants. ISME Journal, 7(12), 2248–2258. https://doi.org/10.1038/ismej.2013.119

Ullah, S., & Bano, A. (2015). Isolation of plant-growth-promoting rhizobacteria from rhizospheric soil of halophytes and their impact on maize (*Zea mays L.*) under induced soil salinity. Canadian Journal of Microbiology, 61(4), 307–313. https://doi.org/10.1139/cjm-2014-0668

Vanwonterghem, I., Evans, P. N., Parks, D. H., Jensen, P. D., Woodcroft, B. J., Hugenholtz, P., & Tyson, G. W. (2016). Methylotrophic methanogenesis discovered in the archaeal phylum *Verstraetearchaeota*. Nature Microbiology, 1(12), 1–9. https://doi.org/10.1038/nmicrobiol.2016.170

Von Bothmer, R., & Komatsuda, T. (2011). Barley Origin and Related Species. In S. E. Ullrich (Ed.), Barley: Production, Improvement, and Uses (pp. 14–62). Wiley-Blackwell. https://doi.org/10.1002/9780470958636.ch2

Vu, H. N., Subuyuj, G. A., Vijayakumar, S., Good, N. M., Martinez-Gomez, N. C., & Skovran, E. (2016). Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. Journal of Bacteriology, 198(8), 1250–1259. https://doi.org/10.1128/JB.00937-15

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E., Stackebrandt, E., Starr, M. P., & Truper, H. G. (1987). Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. International Journal of Systematic and Evolutionary Microbiology, 37(4), 463–464. https://doi.org/10.1099/00207713-37-4-463

Weyens, N., Beckers, B., Schellingen, K., Ceulemans, R., van der Lelie, D., Newman, L., Taghavi, S., Carleer, R., & Vangronsveld, J. (2015). The

Potential of the Ni-Resistant TCE-Degrading *Pseudomonas putida* W619-TCE to Reduce Phytotoxicity and Improve Phytoremediation Efficiency of Poplar Cuttings on A Ni-TCE Co-Contamination. International Journal of Phytoremediation, 17(1), 40–48. https://doi.org/10.1080/15226514.2013.828016

Whiticar, M. J. (2020). The Biogeochemical Methane Cycle. In Hydrocarbons, Oils and Lipids: Diversity, Origin, Chemistry and Fate (pp. 1–78). https://doi.org/10.1007/978-3-319-54529-5_5-1

Williams, P. A., Coates, L., Mohammed, F., Gill, R., Erskine, P. T., Coker, A., Wood, S. P., Anthony, C., & Cooper, J. B. (2005). The atomic resolution structure of methanol dehydrogenase from *Methylobacterium extorquens*. Acta Crystallographica Section D: Biological Crystallography, 61(1), 75–79. https://doi.org/10.1107/S0907444904026964

Wise, R. R., & Naylor, A. W. (1987). Chilling-Enhanced Photooxidation. Plant Physiology, 83(2), 278–282. https://doi.org/10.1104/pp.83.2.278

Xu, C. M., Zhao, B., Wang, X. D., & Wang, Y. C. (2007). Lanthanum relieves salinity-induced oxidative stress in *Saussurea involucrata*. Biologia Plantarum, 51(3), 567–570. https://doi.org/10.1007/s10535-007-0124-7

Yao, L., Wu, Z., Zheng, Y., Kaleem, I., & Li, C. (2010). Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. European Journal of Soil Biology, 46(1), 49–54. https://doi.org/10.1016/j.ejsobi.2009.11.002

Yeo, A. R., Lee, K.-S., Boursier, P. J., & Flowers, T. J. (1991). Short- and Long-Term Effects of Salinity on Leaf Growth in Rice (*Oryza sativa L.*). In Source: Journal of Experimental Botany (Vol. 42, Issue 240).

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., Da Rocha, U. N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B. P., Firestone, M. K., Northen, T. R., & Brodie, E. L. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. Nature Microbiology, 3(4), 470–480. https://doi.org/10.1038/s41564-018-0129-3

Zhang, H., Kim, M. S., Sun, Y., Dowd, S. E., Shi, H., & Paré, P. W. (2008). Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Molecular Plant-Microbe Interactions, 21(6), 737–744. https://doi.org/10.1094/MPMI-21-6-0737

CHAPTER 2.

Effect of *Hartmannibacter diazotrophicus* and lanthanum on the plant growth and microbial communities of barley grown under salt stress

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Effect of *Hartmannibacter diazotrophicus* and lanthanum on the plant growth and microbial communities of barley grown under salt stress

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Abstract

Increasing agriculture soil salinity caused by intensive fertilization or deficient irrigation negatively affects agricultural crop production. *Hartmannibacter diazotrophicus* E19^T was isolated from a salt meadow and exhibited plant growth promoting activities. Substrates provided from plants like methanol support associated microorganism and genome analysis of *H. diazotrophicus* identified the gene *xox*F which encodes for methanol dehydrogenase protein and confers the ability to grow on methanol only in the presence of lanthanum (La). For elucidation of lanthanum on plant growth promotion effects of *H. diazotrophicus* on barley under salt stress conditions (NaCl 3%) seed inoculation was performed in the presence of La. For a greenhouse experiment soil was amended with and without La (8 mg kg^{-1}) and barley seeds were inoculated with alive or dead *H. diazotrophicus.* Plant growth*, H. diazotrophicus* colonization and bacterial community composition and diversity of rhizosphere and roots were studied. Significant differences of fresh and dry weight biomass of leaves and roots were obtained with *H. diazotrophicus* in the presence and absence of La^{3+}. Treatments with La amended soil (without bacterial inoculation) also evidenced significant differences in leaves and roots biomass under salt stress compared to dead biomass addition of the respective bacterial strain and control. Presence of *H. diazotrophicus* in roots was 4.3 10^6 target copies gdw^{-1} as quantified by realtime PCR using a specific primer set for *H. diazotrophicus* based on the 16S rRNA gene. Similar target numbers were obtained with a primer set for the La-dependent methanol dehydrogenease gene (xoxF). Bacterial community analysis on roots and rhizosphere revealed *Rhodanobacteriaceae* (29.3%) as most dominant together with other families such as *Acidobacteriaceae* and *Caulobacteraceae*. Interestingly the alpha diversity analysis indicated higher indices in treatments with *H. diazotrophicus*, and in presence of La. According to nonmetric multidimensional scaling (NMDS) most similar communities were found in treatments of *H. diazotrophicus* and *H. diazotrophicus* with La; most different was the community in control soil. Differential abundance analysis showed *Rhodanobacter* as most frequent ranked genus in all treatments

This study reasserts the role of *H. diazotrophicus* in growth promotion of barley plants under salt stress and the importance of lanthanum for methylotrophic bacteria xoxF4-type.

Keywords: *Hartmannibacter diazotrophicus*, Salt stress, PGPR, Lanthanum, Methylotrophic Bacterium, XoxF

Introduction

Salinization is a problem which affects according to FAO (United Nations Food and Agriculture Organization) already 22% of agricultural lands (Kolodyazhnaya et al., 2009). As a result of the greenhouse effect this value is increasing constantly due to low precipitation and the need of more irrigation. It has been predicted, that more than 50% of the cropland might be salinized by the year 2050 (Ashraf, 1994; Vinocur and Altman, 2005). The soil salinity has a negative impact in agricultural land decreasing crop yield. High concentration of salt ions such as Na^{+}, K^{+}, Cl^{-}, Ca^{+} lead to osmotic stress and inhibits plant growth (Numan et al., 2018; Shrivastava and Kumar, 2015). The constant growing of world population requires to develop strategies for soil recovering and mitigation of salinity for a further increase of crop yield.

One strategy to alleviate salinity stress for crop plants is the application of plant growth promoting rhizobacteria (PGPR) which can be inoculated to crop seeds and by different mechanisms enable better plant growth in saline soil. These mechanism include induction of systemic tolerance (IST), production of ACC-deaminase, support with nutrient uptake and resistance to phytopathogens (Suarez et al., 2015; Yang et al., 2009; Zarea et al., 2012). *Hartmannibacter diazotrophicus* $E19^{T}$ was isolated from a salt meadow and was shown to promote barley growth under salt stress (Suarez et al., 2015). Recently the genome of *H. diazotrophicus* was sequenced and candidate genes for potential plant growth promoting activities were recognized (Suarez et al. 2019) such as *nif* genes involved in nitrogen fixation, genes for acid and alkaline phosphatase associated to phosphorus metabolism, genes for protein expression of *ton*B, *exb*B, and *exb*D related to iron acquisition and also board clusters of genes involved in salt tolerance.

Also three CDSs encoding for methanol dehydrogenase genes were found (Suarez et al. 2019) that as previously shown (Lv and Tani, 2018) belongs to *xoxF* type of the methanol dehydrogenases, that required for its activity the presence of rare metals as a cofactors such as lanthanum (La^{3+}) and Cesium (Ce^{+3}) (Chu and Lidstrom, 2016; Fitriyanto et al., 2011; Lv et al., 2017; Pol et al., 2014). *H. diazotrophicus* expressed only this type and cannot oxidize methanol without lanthanides (Lv et al., 2017).

Methanol is a byproduct of pectine demethylation during the process of cell walls maturation in plants which is exudates in leaves (Fall and Benson, 1996; Galbally and Kirstine, 2002) and was also suggested in roots (Sy et al., 2005). In the root zone and below the *xoxF* type methanol dehydrogenases was found to be one of the most abundant expressed bacterial proteins (Butterfield et al. 2016, Li et al. 2019) and thus methanol could be an important carbon source of *H. diazotrophicus* on the root surface.

In this work we tested the following working hypothesis: (I) Performance of *H. diazotrophicus* as plant growth promoting rhizobacterium on barley plants under salt stress is enhanced in presence of lanthanum. (II) Presence of lanthanum without seed inoculation with *H. diazotrophicus* has no effect on

plant growth. (III) Availability of lanthanum improves rhizosphere abundance of *H. diazotrophicus.* (IV) Bacterial communities are affected by strain E19^T inoculation and lanthanum application in rhizosphere and roots. For testing our hypothesis, a greenhouse experiment was performed with barley under saline soil conditions and soil amendments with/without lanthanum and with/without seed inoculation of barley. Plant biomass, rhizosphere abundance of *H. diazotrophicus,* and effect of amendments of rhizosphere bacterial community were determined.

Material and Methods

Hartmannibacter diazotrophicus E19^T was recultured from a glycerin stock from the culture collection of the Institute of Applied Microbiology, JLU Giessen.

Growth of *H. diazotrophicus* on methanol

For confirmation of lanthanum dependent methanol growth of strain E19^T of our stock culture, a growth test was performed to proof enzymatic activity of the La-containing methanol dehydrogenase of *H. diazotrophicus.* A modified liquid freshwater media (FWM) (Widdel and Bak, 1992), containing 1.0 g NaCl, 0.4 g MgCl x 6H_2O, 0.15 g $CaCl_2$ x 2H_2O, 0.5 g KCl, 0.2 g KH_2PO_4, 0.25 g NH_4Cl, 1.4 g Na_2SO_4, 1.0 ml trace element solution containing no lanthanum (SL10), 50 ml Phosphate buffer 0.4 M pH 7.0, 1.0 ml vitamin B12-solution, 1.0 ml vitamin-5 solution, 1.0 ml thiamine solution, 1.0 ml riboflavine solution in 1.0 liter deionized water, the pH was adjusted to 6.5. Methanol 5% v/v as carbon source and 30 µM $LaCl_3$ was added to this liquid culture medium, as well as *H. diazotrophicus* as inoculum which has been grown on glucose. As further growth tests 5 mM glucose (as a positive control of growth), no carbon source and methanol without lanthanum were included. The cultures were incubated at 28 °C on a shaker with 120 rpm. Growth was monitored by measurements of optical density at 600 nm. Another growth experiment was performed with different concentrations of $LaCl_3$ (0, 30,60,90,120 µM), methanol 5% v/v under slightly saline conditions with 1% NaCl in the above described medium in order to test the optimal lanthanum concentration for growth.

Growth experiment with barley in presence of *H. diazotrophicus*

The effect of *H. diazotrophicus* E19^T on growth of barley seeds was studied in pots filled with soil substrate under salt stress (NaCl 3%). Pots were arranged in a randomized complete block design (RCBD; McKillup, 2011), with six treatments and five replications per treatment. The treatments were (I) E19^T, (II) E19^T with La, (III) E19^T dead biomass, (IV) E19^T dead biomass with La, and (V) non-inoculated seed control. Two suspensions of *H. diazotrophicus* were obtained by growing the bacteria in FWM liquid medium with glucose or FWM with addition of methanol and 30 µM $LaCl_3$ until 10^7 CFU ml^{-1}. The suspension was centrifuged and suspended in 30 mM $MgSO_4$. Seeds of barley were disinfected with 70% ethanol, 2.5% bleach, washed five times with sterile water and incubated for 1 h in the suspension of *H. diazotrophicus* grown in FWM with glucose for treatments of strain E19^T without La or FWM with

methanol for treatments of strain E19^T with La with gentle shaking. Further control assays consisted of barley seeds inoculated with an autoclaved aliquot of *H. diazotrophicus* E19^T to assess the effect of dead bacteria and an assay with non-inoculated seeds.

Plastics pots (13 x 10 cm) were filled with ~ 750 ml (~140 g dry weight) non-sterile soil (Fruhstorfer Erde Hawita typ P, Table. S1). Soil water holding capacity (WHC) was determinate to be 225 ml and each pot was watered with 170 ml water (~75% WHC) in order to keep the soil substrate moistened. Ten barley seeds were placed on the filled soil pots and were covered with 1 cm layer of soil substrate. The experiments were carried out in a greenhouse with 12 h of light, 21 °C of temperature. After germination the pots were rarefied to 6 plants per pot.

In order to establish salt stress, the pots were irrigated three times during sowing, germination and rarefaction phases with 136 mM NaCl solutions in water until a salinity of 3.0% was reached. To evaluate the sorption of La^{3+} for the used substrate, the substrate without plants was flushed with solutions containing different La^{3+} concentration and the concentration of La^{3+} was determined by ICP-MS analysis in the runout (Table S2). Afterwards for the plant experiment a solution $LaCl_3$ in water was added into the soil during seeding to obtain a concentration of 8 mg La kg^{-1}.

Plants were monitored for 35 day. Five plants per pot were separated for harvest of leaves and roots and collected in paper bags for determination of fresh weights. From root biomass soil was washed out thoroughly and air dried before fresh weights were determined. For dry biomass leaves and roots were dried at 80 °C for 48 h and subsequently their weights were recorded.

Data normality was evaluated by Anderson-darling-test (Fig. S3); statistical differences of plants fresh and dry weight between treatments were analyzed by ANOVA followed by Tukey post-hoc tests at $P<0.05$ using the Minitab 19 Statistical Software (2019).

DNA extraction from rhizosphere and roots

From each pot one plant was separated and carefully the soil loosely adhering to the roots was removed by gentle shaking, collected in sterile centrifuge tubes and frozen to -80 °C. Additionally from the same plant one long root was cut out, remaining soil was removed by gentle shaking, afterwards root was collected in DNA-free tube and stored at -80 °C. For DNA extraction samples of rhizosphere were defrosted and kept in ice box. Approximately, 300 mg rhizosphere soil was transferred to tubes with ceramic beads and DNA was extracted using NucleoSpin ® Soil kit (Macherey- Nagel, Düren, Germany). Roots samples were disrupted to fine powder with a mortar and pestle using liquid nitrogen. Mortar and pestle were before use washed with soap, then rinsed with deionized water, flame treated with 100% ethanol and in last step the tools were wiped with ROTI®Nucleic Acid-free solution (ROTH, Germany). From the powder 300 mg was transferred to tubes with ceramics beads. To these tubes with powdered roots and ceramic beads 1 ml extraction buffer (5 g l^{-1} SDS, 0.2 M sodium phosphate buffer (pH 8), 50 mM EDTA and 0.1 M NaCl, pH 8) was added and shaken for 2 min at maximum speed in a cell mill MM200 (Retsch Haan, Germany). Then samples were centrifuged at 4 °C and 10.000 g for 5 min in a microcentrifuge (Heraeus Fresco, Thermo Fisher Scientific Inc., Waltham, USA). The supernatant was collected in a new tube, added 5 µl RNase A (10 mg ml^{-1}) and incubated at 37 °C for 30 min.

DNA from roots was extracted using a modified phenol-chloroform methodology (Bürgmann et al., 2001). After RNAse A treatment 500 µl of phenol/chloroform/isoamyl alcohol (25:24:1) were added and mixed by inverting the tubes 5 times. The tubes were centrifuged again at 10.000 g for 5 min at 4 °C; aqueous phase was collected in a new DNA-free tube and chloroform/isoamyl alcohol (24:1) was added, mixed by gentle inverting and centrifuged again at 10.000 g for 5 min at 4 °C. Aqueous phase was recovered and DNA was precipitated with 1 ml of PEG [20% Poly (ethylene glycol) and 2.5 M NaCl], tubes were kept in ice box at 4 °C for 30 min and centrifuged again at 10.000 g for 5 min at 4 °C; PEG was discarded and DNA pellets were washed with ice cold 75% ethanol, dried out, dissolved in nuclease free water. DNA extracts of rhizosphere and roots were used for quantification of strain E19^T by real-time PCR and taxonomic metabarcode sequencing by Ion Torrent.

Quantification of *H. diazotrophicus* by real-time-PCR (qPCR)

A specific primer set (E19_F_932: 5'-GTCCGGCTATCCAGAGAGAT-3'; E19_R_1261: 5'-ATTAGCTGACCCTCGAGGGT-3') targeting the 16S rRNA gene of *H. diazotrophicus* has been developed through alignment of 16S rRNA gene of strain E19^T and the closest relatives. The sequences obtained from the SILVA database (Quast et al., 2013) were aligned and merged with the database LTPs111 (Feb 2013) (Yilmaz et al., 2014) using the ARB version 5.2 program (Ludwig et al., 2004). The specificity of the primer pair was checked using the online program Probe Check (Loy et al., 2008) and also by cloning and sequencing of the PCR products amplified from DNA from bulk and rhizospheric soil of plants in two different environments. Likewise, a primer pair set (E19_F_*xoxF*: 5'-CGCTGCCATCTCACTGCCTA-3'; E19_R_*xoxF*: 5'-ACTGGTCGCCTTCCCATGTG-3') targeting the *xoxF*-gene in strain E19^T was designed on primerBLAST tool of NCBI (Ye et al., 2012) using NZ_LT960614, region: 2806010-2807833 sequence of the GenBank; the specificity of the primers were confirmed as well with primerBLAST.

For cloning, PCR products were cleaned using NucleoSpin gel PCR clean-up (Macherey-Nagel) and ligated by TA-cloning to a vector plasmid pGEM-T (Promega GmbH, Mannheim, Germany); competent cells of *Escherichia coli* JM 109 (Promega) were transformed with the plasmids thereafter plasmids from transformed cells were isolated as described in Kampmann et al., 2012. The purified plasmids with insert were quantified using the fluorescence of DNA-quantification Helixyte Green kit (Biomol, Hamburg, Germany), copy numbers of standard fragments were calculated according to Kampmann et al. (2012) and used as a standards for real-time PCR curve in 10-fold dilutions from 10^6 to 10^1 of strain E19^T 16S rRNA gene copies.

Real-time PCRs were carried out in 10-µl reaction mixture volumes using a 72-well rotor in a Rotor-Gene 3000 cycler (Corbett Research, Sydney, Australia). All samples including the standard curve were run in quadruplicates starting with a preheating step at 94 °C for 2 min followed by 40 cycles of thermal cycling protocol: 45 s at 94 °C, 45 s at 63 °C, 45 s at 72 °C and 15 s at 84 °C. After the last amplification step, a melting curve was obtained by heating the amplification product from 60 °C to 95 °C and SybrGreen fluorescent (SYBR-Green JumpStart Ready Mix, Sigma-Aldrich, St. Louis, USA)

measurement was performed with Qiagen Software Rotor Gene Q 2.3 (Qiagen, Hilden, Germany). The copy numbers were determined using the reference curve plotting the log of starting target amount versus threshold cycle, from the equation of reference DNA plots the initial concentration of each unknown sample was calculated. Data normality was tested using the method of Anderson-Darling and analysed by ANOVA with Tukey´s post-hoc at $P<0.05$ test in Minitab.

Ion torrent sequencing

For sequencing of rhizosphere and root bacterial community a procedure according to Kaplan et al., (2019) was followed; PCR reactions were performed for the amplification of the 16S rRNA gene using a general bacterial primer sets (520_F: 5'-AYTGGGYDTAAAGNG-3'; 926_R: 5'-CCGTCAATTYYTTTRAGTTT-3') (Claesson et al., 2009; Engelbrektson et al., 2010) targeting hypervariable regions V4 and V5 of bacteria. Purified PCR products of samples were transferred to a 314 chip and a 318 chip (Ion PGM Hi-Q Sequencing kit , Ion 314v2 chip & Ion 318v2 chip) (Life Technologies) and sequenced using Ion PGM Sequencer (Life Technologies).

Sequence data obtained were analyzed using the microbiome analysis package QIIME2 (version 2020.6) (Bolyen et al., 2019) . After importing the sequencing in the QIIME2 format the reads were demultiplexed with the tool cutadapt (Martin, 2011) then quality filtered, chimeras removed, clustered and dereplicate using DADA2 (Callahan et al., 2016). After removing sequences with plastid and mitochondrial origin, the dereplicated sequences were classified using a pre-trained Naive Bayes classifier (Pedregosa et al., 2011) trained with the SILVA 132 database (Quast et al., 2013). Alignment of the sequences were done with MAFFT (Katoh and Standley, 2013) and the phylogenetic tree using the maximum-likelihood algorithms was generate using FastTree (Price et al., 2010). Alpha diversity indexes were compared statistically using Minitab 19 and beta biodiversity analysis was compared statistically using the "vegan " package within the R software environment version 3.6.3 (R Core Team, 2020) with R Studio version 1.3.1056 (RStudio Team, 2020). The alpha diversity indexes Observed ASVs and Shannon were calculated to estimate species diversity, normality data test was performed as aforementioned and ANOVA test along with Tukey at $P<0.05$ was used to analyze the effects of the treatments in roots and rhizosphere soil and their interaction with microbial alpha diversity. For beta diversity analysis a Non-metric multidimensional scaling (NMDS) was performed to reproduce the influence among the treatments in roots and rhizosphere soil on the Bray-Curtis dissimilarity matrix by applying the Adonis function permutational multivariate analysis of variance (ADONIS) (permutations = 999). Differential abundance testing was performed using the QIIME2-plugin Songbird (Morton et al., 2019) following the procedure described by Estaki et al. (2020). The method applies a multinomial regression model to estimate differential ranks of taxa across the treatments.

RESULTS

Lanthanide-dependent methylotrophic activity of *H. diazotrophicus*

Fresh water medium supplemented with 0.5% (v/v) methanol in presence of La^{3+} was used to test the growth of *H. diazotrophicus* using methanol as carbon source (Fig. S1). It is evident that the presence of La^{3+} promote growth of *H. diazotrophicus* and is required for growth on methanol as source of carbon. In addition, growth was evaluated under slightly saline conditions with 1% NaCl and different concentrations of lanthanum (30, 60, 90, 120 µM) and methanol 5% (v/v) to evaluate the optimal La^{3+} concentration. Results indicated that 60 µM La^{3+} was slightly the best concentration for growth of *H. diazotrophicus* (Fig.1).

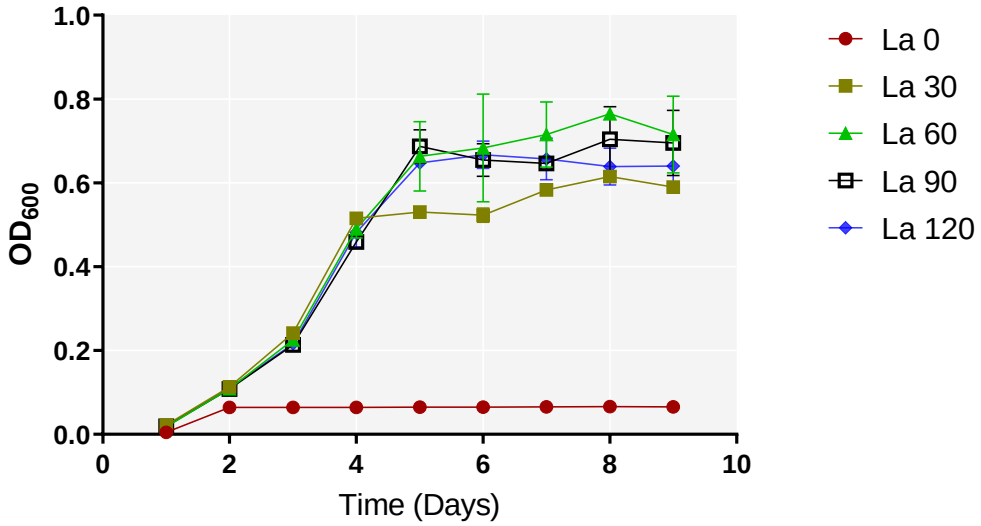


Fig 1. Growth of *H. diazotrophicus* in mineral medium with NaCl 1%, 5% methanol (v/v) under different concentrations of La^{3+} (0, 30, 60, 90,120 µM). Values represent the average of triplicate replications with corresponding standard deviations, the optical density (OD_{600}) was used as growth parameter.

Plant growth promotion activity and colonization of *H. diazotrophicus* E19^T on barley

The effect of *H. diazotrophicus* E19^T inoculation on fresh and dry weights of barley under salt stress conditions of greenhouse experiment was test after 35 days (Fig. 2), normality test on the data using the Anderson-Darling test revealed all of the data sets were normally distributed (Table. S3). Overall, the tendency is similar for fresh or dry weight of plants inoculated with strain E19^T. A significant difference was revealed in fresh ($F_{5,20}$=5.45, P=0.003), ($F_{5,20}$=11.35, P<0.001) and dry weight ($F_{5,20}$=57.78, P<0.001) ($F_{5,20}$=15.91, P<0.001) of leaves and roots. The biomass was higher in plants which have been inoculated with strain E19^T and also in presence of La^{3+} compared to treatments with dead biomass (DB), dead biomass with lanthanum (DB+La) and the corresponding control (non-inoculated plants). Plants inoculated with *H. diazotrophicus* showed an increase in leaves biomass by 5, 5 and 18% and in roots biomass by 38, 36 and 49% against DB, DB+La and the control (Tab. S4) (Tukey HSD, P< 0.05; Fig. 2). Similarly, plants inoculated with strain E19^T combined with lanthanum

showed increases for leaves biomass by 17, 18, 29% and roots biomass by 48, 46, 58% compared to DB, DB+La and control.

Plants grown in presence of only lanthanum showed also a good performance with increased leaves and root dry biomass (Tukey HSD, P< 0.05; Fig. 2) by 7, 7, 20% and 49 ,48, 60% compared to DB, DB+La, and control respectively. Plants inoculated with dead biomass of *H. diazotrophicus* with and without lanthanum had no significant effect in plant growth under salt stress compared to non-inoculated plants.

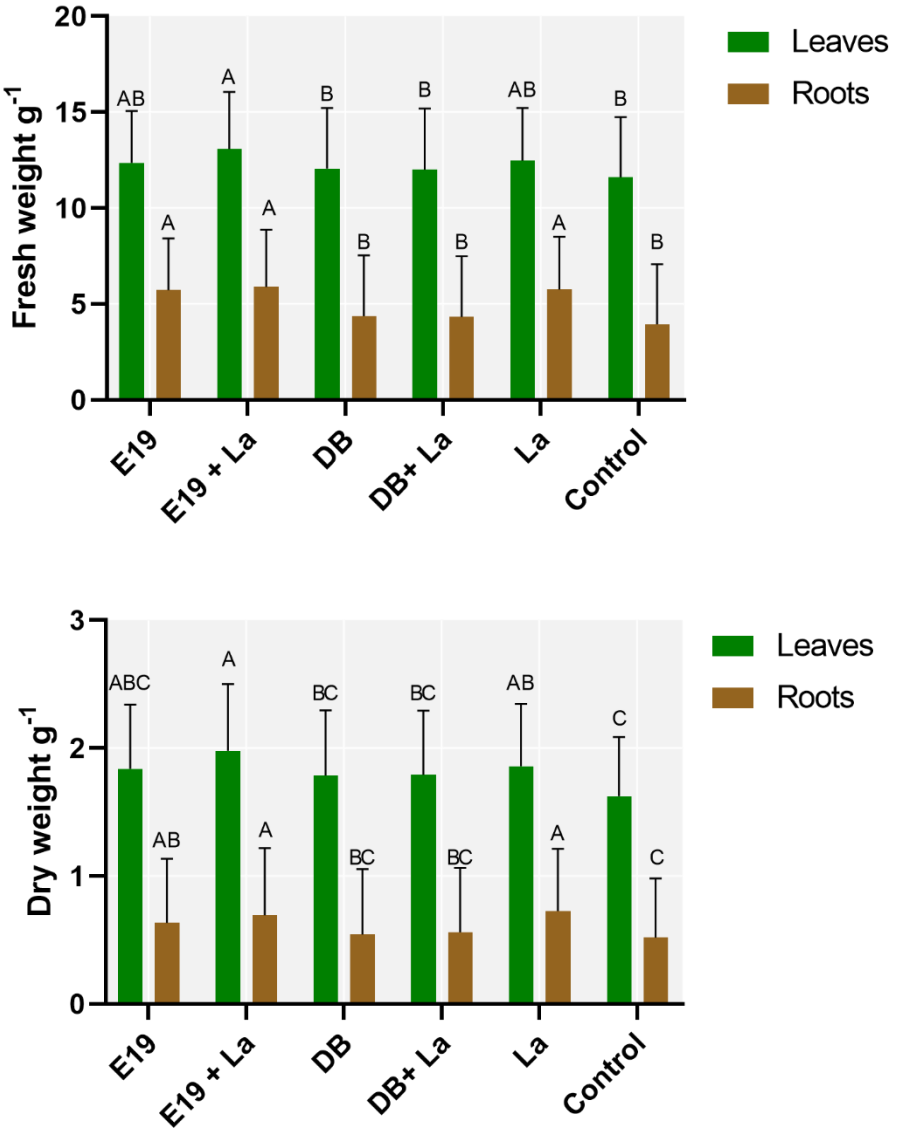


Fig 2. Fresh and dry weight of leaves and roots (mean ± SD, n = 5) of barley plants with inoculation of *H. diazotrophicus* ($E19^T$), in combination with La^{3+} ($E19^T$ + La), dead biomass of $E19^T$ (DB) and mixed with La^{3+} (DB + La^{3+}), single La^{3+} (La) and control without amendments. Each value is the average of five replicates. Bars with the same letter are not significantly different (Tukey HSD, P< 0.05) among treatments after ANOVA.

Quantification of *H. diazotrophicus* on roots and rhizosphere of barley

Survival and growth of *H. diazotrophicus* on roots and rhizosphere of barley plants 35 days after seed inoculation were determined using a specific qPCR assay. Specificity was confirmed *in silico* with the online tool TestPrime from Silva (Klindworth et al., 2013) and *in situ* by cloning environmental 16S rRNA genes and subsequent sequencing of the plasmid insert which recovered the expected sequence (Data not shown).

A clear difference was observed between E19^T colonization in rhizosphere ($F_{5,20}$=5.33, P=0.003) and roots ($F_{5,20}$=6.78, P=0.001) with much higher DNA target numbers of the root (Fig. 3). In rhizosphere quantification of *H. diazotrophicus* gene copies were twice in treatment with *H. diazotrophicus* in comparison to the bacterial treatment with lanthanum (E19^T+La); whereas results for *H. diazotrophicus* gene copies in/on roots (Tukey HSD, P<0.05, Fig. 4 A, B) were similar on both treatments (E19^T) and (E19^T+La). Furthermore, the new primer for detection and quantification of E19^T *xoxF-gene* (encoding the La-dependent methanol dehydrogenase) was tested, its specificity was evaluated *in silico* by PrimerBlast from NCBI and *in situ* as described for strain E19^T 16S rRNA gene. The *xoxF*-gene involved in methylotrophic activity was detected on root samples with gene copies numbers of *the xoxF-gene* in a similar tendency ($F_{5,20}$=3,18, P=0.024) to that observed for strain E19^T 16S gene in/on roots (Fig. S2).

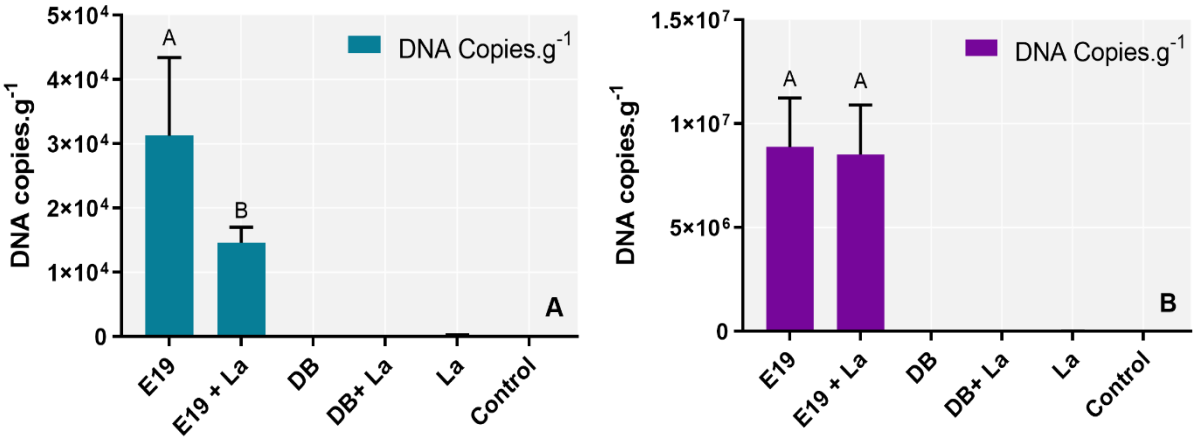
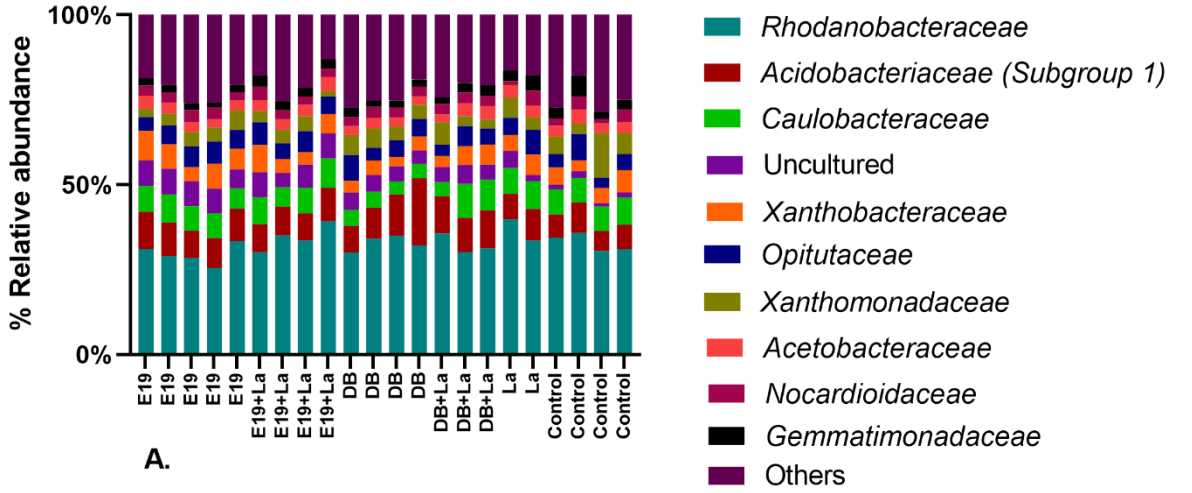


Fig 3. Quantification of strain E19^T in rhizosphere soils (A) and roots (B) of barley 35 days after seed inoculation and plant growth. A specific primer for strain E19^T targeting the 16S rRNA gene was used for qPCR. Each value is the average (± SD) of five replicates measured quadruple. Bars with the same letter are not significantly different (Tukey HSD; P<0.05) among treatments after ANOVA. E19 refers to samples with addition of *H. diazotrophicus* E19^T, E19+La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB+La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition.

Effect of seed inoculation with *H. diazotrophicus* on rhizosphere and root microbial community

Total rhizosphere and root microbial community consisted of 45 phyla, 98 classes, 255 orders, 451 families and 845 genera. At phyla level most dominant in roots and rhizosphere were members of *Proteobacteria* with a relative abundance of 62%. In roots other dominant phyla were *Actinobacteria* (20.5%), *Acidobacteria* (6.9%), *Verrucomicrobia* (4.0%), *Bacteroidetes* (3.6%) and others less abundant phyla (5.24%) comprising the phyla *Gemmatimonadetes*, "*Candidatus* Patescibacteria", *Chloroflexi*, *Firmicutes*, *Lentisphaerae*, "*Candidatus* Dependentiae", *Armatimonadetes*, *Cyanobacteria* among others. In rhizosphere community besides *Proteobacteria* also *Acidobacteria* (14.6%), *Verrucomicrobia* (5.6%), *Actinobacteria* (4%), *Bacteroidetes* (3.3%) and others (6.2%) were found. The phylum *Tenericutes* was found only in rhizosphere. At classes level *Gammaproteobacteria* and *Alphaproteobacteria* were most abundant in roots with 40.6% and 18.3% and rhizosphere with 43.4% and 21.9% respectively (Fig. S3). At the order level, *Xanthomonadales* was the most abundant either in rhizosphere or roots with 35.7%. Family-level analysis revealed that with a mean relative abundance of 29.3% the family *Rhodanobacteraceae* was the most dominant in both habitats (rhizosphere and roots), other families such as *Nocardioidaceae*, *Xanthomonadaceae*, *Caulobacteraceae*, *Opitutaceae*, *Xanthobacteraceae*, *Acidobacteriaceae (Subgroup.1)*, an uncultured family belonging to the order *Vicinamibacterales*, *Solimonadaceae* and *Acetobacteraceae* also were founded dominant in roots, whereas in rhizosphere; *Acidobacteriaceae (Subgroup.1)*, *Caulobacteraceae*, an uncultured family belonging to the order *Vicinamibacterales*, *Xanthobacteraceae*, *Opitutaceae*, *Xanthomonadaceae*, *Acetobacteraceae*, *Nocardioidaceae* and *Gemmatimonadaceae* were the most dominant families (Fig. 4).



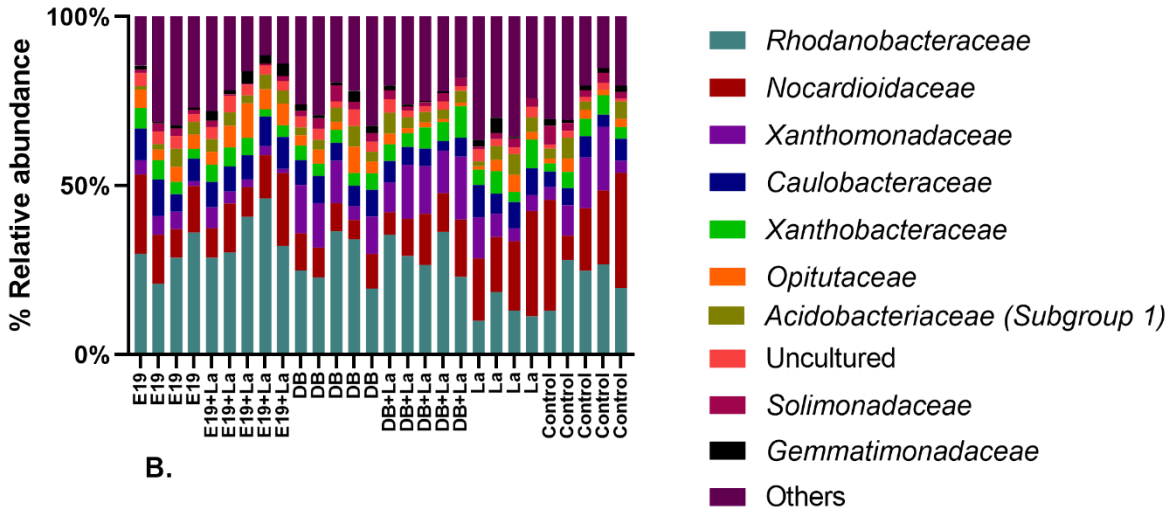


Fig 4. Phylogenetic distribution at family level of bacterial community from rhizosphere (A) and roots (B). E19 refers to samples with addition of *H. diazotrophicus* E19^T, E19^T + La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB + La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition. Only the ten most abundant classes are shown, while the further 220 (Rhizosphere) and 159 (roots) families were collapsed into “Others”.

Alpha and Beta-diversity

The alpha diversity of the bacterial community was evaluated using several diversity indicators. Bacterial taxonomic diversity on rhizosphere and roots showed significant changes among the treatments in alpha-diversity indices observed ASVs ($F_{5,16}$=6.45, P=0.002) ($F_{5,23}$=3.47, P=0.0017) and Shannon only in rhizosphere ($F_{5,16}$=4.99, P=0.006) ($F_{5,23}$=1.76, P=0.161). In rhizosphere most significant differences of alpha diversity were found for the treatments with E19^T and E19^T with lanthanum (Tukey HSD P<0.05; Fig. 5). Roots showed a similar trend (Fig. 5) where the most significant differences in alpha diversity were found for E19^T, dead biomass and E19^T with lanthanum. The inoculation of barley seeds with E19^T evidently showed changes of alpha diversity metrics for bacterial root and rhizosphere community in comparison to control seeds without inoculation.

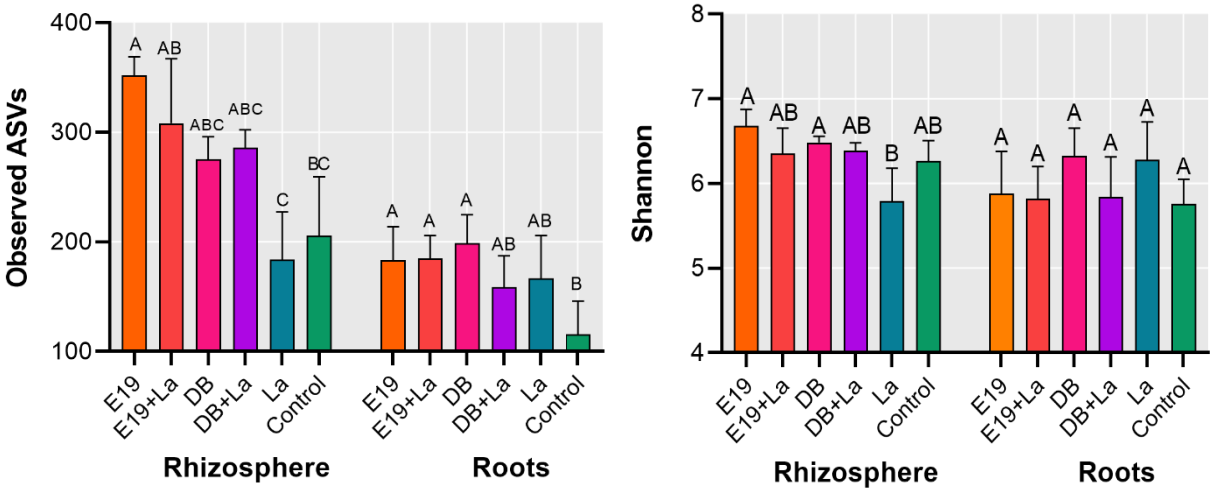


Fig 5. Alpha diversity indices of the different treatments based on ASVs of bacterial community from rhizospheric soil and roots. Boxes with the same letter are not significantly different (Tukey HSD P<0.05) among treatments after ANOVA. E19 refers to samples with addition of *H. diazotrophicus* E19^T, E19 + La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB + La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition.

According to the Bray-Curtis dissimilarities, nonmetric multidimensional scaling (NMDS) was employed to depict the beta diversity of the bacterial community (Fig. 6). The variation of communities in rhizosphere (Adonis, $F_{5,16}$=3.82, P<0.001)) and roots (Adonis $F_{5,22}$=2.93, P<0.001) of barley plants was significant between the different treatments. In both rhizosphere and roots similar bacteria communities were found in treatments with E19^T and E19^T with lanthanum; most different was the community in control soil. Other cluster correspond to the treatments of dead biomass and death biomass with lanthanum.

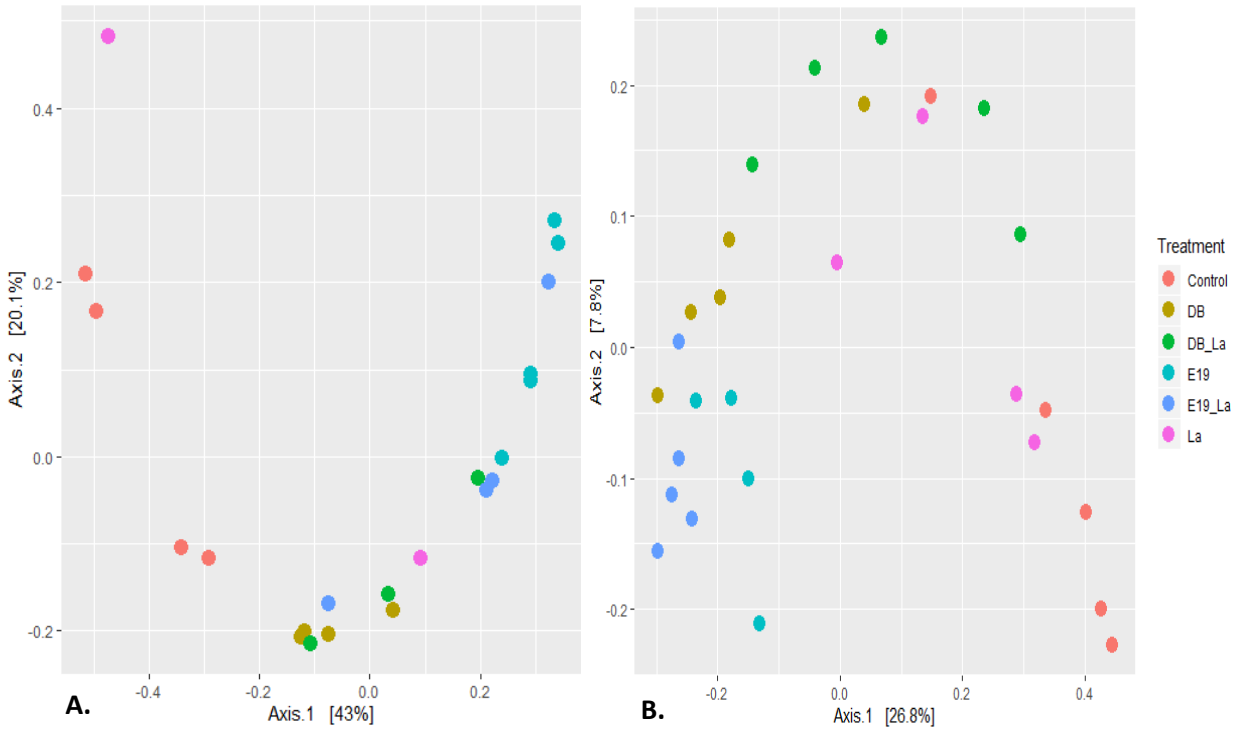


Fig 6. Non-Metric multidimensional scaling (NMDS) plot of β-diversity by Bray-Curtis dissimilarities for barley microbiota in (A) Rhizosphere (Adonis (P<0.001)) and (B) Roots (Adonis (P<0.001)). The number of permutations was 999. E19 ^{T} refers to samples with addition of *H. diazotrophicus* E19^{T}, E19 ^{T} + La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB + La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition.

Differential Abundance Ranking

Differential abundance analysis was tested on the genus level to identify the most associated genus by the different treatments using the plugin Songbird (Morton et al., 2019) for QIIME2 computing relative differentials ranks. The genus with higher differential ranking associated to the different treatments in rhizosphere and roots are showed in Table 1, and the top ten of genus are included in Table S5 and S6. As a whole *Rhodanobacter* was the most strongly associated genus between the treatments whereas *Granulicella*, *Aquicella*, uncultured bacteria of order *Vicinamibacterales* were associated to rhizosphere and *Alkanibacter*, uncultured bacteria of order *Vicinamibacterales* to roots respectively (Tables S5 and S6) .

Table 1. Top-ranked genus abundance per treatment obtained from a multinomial regression rank test. Strain E19^{T} refers to samples with addition of *H. diazotrophicus* E19^{T}, E19^{T} + La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB + La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition.

	Genus Ranked	
Treatment	**Rhizosphere**	**Roots**
E19^T	*Rhodanobacter sp.*	*Tistrella sp.*
E19^T+La	*Alkanibacter sp.*	*Dongia sp.*
DB	*Rhodanobacter sp.*	*Rhodanobacter sp.*
DB+La	*Caulobacter sp.*	*Rhodanobacter sp.*
La	*Solirubrobacter sp.*	Family *Blastocatellaceae*-Uncultured bacteria
Control	*Rhodanobacter sp.*	*Nocardioides sp.*

DISCUSSION

Rare earth elements (REE) were long time overseen as trace metals for enzyme activity of microorganisms. Since the publications of Hibi et al. (2011), Fitriyanto et al. (2011) and Pol et al. (2014) the importance of the REE for the lanthanides depending methanol metabolisms in bacteria in different environments (Howat et al., 2018; J. Huang et al., 2019) become evident. Therefore, we postulate that *H. diazotrophicus* as plant growth promoting bacteria and able to grow with methanol expressing a La-dependent methanol dehydrogenase is supported/favored in root colonization in the presence of La.
The results obtained in this work support some of the hypotheses stated. Barley seed inoculation with strain E19^T in presence of La enhanced plant biomass more that the treatment with E19^T but without La (hypothesis I). Fresh and dry weight of both leaves and roots grown under salt stress were highest after E19^T inculcation and presence of La. However the difference of leave and root biomass in the presence of strain E19^T supplemented with La were not significant different compare to E19^T, thus this hypothesis was not supported by our data. The untreated control plants and plants after seed inoculation of dead biomass of strain E19^T were significantly lower compare to treatments with alive strain E19^T and/or lanthanum. The observed growth stimulation of barley plants inoculated with strain E19^T confirmed results of Suarez et al. (2015) who also reported significantly increased dry biomass of roots and leaves of barley plants after inoculation with strain E19^T under salt stress conditions.

The second working hypothesis (presence of lanthanum has no effect on plant growth) was not fulfilled, interestingly, in the presence of lanthanum dry and fresh weights were increased compared to control treatment. Previous studies reported that lanthanum might play a role in regulation of the plant antioxidant defense system, increasing its activity removing reactive oxygen species like oxygen peroxide (H_2O_2) , superoxide ($O^{2\bullet-}$), and therefore diminishing the salt stress (Huang and Shan, 2018). Huang and Shan (2018) demonstrated a positive effect in plant height, stem diameter and dry weight after application of $LaCl_3$ to tomato seedlings under salt stress conditions. Other studies carried out by Xu et al. (2007) and Liu et al. (2016) indicated that lanthanum application alleviated the oxidative damage in maize leaves and promoted the growth of *Salvia involucrata* under salt stress protecting the photosynthetic system from damage. An additional effect could be that other plant growth promoting

bacteria were stimulated with lanthanum. Rare-earth element depending alcohol dehydrogenases seems to be widely distributed (Howat et al., 2018; Huang et al., 2019) and rare earth depending enzyme activity were also found for another substrate (glycerol) in *Pseudomonas putida* known as well by its activity as PGPR (Wehrmann et al., 2020).
Plants inoculated with dead bacteria did not improve barley growth, which supports work of Suarez et al. (2015) who stated that salt stress tolerance of barley is due to PGPR activity of bacteria and not an effect of nutrients provided in the dead biomass. Treatments of barley seeds inoculated with dead biomass with and without the presence of lanthanum were the same and the effect of lanthanum supporting plant growth disappeared. This observation could be explained by microprecipitation of lanthanum with the dead bacterial biomass. A similar observation was described by Kazy et al. (2006) where inactive biomass of *Pseudomonas sp.* accumulated high amount of lanthanum in a mechanism of La-biosorption.

Our third hypothesis that lanthanum improves strain E19^T rhizosphere abundance was not supported since the abundance of E19^T in rhizosphere was lower in presence of lanthanum compared to no lanthanum treatment (Fig. 3 A). We suggest that lanthanum did not improve growth of strain E19^T in the rhizosphere likely due to a rare earth element (REE) switch; a regulatory mechanism which modulate the expression of methanol dehydrogenase (*xox*F) (Yu and Chistoserdova, 2017) conditioning the primary substrate consumption in strain E19^T to methanol uptake. In pure culture E19^T showed La-dependent growth on methanol (Fig. 1). If methanol concentrations however would be very low in the rhizosphere, possibly also because of substrate competition of other methylotrophic bacteria growth of strain E19^T might occur based on other low molecular weight compounds like glucose, arabinose, mannose which have been described as typical rhizosphere compounds (Derrien et al., 2004; Sasse et al., 2018). These substrates can be utilized also in absence of lanthanum.

On roots strain E19^T was able to grow successfully and high copies numbers of bacteria were determined compared to rhizosphere (roots 4.3 x 10^6 copy numbers vs. rhizosphere 3.2 x 10^4 copy numbers gdw^{-1}). Treatment with lanthanum did not affect growth of strain E19^T on roots, methanol production during root growth through pectin demethylation of wall cells as described by Fall and Benson, 1996 and Galbally and Kirstine (2002) might attract E19^T. However also other root exudates enable growth of strain E19^T independent of La.
Similarly high copy numbers of E19^T *xoxF* gene were quantified (roots 4.8 x 10^6 copy numbers g^{-1}), the ecological role of *xoxF* in establishment of bacteria in/on plants roots has not been up to date elucidated, however studies of *Methylobacterium* groups on phyllosphere of *Arabidopsis thaliana*, showed a high expression of *xoxF* over *mxaF* suggesting *xoxF* gene can play an important role during plant colonization (Delmotte, 2009).

Further studies are required to clarify how strain E19^T can settle on roots and establish a synergistic interaction with the plant. Various strategies for roots colonization have been described for different PGPR by Compant et al. (2010) and Gamez et al. (2019). For *Pseudomonas fluorescens* the presence of an exudated mucigel of roots (Hansen et al., 1997) has been described for attraction.

Our fourth hypothesis was confirmed, and significant differences were found in alpha diversity indices and beta diversity metrics. Seed inoculation with strain E19^T influenced bacterial alpha diversity possibly due to the ability of strain E19^T to stimulate exudates release from roots which subsequently enriches the microbial community diversity. Similar studies have reported that inoculation of plants with PGPR modified the composition and/or function of the rhizosphere bacterial community. Bhattacharyya et al. (2018), founded that inoculation of cabbage with *Proteus vulgaris* induced changes in community abundance. Inoculation of maize plants with *Azospirillum lipoferum* shaped the composition of the indigenous rhizobacterial community changing exudate patterns that result in the variability of the bacterial community as demonstrated by Baudoin et al. (2009) in a field trial. In our experiment seed treatment with dead biomass of strain E19^T also showed a higher alpha diversity metrics possibly because nutrients from inactivated bacterial biomass might have enriched the bacteria populations. For lanthanum treatments only in roots, observed ASVs index were significant different to control treatment, which might indicate a specific enrichment of methylotrophic microbiota inside the roots using methanol for their grown (Butterfield et al., 2016). Interestingly, the Observed ASVs and Shannon index are higher in rhizosphere than in roots, the differences in the habitats could imply more restrictions for the growth and proliferation of specific adapted bacteria recruited by the plant in the roots (Compant et al., 2010). Beta diversity showed that all the treatments evidenced changes in bacterial communities and the differences are linked to the effects described for alpha diversity analyses.

The taxonomical composition at the phylum level indicated that the most abundant phyla founded were *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, and *Bacteroidetes* which have also been detected by Ma and Gong (2013) in a soil affected by salt. On the taxonomic order level *Xanthomonadales* represent the abundant order between the treatments which coincide with the study carried out by Valenzuela-Encinas et al. (2009) who described the bacterial composition in three saline soil samples and found bacterial members belonging to the order *Xanthomonadales*. At the family level the dominant family was *Rhodanobacterace*.

A multinomial regression designed for differential abundance analysis was used to identify the most abundant bacterial genera associated to the treatments; from the top ten most abundant genus (Table. S5 and S6) we observed that *Rhodanobacter* was by far the most dominant associated to the treatments in both rhizosphere and roots, in rhizosphere were also predominant the genera *Granulicella* and *Caulobacter*, while for roots an uncultured bacterial sequence of order Vicinamibacterales, no genera was uniquely present in either the soil or root samples.

The first *Rhodanobacter* isolate was described as new genera and species able to catalyze the first steps of lindane (ɣ-HCH) degradation and was isolated from soil (Nalin et al., 1999). Many other *Rhodanobacter* species have been isolated later from soil including rhizosphere soil (Huo et al., 2018; Won et al., 2015) and the rhizoplane (Madhaiyan et al., 2014). DNA hydrolyzing activity was described for *Rhodanobacter hydrolyticus* (Dahal et al., 2018) which might contribute to abundance under treatments with dead biomass of E19^T. *Granulicella* is the largest group from family *Acidobacteriaceae*, most of *Granulicella* species were isolated from peat bogs and tundra soils (Yamada et al., 2014); some species can tolerate high NaCl concentrations being able to growth at concentrations of 3.5% (W/V)

(Pankratov and Dedysh, 2010) what allowed them the develop in the saline conditions settled for our study. Species of genus *Caulobacter* have a particular mode of reproduction where the division generate one non motile prostheca cell and one cell with a polar flagellum (Staley, 1968), and can be found in environments, such as soil, water, rhizosphere soil, roots (Gao et al., 2018; Jin et al., 2013; Sun et al., 2017; Yang et al., 2020). A study reported by Benidire et al. (2020) of microbiota structure of *Vicia faba* after inoculation with PGPR in the presence or absence of saline stress showed *Caulobacter* as one of the main groups in root colonization in absence of saline stress, which coincide with not dominance of *Caulobacter* in roots in our study across the treatments, moreover the plant growth promotion activity have been also examined, a novel specie of *Caulobacter* isolated from maize roots has demonstrated to promote lateral root formation in the root, and increased size and number of shoots in plants of *Arabidopsis thaliana* as described by Luo et al. (2019).

The order *Vicinamibacterales* and family *Vicinamibacteraceae* which include two species *Vicinamibacter silvestris* and *Luteitalea pratensis*, members of this order have been described as gram-negative, non-spore-forming and chemoheterotrophic (Dedysh and Yilmaz 2018); sequences of bacteria belonging to order *Vicinamibacterales* also were retrieved in a study of microbial communities of a soil cultivated with napier grass and amended with biochar (Yu et al., 2020).

In conclusion, this study is the first study carried out to understand the interaction between a plant and a xoxF-type methylotrophic bacterium with PGPR activity in presence of a rare metal like lanthanum and corroborated that seed inoculation with *H. diazotrophicus* E19^T promoted the growth on barley plants under saline conditions. Novel results include the (i) bacterial diversity in rhizosphere and roots of barley plants were higher in plants after seed inoculation with strain E19^T, and (ii) lanthanum treatment had no incidence improving PGPR activity of strain E19^T. Further studies are required to elucidate the action mechanism of strain E19^T on growth of barley.

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Conflict of interest

The authors declare that they have no conflict of interest.

Bibliography

Ashraf, M. (1994). Breeding for Salinity Tolerance in Plants. Critical Reviews in Plant Sciences, 13(1), 17–42.

Baudoin, E., Nazaret, S., Mougel, C., Ranjard, L., & Moënne-Loccoz, Y. (2009). Impact of inoculation with the phytostimulatory PGPR *Azospirillum lipoferum* CRT1 on the genetic structure of the rhizobacterial community of field-grown maize. Soil Biology and Biochemistry, 41(2), 409–413. https://doi.org/10.1016/j.soilbio.2008.10.015

Benidire, L., El Khalloufi, F., Oufdou, K., Barakat, M., Tulumello, J., Ortet, P., Heulin, T., & Achouak, W. (2020). Phytobeneficial bacteria improve saline stress tolerance in *Vicia faba* and modulate microbial interaction network. Science of The Total Environment, 729, 139020. https://doi.org/10.1016/J.SCITOTENV.2020.139020

Bhattacharyya, D., Duta, S., Yu, S. M., Jeong, S. C., & Lee, Y. H. (2018). Taxonomic and functional changes of bacterial communities in the rhizosphere of Kimchi cabbage after seed bacterization with *Proteus vulgaris* JBLS202. Plant Pathology Journal, 34(4), 286–296. https://doi.org/10.5423/PPJ.OA.03.2018.0047

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. In Nature Biotechnology (Vol. 37, Issue 8, pp. 852–857). Nature Publishing Group. https://doi.org/10.1038/s41587-019-0209-9

Bürgmann, H., Pesaro, M., Widmer, F., & Zeyer, J. (2001). A strategy for optimizing quality and quantity of DNA extracted from soil. Journal of Microbiological Methods, 45(1), 7–20. https://www.sciencedirect.com/science/article/pii/S0167701201002135

Butterfield, C. N., Li, Z., Andeer, P. F., Spaulding, S., Thomas, B. C., Singh, A., Hettich, R. L., Suttle, K. B., Probst, A. J., Tringe, S. G., Northen, T., Pan, C., & Banfield, J. F. (2016). Proteogenomic analyses indicate bacterial methylotrophy and archaeal heterotrophy are prevalent below the grass root zone. PeerJ, 4, e2687. https://doi.org/10.7717/peerj.2687

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

Chu, F., & Lidstrom, M. E. (2016). XoxF acts as the predominant methanol dehydrogenase in the type I methanotroph *Methylomicrobium buryatense*. Journal of Bacteriology, 198(8), 1317–1325. https://doi.org/10.1128/JB.00959-15

Claesson, M. J., O'Sullivan, O., Wang, Q., Nikkilä, J., Marchesi, J. R., Smidt, H., de Vos, W. M., Ross, R. P., & O'Toole, P. W. (2009). Comparative Analysis of Pyrosequencing and a Phylogenetic Microarray for Exploring Microbial Community Structures in the Human Distal Intestine. PLoS ONE, 4(8), e6669. https://doi.org/10.1371/journal.pone.0006669

Compant, S., Clément, C., & Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. Soil Biology and Biochemistry, 42(5), 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024

Dahal, R. H., Chaudhary, D. K., & Kim, J. (2018). *Rhodanobacter hydrolyticus* sp. nov., a novel DNA- and tyrosine-hydrolysing gammaproteobacterium isolated from forest soil. International Journal

of Systematic and Evolutionary Microbiology, 68(8), 2580–2586. https://doi.org/10.1099/IJSEM.0.002881

Dedysh, S. N., & Yilmaz, P. (2018). Refining the taxonomic structure of the phylum Acidobacteria. International Journal of Systematic and Evolutionary Microbiology, 68(12), 3796–3806. https://doi.org/10.1099/IJSEM.0.003062

Derrien, D., Marol, C., & Balesdent, J. (2004). The dynamics of neutral sugars in the rhizosphere of wheat. An approach by 13C pulse-labelling and GC/C/IRMS. Plant and Soil, 267(1–2), 243–253. https://doi.org/10.1007/s11104-005-5348-8

Engelbrektson, A., Kunin, V., Wrighton, K. C., Zvenigorodsky, N., Chen, F., Ochman, H., & Hugenholtz, P. (2010). Experimental factors affecting PCR-based estimates of microbial species richness and evenness. ISME Journal, 4(5), 642–647. https://doi.org/10.1038/ismej.2009.153

Estaki, M., Jiang, L., Bokulich, N. A., McDonald, D., González, A., Kosciolek, T., Martino, C., Zhu, Q., Birmingham, A., Vázquez-Baeza, Y., Dillon, M. R., Bolyen, E., Caporaso, J. G., & Knight, R. (2020). QIIME 2 Enables Comprehensive End-to-End Analysis of Diverse Microbiome Data and Comparative Studies with Publicly Available Data. Current Protocols in Bioinformatics, 70(1). https://doi.org/10.1002/cpbi.100

Fall, R., & Benson, A. A. (1996). Leaf methanol - The simplest natural product from plants. Trends in Plant Science, 1(9), 296–301. https://doi.org/10.1016/1360-1385(96)88175-1

Fitriyanto, N. A., Fushimi, M., Matsunaga, M., Pertiwiningrum, A., Iwama, T., & Kawai, K. (2011). Molecular structure and gene analysis of Ce^{3+}-induced methanol dehydrogenase of *Bradyrhizobium* sp. MAFF211645. Journal of Bioscience and Bioengineering, 111(6), 613–617. https://doi.org/10.1016/j.jbiosc.2011.01.015

Galbally, I. E., & Kirstine, W. (2002). The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry, 43, 195–229. https://doi.org/10.1023/A:1020684815474

Gamez, R. M., Massimiliano, C., Montes, M., Ramírez, S., Schnell, S., & Armestar Rodríguez, F. (2019). Screening, plant growth promotion and root colonization pattern of two rhizobacteria (*Pseudomonas fluorescens* Ps006 and *Bacillus amyloliquefaciens* Bs006) on banana cv. Williams (*Musa acuminata Colla*). Microbiological Research, 220, 12–20. https://doi.org/10.1016/j.micres.2018.11.006

Gao, J. lian, Sun, P., Sun, X. hong, Tong, S., Yan, H., Han, M. lin, Mao, X. jie, & Sun, J. guang. (2018). *Caulobacter zeae* sp. nov. and *Caulobacter radicis* sp. nov., novel endophytic bacteria isolated from maize root (*Zea mays L.*). Systematic and Applied Microbiology, 41(6), 604–610. https://doi.org/10.1016/J.SYAPM.2018.08.010

Hansen, M., Kragelund, L., Nybroe, O., & Sorensen, J. (1997). Early colonization of barley roots by *Pseudomonas fluorescens* studied by inmunofluorescence technique and confocal laser scanning microscopy. FEMS Microbiology Ecology, 23, 353–360. https://doi.org/10.1111/j.1574-6941.1997.tb00416.x

Hibi, Y., Asai, K., Arafuka, H., Hamajima, M., Iwama, T., & Kawai, K. (2011). Molecular structure of La^{3+}-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. Journal of Bioscience and Bioengineering, 111(5), 547–549. https://doi.org/10.1016/j.jbiosc.2010.12.017

Howat, A. M., Vollmers, J., Taubert, M., Grob, C., Dixon, J. L., Todd, J. D., Chen, Y., Kaster, A. K., & Murrell, J. C. (2018). Comparative genomics and mutational analysis reveals a novel XoxF-utilizing methylotroph in the *roseobacter* group isolated from the marine environment. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2018.00766

Huang, G., & Shan, C. (2018). Lanthanum improves the antioxidant capacity in chloroplast of tomato seedlings through ascorbate-glutathione cycle under salt stress. Scientia Horticulturae, 232(October 2017), 264–268. https://doi.org/10.1016/j.scienta.2018.01.025

Huang, J., Yu, Z., Groom, J., Cheng, J.-F., Tarver, A., Yoshikuni, Y., & Chistoserdova, L. (2019). Rare earth element alcohol dehydrogenases widely occur among globally distributed, numerically abundant and environmentally important microbes. The ISME Journal, 13(8), 2005–2017. https://doi.org/10.1038/s41396-019-0414-z

Huo, Y., Kang, J.-P., Park, J.-K., Li, J., Chen, L., & Yang, D.-C. (2018). Rhodanobacter ginsengiterrae sp. nov., an antagonistic bacterium against root rot fungal pathogen *Fusarium solani*, isolated from ginseng rhizospheric soil. Archives of Microbiology 2018 200:10, 200(10), 1457–1463. https://doi.org/10.1007/S00203-018-1560-9

Jin, L., Lee, H.-G., Kim, H.-S., Ahn, C.-Y., & Oh, H.-M. (2013). *Caulobacter daechungensis* sp. nov., a stalked bacterium isolated from a eutrophic reservoir. International Journal of Systematic and Evolutionary Microbiology, 63(Pt_7), 2559–2564. https://doi.org/10.1099/IJS.0.048884-0

Kampmann, K., Ratering, S., Kramer, I., Schmidt, M., Zerr, W., & Schnell, S. (2012). Unexpected stability of *Bacteroidetes* and *Firmicutes* communities in laboratory biogas reactors fed with different defined substrates. Applied and Environmental Microbiology, 78(7), 2106–2119. https://doi.org/10.1128/AEM.06394-11

Kaplan, H., Ratering, S., Felix-Henningsen, P., & Schnell, S. (2019). Stability of in situ immobilization of trace metals with different amendments revealed by microbial 13 C-labelled wheat root decomposition and efflux-mediated metal resistance of soil bacteria. Science of the Total Environment, 659, 1082–1089. https://doi.org/10.1016/j.scitotenv.2018.12.441

Katoh, K., & Standley, D. M. (2013). MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. Mol. Biol. Evol., 30(4), 772–780. https://doi.org/10.1093/molbev/mst010

Kazy, S. K., Das, S. K., & Sar, P. (2006). Lanthanum biosorption by a *Pseudomonas* sp.: Equilibrium studies and chemical characterization. Journal of Industrial Microbiology and Biotechnology, 33(9), 773–783. https://doi.org/10.1007/s10295-006-0108-1

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Research, 41(1), e1. https://doi.org/10.1093/nar/gks808

Kolb, S. (2009). Aerobic methanol-oxidizing Bacteria in soil. FEMS Microbiology Letters, 300(1), 1–10. https://doi.org/10.1111/j.1574-6968.2009.01681.x

Kolodyazhnaya, Y. S., Kutsokon, N. K., Levenko, B. A., Syutikova, O. S., Rakhmetov, D. B., & Kochetov, A. V. (2009). Transgenic plants tolerant to abiotic stresses. Cytology and Genetics, 43(2), 132–149. https://doi.org/10.3103/S0095452709020108

Liu, R. Q., Xu, X. J., Wang, S., & Shan, C. J. (2016). Lanthanum improves salt tolerance of maize seedlings. Photosynthetica, 54(1), 148–151. https://doi.org/10.1007/s11099-015-0157-7

Loy, A., Arnold, R., Tischler, P., Rattei, T., Wagner, M., & Horn, M. (2008). probeCheck – a central resource for evaluating oligonucleotide probe coverage and specificity. Environmental Microbiology, 10(10), 2894–2898. https://doi.org/10.1111/j.1462-2920.2008.01706.x

Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, A., Buchner, A., Lai, T., Steppi, S., Jacob, G., Förster, W., Brettske, I., Gerber, S., Ginhart, A. W., Gross, O., Grumann, S., Hermann, S., Jost, R., König, A., … Schleifer, K. H. (2004). ARB: A software environment for sequence data. Nucleic Acids Research, 32(4), 1363–1371. https://doi.org/10.1093/nar/gkh293

Luo, D., Langendries, S., Mendez, S. G., Ryck, J. De, Liu, D., Beirinckx, S., Willems, A., Russinova, E., Debode, J., & Goormachtig, S. (2019). Plant Growth Promotion Driven by a Novel *Caulobacter* Strain. Https://Doi.Org/10.1094/MPMI-12-18-0347-R, 32(9), 1162–1174. https://doi.org/10.1094/MPMI-12-18-0347-R

Lv, H., Masuda, S., Fujitani, Y., Sahin, N., & Tani, A. (2017). *Oharaeibacter diazotrophicus* gen. nov., sp. nov., a diazotrophic and facultatively methylotrophic bacterium, isolated from rice rhizosphere. International Journal of Systematic and Evolutionary Microbiology, 67(3), 576–582. https://doi.org/10.1099/ijsem.0.001660

Lv, H., & Tani, A. (2018). Genomic characterization of methylotrophy of *Oharaeibacter diazotrophicus* strain SM30T. Journal of Bioscience and Bioengineering, 126(6), 667–675. https://doi.org/10.1016/j.jbiosc.2018.05.023

Ma, B., & Gong, J. (2013). A meta-analysis of the publicly available bacterial and archaeal sequence diversity in saline soils. World Journal of Microbiology and Biotechnology, 29(12), 2325–2334. https://doi.org/10.1007/s11274-013-1399-9

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., & Kwon, S.-W. (2014). *Rhodanobacter glycinis* sp. nov., a yellow-pigmented gammaproteobacterium isolated from the rhizoplane of field-grown soybean. International Journal of Systematic and Evolutionary Microbiology, 64(Pt_6), 2023–2028. https://doi.org/10.1099/IJS.0.055525-0

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.Journal, 17(1), 10. https://doi.org/10.14806/ej.17.1.200

McKillup, S. (2011). Statistics Explained: An Introductory Guide for Life Scientists (2nd ed.). Cambridge University Press. https://books.google.de/books?id=tb4x9uIj3VAC

Morton, J. T., Marotz, C., Washburne, A., Silverman, J., Zaramela, L. S., Edlund, A., Zengler, K., & Knight, R. (2019). Establishing microbial composition measurement standards with reference frames. Nature Communications, 10(1), 1–11. https://doi.org/10.1038/s41467-019-10656-5

Nalin, R., Simonet, P., Vogel, T. M., & Normand, P. (1999). *Rhodanobacter lindaniclasticus* gen. nov., sp. nov., a lindane-degrading bacterium. International Journal of Systematic Bacteriology, 49 Pt 1(1), 19–23. https://doi.org/10.1099/00207713-49-1-19

Numan, M., Bashir, S., Khan, Y., Mumtaz, R., Shinwari, Z. K., Khan, A. L., Khan, A., & AL-Harrasi, A. (2018). Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: A review. Microbiological Research, 209(December 2017), 21–32. https://doi.org/10.1016/j.micres.2018.02.003

Pankratov, T. A., & Dedysh, S. N. (2010). *Granulicella paludicola* gen. nov., sp. nov., *Granulicella pectinivorans* sp. nov., *Granulicella aggregans* sp. nov. and *Granulicella rosea* sp. nov., acidophilic, polymer-degrading acidobacteria from Sphagnum peat bogs. International Journal of Systematic and Evolutionary Microbiology, 60(12), 2951–2959. https://doi.org/10.1099/IJS.0.021824-0

Pedregosa, F., Varoquaux, G., Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., & Duchesnay, É. (2011). Scikit-learn: Machine learning in Python. Journal of Machine Learning Research, 12, 2825–2830. http://scikit-learn.org.

Pol, A., Barends, T. R. M., Dietl, A., Khadem, A. F., Eygensteyn, J., Jetten, M. S. M., & Op den Camp, H. J. M. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environmental Microbiology, 16(1), 255–264. https://doi.org/10.1111/1462-2920.12249

Price, M. N., Dehal, P. S., & Arkin, A. P. (2010). FastTree 2 - Approximately maximum-likelihood trees for large alignments. PLoS ONE, 5(3), e9490. https://doi.org/10.1371/journal.pone.0009490

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2013). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Research, 41(D1), 590–596. https://doi.org/10.1093/nar/gks1219

Sasse, J., Martinoia, E., & Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends in Plant Science, 23(1), 25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi Journal of Biological Sciences, 22(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. Journal of Bacteriology, 95(5), 1921–1942. https://doi.org/10.1128/JB.95.5.1921-1942.1968

Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A. M. Z., Geissler-Plaum, R., & Schnell, S. (2015). Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare L.*) under salt stress. Applied Soil Ecology, 95, 23–30. https://doi.org/10.1016/j.apsoil.2015.04.017

Sun, L.-N., Yang, E.-D., Hou, X.-T., Wei, J.-C., Yuan, Z.-X., & Wang, W.-Y. (2017). *Caulobacter rhizosphaerae* sp. nov., a stalked bacterium isolated from rhizosphere soil. International Journal of Systematic and Evolutionary Microbiology, 67(6), 1771–1776. https://doi.org/10.1099/IJSEM.0.001860

Sy, A., Timmers, A. C. J., Knief, C., & Vorholt, J. a. (2005). Methylotrophic metabolism is advantageous for *Methylobacterium extorquens* during colonization of *Medicago truncatula* under competitive conditions. Applied and Environmental Microbiology, 71(11), 7245–7252. https://doi.org/10.1128/AEM.71.11.7245

Valenzuela-Encinas, C., Neria-González, I., Alcántara-Hernández, R. J., Estrada-Alvarado, I., Zavala-Díaz de la Serna, F. J., Dendooven, L., & Marsch, R. (2009). Changes in the bacterial populations of the highly alkaline saline soil of the former lake Texcoco (Mexico) following flooding. Extremophiles, 13(4), 609–621. https://doi.org/10.1007/s00792-009-0244-4

Vinocur, B., & Altman, A. (2005). Recent advances in engineering plant tolerance to abiotic stress : achievements and limitations. Current Opinion in Biotechnology, 16, 123–132. https://doi.org/10.1016/j.copbio.2005.02.001

Wehrmann, M., Toussaint, M., Pfannstiel, J., Billard, P., & Klebensberger, J. (2020). The cellular response to lanthanum is substrate specific and reveals a novel route for glycerol metabolism in *Pseudomonas putida* kt2440. MBio, 11(2), 2020. https://doi.org/10.1128/mBio.00516-20

Widdel, F., & Bak, F. (1992). Gram-Negative Mesophilic Sulfate-Reducing Bacteria. In The Prokaryotes (pp. 3352–3378). Springer New York. https://doi.org/10.1007/978-1-4757-2191-1_21

Won, K., Singh, H., Ngo, H. T. T., Son, H., Kook, M., Kim, K., & Yi, T. (2015). *Rhodanobacter koreensis* sp. nov., a bacterium isolated from tomato rhizosphere. International Journal of Systematic and Evolutionary Microbiology, 65(Pt_4), 1180–1185. https://doi.org/10.1099/IJS.0.000077

Xu, C. M., Zhao, B., Wang, X. D., & Wang, Y. C. (2007). Lanthanum relieves salinity-induced oxidative stress in *Saussurea involucrata*. Biologia Plantarum, 51(3), 567–570. https://doi.org/10.1007/s10535-007-0124-7

Yamada, K., Okuno, Y., Meng, X.-Y., Tamaki, H., Kamagata, Y., & Hanada, S. (2014). *Granulicella cerasi* sp. nov., an acidophilic bacterium isolated from cherry bark. International Journal of Systematic and Evolutionary Microbiology, 64(Pt_8), 2781–2785. https://doi.org/10.1099/IJS.0.058636-0

Yang, J., Joseph W., K., & Ryu, C.-M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. Trends in Plant Science, 14(1), 1–4. https://doi.org/10.1038/458702c

Yang, Y., Jin, C.-Z., Jin, F.-J., Li, T., Lee, J.-M., Kim, C.-J., Lee, H.-G., & Jin, L. (2020). *Caulobacter soli* sp. nov., isolated from soil sampled at Jiri Mountain, Republic of Korea. International Journal of Systematic and Evolutionary Microbiology, 70(7), 4158–4164. https://doi.org/10.1099/IJSEM.0.004264

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics, 13, 134. https://doi.org/10.1186/1471-2105-13-134

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Ludwig, W., Pruesse, E., Quast, C., Schweer, T., & Glo, F. O. (2014). The SILVA and ‘“ All-species Living Tree Project (LTP)”’ taxonomic frameworks. Nucleic Acids Research, 42(November 2013), 643–648. https://doi.org/10.1093/nar/gkt1209

Yu, J., Pavia, M. J., Deem, L. M., Crow, S. E., Deenik, J. L., & Penton, C. R. (2020). DNA-Stable Isotope Probing Shotgun Metagenomics Reveals the Resilience of Active Microbial Communities to Biochar Amendment in Oxisol Soil. Frontiers in Microbiology, 11. https://doi.org/10.3389/FMICB.2020.587972

Yu, Z., & Chistoserdova, L. (2017). Communal Metabolism of Methane and. Journal of Bacteriology, 199(22), 1–12.

Zarea, M. J., Hajinia, S., Karimi, N., Mohammadi Goltapeh, E., Rejali, F., & Varma, A. (2012). Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. Soil Biology and Biochemistry, 45, 139–146. https://doi.org/10.1016/j.soilbio.2011.11.006

Biology and Fertility of Soils- Supplementary Data

Effect of *Hartmannibacter diazotrophicus* and lanthanum on the plant growth and microbial communities of barley grown under salt stress

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Table S1. Physico-chemical properties of the substrate Fruhstorfer Erde Hawita typ P (**HAWITA Gruppe GmbH,** Vechta, Germany), the substrate used for the plant assays.

Physico-chemical parameter	Value
pH ($CaCl_2$)	5.9
KCl	1.0 g. l^{-1}
Nitrogen ($CaCl_2$)	120 mg l^{-1}
Phosphate (CAL)	120 mg l^{-1}
Potassium (CAL)	120 mg l^{-1}
Magnesium ($CaCl_2$)	120 mg l^{-1}

Table S2. Sorption test of Lanthanum by ICP-MS analysis on non-sterile soil in triplicate replications (Fruhstorfer Erde Hawita typ P)

Sample	La (isotop 139) mg l^{-1}
Stock 20 ppm	20.09
Stock 2 ppm	2.81
Typ P 2 ppm	0.81
Typ P 2 ppm	0.22
Typ P 2 ppm	0.28
Typ P 20 ppm	4.43
Typ P 20 ppm	4.08
Typ P 20 ppm	4.71

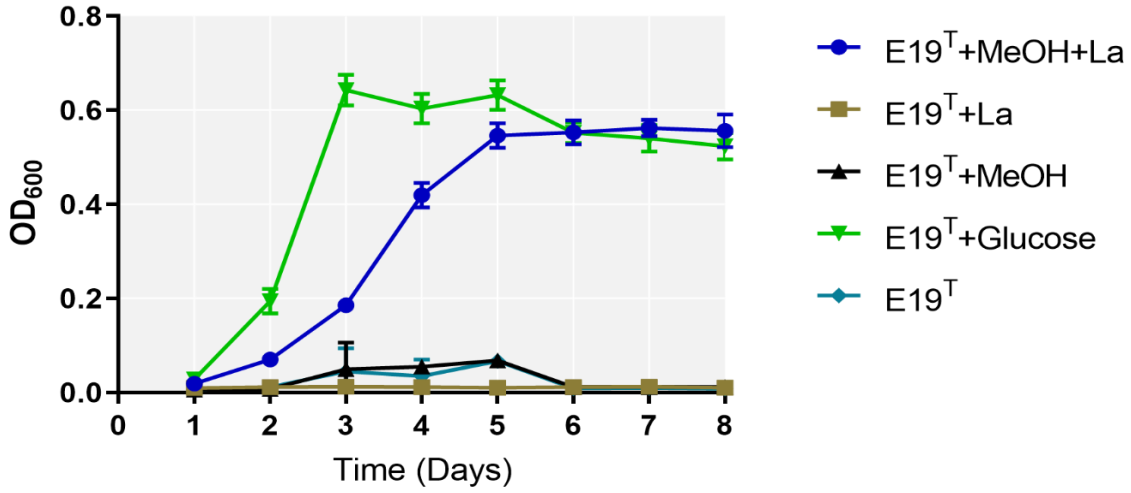

Figure S1. Growth of *H. diazotrophicus* E19^T in freshwater media with La^{3+} (lanthanum 30 µM) and MeOH (methanol 5%v/v). Values represent the average of triplicate replications with corresponding standard deviations, the optical density (OD_{600}) was used as growth parameter

Table S3. Data sets evaluated by normality Anderson-Darling test prior to ANOVA analysis. Data sets with P-values less than alpha 0.05 (P<0.05) not come from a normal distribution.

Data	Anderson Darling-Value	P-value
Fresh Weigh Shoots	0.201	0.869
Fresh Weigh Roots	0.201	0.869
Dry Weigh Shoots	0.669	0.073
Dry Weigh Roots	0,504	0.189
qPCR-Copies/g Rhizosphere	0.213	0.757
qPCR Copies/g Roots	0.656	0.065
Alpha diversity Observed ASVs-Rhizosphere	0.567	0.125
Alpha diversity Shannon-Rhizosphere	0.809	0.325
Alpha diversity Observed ASVs-Roots	0.586	0.117

Table S4. Fresh and dry weight values of leaves and roots of barley plants with inoculation of *H. diazotrophicus* ($E19^T$), supplemented with La^{3+} ($E19^T$ + La), dead biomass of $E19^T$ (DB) and mixed with La^{3+} (DB + La^{3+}), single La^{3+} (La) and control without amendments. Average ± standard error from 5 replications.

Growth Parameter	$E19^T$	$E19^T$ + La	DB	DB + La	La	Control
Leaves fresh weight (g^{-1})	12.35 ± 2.70	13.07 ± 2.98	12.06 ± 3.15	12.02 ± 3.16	12.48 ± 2.74	11.61 ± 3.12
Leaves dry weight (g^{-1})	1.84 ± 0.50	1.98 ± 0.52	1.79 ± 0.51	1.79 ± 0.50	1.86 ± 0.48	1.62 ± 0.46
Roots fresh weight (g^{-1})	5.72 ± 2.70	5.89 ± 2.98	4.38 ± 3.15	4.33 ± 3.16	5.76 ± 2.74	3.94 ± 3.12
Roots dry weight (g^{-1})	0.63 ± 0.50	0.69 ± 0.52	0.54 ± 0.51	0.56 ± 0.50	0.73 ± 0.48	0.52 ± 0.46

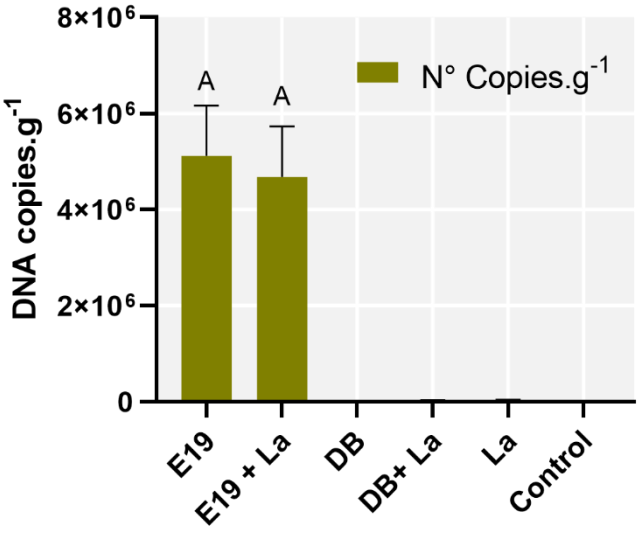

Figure S2. Quantification *xoxF*-gene of strain $E19^T$ in roots of barley 35 days after seed inoculation and plant growth. Each value is the average (± SD) of five replicates measured quadruple. Bars with the same letter are not significantly different (Tukey HSD; $P<0.05$) among treatments after ANOVA. E19 refers to samples with addition of *H. diazotrophicus* $E19^T$, E19+La to samples with addition of *H. diazotrophicus* and lanthanum, DB to sample with addition of dead biomass, DB+La to sample with addition of dead biomass and lanthanum, La to sample with addition of lanthanum, Control to samples without addition.

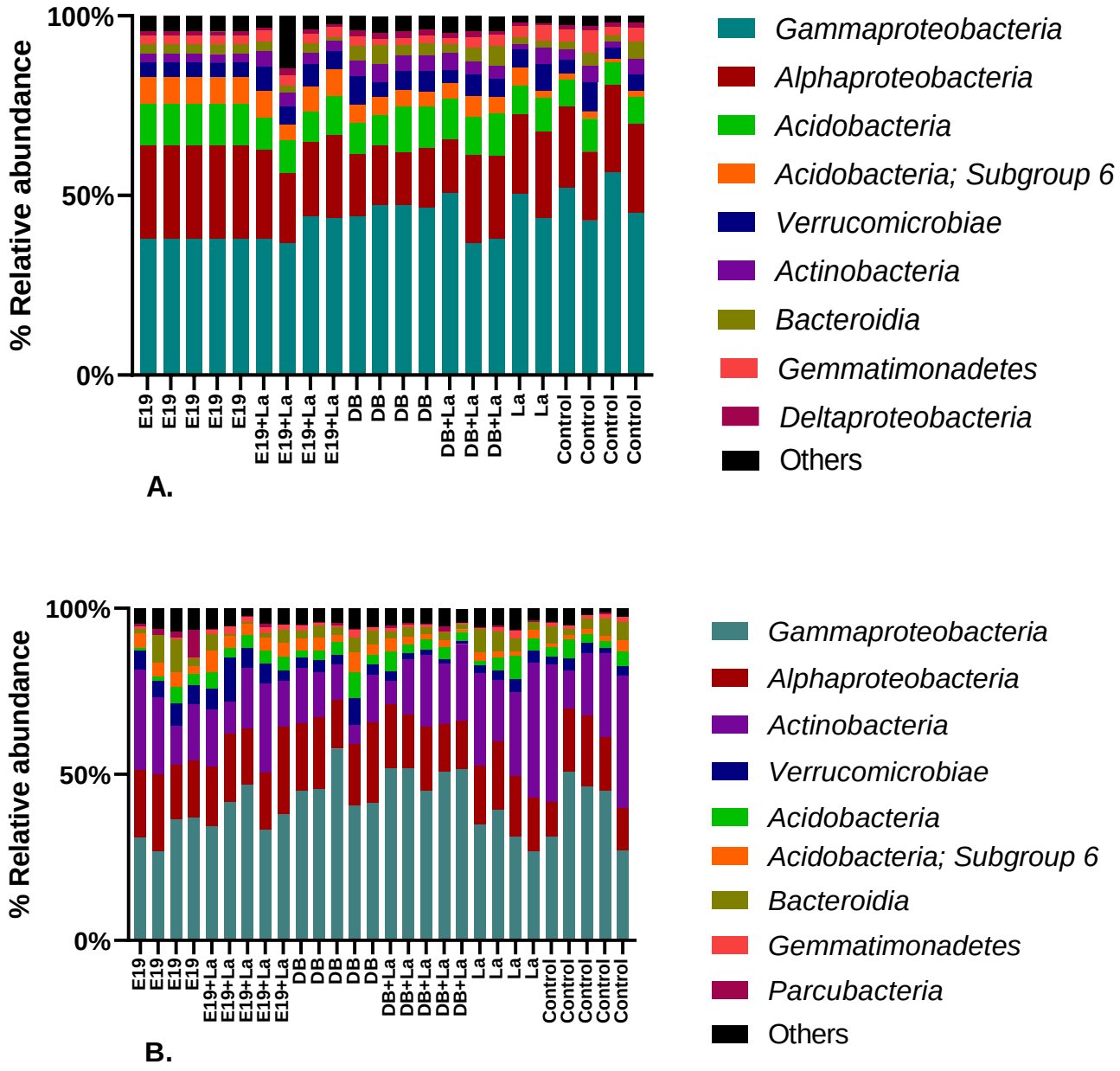

Figure S3. Phylogenetic distribution at class level of bacterial community from rhizosphere (A) and roots (B). Only the ten most abundant classes are shown, while the further 44 (Rhizosphere) and 35 (roots) classes were collapsed into “Others”.

Table. S5 Top ten ranked taxa genus per treatment in rhizosphere.

Treatment E19	Taxonomy
Feature ID	
722f3de083fe8bcbfbd08cb6e2f5f0fe	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
cfd3c6d979b698047f6b5d54290932a0	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
9cfe32c6dad0f09b722dc51ac417823c	*Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; Aquicella*
82996ae173075afd94a55ba684a5ea59	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae; Opitutus*
e641e131483e297b5e69ef6fcb901bea	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
6aa42a3bb44fb31e743bb20bddeafea4	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Caulobacter*
5c502159d8025e710f37f509c20021e7	*Acidobacteriota; Acidobacteriae; Solibacterales; Solibacteraceae; Candidatus_Solibacter*
abee41022a9cce3620183c485f494d2d	*Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; Rhodovastum*
288b3b0cb1cae1e302ceafc49de2f2a9	*Proteobacteria; Alphaproteobacteria; Dongiales; Dongiaceae; Dongia*
4557e11e97b0be54e2890bbc1ab17aa4	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Bradyrhizobium*
Treatment E19+La	
d8fe8fce20f5033355c8b591dc70fa78	*Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Alkanibacter*
e641e131483e297b5e69ef6fcb901bea	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
12ddf670a2a1a4c7b562b286ba5367ac	*Proteobacteria; Alphaproteobacteria; Reyranellales; Reyranellaceae; Reyranella*
722f3de083fe8bcbfbd08cb6e2f5f0fe	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
8ebaa5061aa34b1fbfa1eec3ed47983b	*Acidobacteriota; Acidobacteriae; Solibacterales; Solibacteraceae; Candidatus_Solibacter*
5493a905ede49d0f9141effc82aae995	*Actinobacteriota; Actinobacteria; Micrococcales; Microbacteriaceae*
f8cf9ff7ffc137d8486a1a6dc047bb3f	*Dependentiae; Babeliae; Babeliales; Vermiphilaceae; Vermiphilaceae*
abbbbce464d2e6b809867604f6876b96	*Patescibacteria; Parcubacteria; Candidatus_Kaiserbacteria; Candidatus_Kaiserbacteria; Candidatus_Kaiserbacteria*
dd7beb41e3d66aa46d69907586418e21	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Caulobacter*
a5d3593d869d7e1e9633b970b449b9bc	*Actinobacteriota; Actinobacteria; Micrococcales; Promicromonosporaceae; Promicromonospora*
Treatment Death biomass	
220b5a09598e39a9f564e1e86007a46c	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae*
4cc8857431d97ba771ed1d941b15875d	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
e8594d94fae64ee074836846f8efa5df	*Actinobacteriota; Actinobacteria; Streptomycetales; Streptomycetaceae; Streptomyces*
2ee5b26882e6c8a61f3a984af852280a	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
d8fe8fce20f5033355c8b591dc70fa78	*Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Alkanibacter*
e3d8616809b19796ee96b370f8af563c	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae; Lacunisphaera*
eb4541bfc1922a99b0bc7ef1e6c06d79	*Sumerlaeota; Sumerlaeia; Sumerlaeales; Sumerlaeaceae; Sumerlaea*
179f89ab409c974dbe519f00812e5434	*Bdellovibrionota; Oligoflexia; 0319-6G20; 0319-6G20; 0319-6G20*
f1e170d5f3c8736838136a76df08d8b3	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Luteimonas*
a5d3593d869d7e1e9633b970b449b9bc	*Actinobacteriota; Actinobacteria; Micrococcales; Promicromonosporaceae; Promicromonospora*
Treatment .DB+La	
dd7beb41e3d66aa46d69907586418e21	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Caulobacter*
9cfe32c6dad0f09b722dc51ac417823c	*Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; g_Aquicella*
4cc8857431d97ba771ed1d941b15875d	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
8c6b0c63ca6922b21c76c66539a60923	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Rhodopseudomonas*
fb82af326f24c8e2492d613ac2e15271	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae*
e8594d94fae64ee074836846f8efa5df	*Actinobacteriota; Actinobacteria; Streptomycetales; Streptomycetaceae; Streptomyces*
2ee5b26882e6c8a61f3a984af852280a	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
85c64dfcc7e38e56239cbc7d661d6816	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Kribbella*
179f89ab409c974dbe519f00812e5434	*Bdellovibrionota; Oligoflexia; 0319-6G20; 0319-6G20; 0319-6G20*
d9c290dad797d0636e94fa8c5cdb0c49	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium*
Treatment La	
28502a411927dadb7af7eea937c8a905	*Actinobacteriota; Thermoleophilia; Solirubrobacterales; Solirubrobacteraceae; Solirubrobacter*
c7f519f50a9112eed281131d9329ca32	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Devosiaceae; Devosia*
fe9f0ebd7aab56ce96f498bced86bf80	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae*
9e851dfbc37ff8cc2a47cd21e1cc3fe7	*Proteobacteria; Gammaproteobacteria; Legionellales; Coxiellaceae; g_Aquicella*
80201efe5481555cee510e108c297090	*Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; uncultured*
40f27c637cde80714a2313cf67d5f692	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium*
9bc8bf2b9ad588a867f23ee077754929	*Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured*
4055f3ab461b2adf7c59a5dfa410e2e6	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
5c502159d8025e710f37f509c20021e7	*Acidobacteriota; Acidobacteriae; Solibacterales; Solibacteraceae; Candidatus_Solibacter*
dc03a075baf013d7de0df3debbb01070	*Proteobacteria; Gammaproteobacteria; Burkholderiales; Burkholderiaceae; Burkholderia-Caballeronia-Paraburkholderia*

Control	
40ae6e0f80204fddef2b030fb08dd3ae	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
8d1be44b544f7271330c4a68697346c0	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae; Opitutus*
ed583e3562a89139a870af286de44084	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
96ac15fdf84ba15d752b4f098f053793	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae*
31af174831d38265000802fb9c54c974	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Rhodopseudomonas*
4557e11e97b0be54e2890bbc1ab17aa4	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Bradyrhizobium*
f1e170d5f3c8736838136a76df08d8b3	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Luteimonas*
6eceb3ef8939eee83bb630c6e9f63769	*Bacteroidota; Bacteroidia; Chitinophagales; Chitinophagaceae; Chitinophaga*
90e2666427144e93bed1cd32da1f2c39	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Kribbella*
2167e8f2ceb12316eff450b006e71ed3	*Gemmatimonadota; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured*

Table. S6 Top ten ranked taxa genus per treatment in roots.

Treatment E19	Taxonomy
Feature ID	
ca9958262efc0ea520e109a45bfa6281	*Verrucomicrobiota; Verrucomicrobiae; Pedosphaerales; Pedosphaeraceae; Pedosphaeraceae*
27a614bf842ae59c6056672d720701fd	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
76a6e03ae98d8836ca380dacd294332d	*Bacteroidota; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Pedobacter*
3cfc18fb09ff6ec6c12d237afd940df5	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
209f831103775cb6da3afb10f447fcb6	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas*
a008a56100a38856379e0285c7221aaf	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Rhodopseudomonas*
ee0036283ef96e94d4b13784cbe2b5c1	*Actinobacteriota; Acidimicrobiia; Microtrichales; Iamiaceae; Iamia*
5689250dae3b42d639e404ae649fc0dd	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Nocardioides*
b00c77a07594dfa8e25854514ccb1888	*Abditibacteriota; Abditibacteria; Abditibacteriales; Abditibacteriaceae; Abditibacterium*
ec41952da0cf83f457a4a4080d8b5e1f	*Patescibacteria; Parcubacteria; Candidatus_Kaiserbacteria; Candidatus_Kaiserbacteria; Candidatus_Kaiserbacteria*
Treatment E19+La	
8cd5233fa58de785d0747e27b1473d12	*Proteobacteria; Alphaproteobacteria; Dongiales; Dongiaceae; Dongia*
5cefece331e3d797138525d689dcbeba	*Proteobacteria; Alphaproteobacteria; Acetobacterales; Acetobacteraceae; uncultured*
d2888eeaf0a056ccdb69c75feef9d049	*Chloroflexi; Dehalococcoidia; S085; S085; S085*
1dd1f1bc267a302964ae649ab647649c	*Planctomycetota; Planctomycetes; Planctomycetales; Schlesneriaceae; Schlesneria*
165eb72f4ed1a5f9d1bbd90d49333bfd	*Gemmatimonadota; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured*
3694db554c4bbc01f2f1134822d19079	*Firmicutes; Sulfobacillia; Sulfobacillales; Sulfobacillaceae; Sulfobacillus*
a65f5796ad14dba33e77b80686a206e6	*Proteobacteria; Alphaproteobacteria; uncultured; uncultured; uncultured*
a6f6fa82a84b34cc25f48c8450e7b249	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
88e745080e90b2f90e54a8b257f94b64	*Sumerlaeota; Sumerlaeia; Sumerlaeales; Sumerlaeaceae; Sumerlaea*
8e31565fcaaa5627529016e16e5af346	*Actinobacteriota; Acidimicrobiia; Microtrichales; Iamiaceae; Iamia*
Treatment Death Biomass	
27a614bf842ae59c6056672d720701fd	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
3cfc18fb09ff6ec6c12d237afd940df5	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
90747717ff3ade88dea933c904d0f6f2	*Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Alkanibacter*
02ca4e3cd35a5ac9e758ffe99c2ebdf5	*Gemmatimonadota; Gemmatimonadetes; Gemmatimonadales; Gemmatimonadaceae; uncultured*
5aee2ce96031a900dc66fb28cc5897d6	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Luteimonas*
ddbe1f93ec71e195ea91b2ecfdd21e23	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Pseudoxanthomonas*
76a6e03ae98d8836ca380dacd294332d	*Bacteroidota; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Pedobacter*
2a8ab5c1bf2d8b56026d183ac75c0f9e	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
c1abf51c267237256993ac5a591a4aca	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Aeromicrobium*
35f62206ebe4a0845822a88e230d78ed	*Proteobacteria; Alphaproteobacteria; Rhodospirillales; uncultured; uncultured*
Treatment DB + La	

27a614bf842ae59c6056672d720701fd	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
354d85911f43d9fbc72d1a58337b883f	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Dyella*
9af5c02f01fbca4fc803869f3bd25b90	*Proteobacteria; Gammaproteobacteria; Burkholderiales; Nitrosomonadaceae; Nitrosospira*
0b37066af79eac2c068a0ddf79cf0fbf	*Proteobacteria; Gammaproteobacteria; Salinisphaerales; Solimonadaceae; Alkanibacter*
3cfc18fb09ff6ec6c12d237afd940df5	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
c4fd969d3b59f48f5ff85ec5487946cc	*Proteobacteria; Alphaproteobacteria; Reyranellales; Reyranellaceae; Reyranella*
eb912b6a8cdf34585299f27b3ea573c3	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Phenylobacterium*
79e6fecfd64ce85be66774789481edb7	*Acidobacteriota; Acidobacteriae; Acidobacteriales; Acidobacteriaceae_(Subgroup_1); Granulicella*
262f9d9c05883a19d56776389266069b	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Luteimonas*
3ac0e153f0a77e63b1e7ab931dd73a68	*Proteobacteria; Gammaproteobacteria; Legionellales; Legionellaceae; Legionella*
Treatment La	
b0f3c9c756d25481280498c7c682b085	*Acidobacteriota; Blastocatellia; Blastocatellales; Blastocatellaceae; uncultured*
bef0ea6a4379edb4874c8fcd55d1aace	*Proteobacteria; Gammaproteobacteria; Burkholderiales; Alcaligenaceae*
8363af5c48b88b9d7e127bd9b3b0ee17	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae; Cephaloticoccus;*
7bc7e6881183ae9794629f048cf63ce5	*Acidobacteriota; Vicinamibacteria; Vicinamibacterales; uncultured; uncultured*
958fc4d7612c3b84c8853f215d2ca2f9	*Bacteroidota; Bacteroidia; Sphingobacteriales; Sphingobacteriaceae; Pedobacter*
a008a56100a38856379e0285c7221aaf	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Rhodopseudomonas*
ca9958262efc0ea520e109a45bfa6281	*Verrucomicrobiota; Verrucomicrobiae; Pedosphaerales; Pedosphaeraceae; Pedosphaeraceae*
cd8a58fe596daa1c15855e36de32aae8	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Nitrobacter*
ce3463c6cab44347c61b5fa79a48bb65	*Bacteroidota; Bacteroidia; Cytophagales; Microscillaceae; uncultured*
ee0036283ef96e94d4b13784cbe2b5c1	*Actinobacteriota; Acidimicrobiia; Microtrichales; Iamiaceae; Iamia*
Control	
e1e80cb02e4f8e3997cd124d3fcdc900	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Nocardioides*
c78b590810def61034e0e1653e3400e6	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Rhodanobacteraceae; Rhodanobacter*
01dc497fa9db2a4238044630a9598628	*Verrucomicrobiota; Verrucomicrobiae; Opitutales; Opitutaceae; Opitutus*
5aee2ce96031a900dc66fb28cc5897d6	*Proteobacteria; Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae; Luteimonas*
209f831103775cb6da3afb10f447fcb6	*Proteobacteria; Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas*
9b5facd4baa2f80a0cb44762b134de10	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Kribbella*
a32cb9f206aac5984238431d2e61ffe6	*Sumerlaeota; Sumerlaeia; Sumerlaeales; Sumerlaeaceae; Sumerlaea*
c1abf51c267237256993ac5a591a4aca	*Actinobacteriota; Actinobacteria; Propionibacteriales; Nocardioidaceae; Aeromicrobium*
7be716d1ee84aac44f591723e811542c	*Proteobacteria; Alphaproteobacteria; Rhizobiales; Xanthobacteraceae; Bradyrhizobium*
f37c3b9161b02a42696ae6c896df2909	*Proteobacteria; Alphaproteobacteria; Reyranellales; Reyranellaceae; Reyranella*

CHAPTER 3.

Spirosoma endbachense sp. nov., isolated from a natural salt meadow

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MICROBIOLOGY SOCIETY

Spirosoma endbachense sp. nov., isolated from a natural salt meadow

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Abstract

A Gram-stain-negative bacterium, designated I-24^T, was isolated from soil of a natural salt meadow. Strain I-24^T was aerobic, non-motile, rod-shaped, catalase-positive, oxidase-positive and grew optimally at pH 7 and 25 °C. Comparative 16S rRNA gene analysis indicated that strain I-24^T has closest similarities to *Spirosoma agri* KCTC 52727^T (95.9%) and *Spirosoma terrae* KCTC 52035^T (95.5%). Strain I-24^T contained summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$) and $C_{16:1}\omega5c$ as the major fatty acids, the predominant respiratory quinone was menaquinone MK-7, and the major polar lipids were phosphatidylethanolamine as well as an unidentified phosphoaminolipid. The draft genome of strain I-24^T consists of 10326072 base pairs with 9153 predicted coding sequences and a G+C content of 47.7 mol%. Clear distinctions between strain I-24^T and *S. agri* KCTC 52727^T or *S. terrae* KCTC 52035^T were shown in the pairwise average nucleotide identity results with values of 76.71 and 74.01%, respectively. Moreover, the digital DNA–DNA relatedness values to these strains were 20.8 and 19.0%. Based on its phenotypic, genotypic and chemotaxonomic characteristics, strain I-24^T represents a novel species of the genus *Spirosoma*, for which the name *Spirosoma endbachense* sp. nov. is proposed. The type strain is I-24^T (DSM 111055^T=KCTC 72613^T).

INTRODUCTION

The family *Cytophagaceae* comprises 25 genera and is considered to be one of the largest families in the phylum *Bacteroidetes* [1]. Among these genera, the genus *Spirosoma* [2–5] has 42 published species of which 40 species have valid names [6] including ten recently described species: *Spirosoma telluris* HMF3257^T, *Spirosoma arboris* HMF4905^T [7], *Spirosoma agri* S7-3-3^T [8], *Spirosoma terrae* 15J9-4^T [9], *Spirosoma jeollabukense* S2-3-6^T [10], *Spirosoma pollinicola* HA7^T [11], *Spirosoma humi* S7-4-1^T [12], *Spirosoma horti* S7-3-19^T [13], *Spirosoma harenae* S7-3-19^T [14] and *Spirosoma pomorum* S7-2-11^T [15]. Species that belong to the genus *Spirosoma* have been isolated from a wide range of mostly oxic environments such as air, dust and water. Characteristics that are predominant in this genus include Gram-negative staining, yellow to orange colony colour, diversity of morphologies such as rods, coils and filaments, phosphatidylethanolamine as the major polar lipid, menaquinone of type 7 (MK-7) as the predominant quinone, summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$), $C_{16:1}\omega5c$, iso-$C_{15:0}$ and $C_{16:0}$ as major fatty acids, and DNA G+C content from 47.2 to 57.0 mol% [8, 9, 13, 15].

ISOLATION AND CULTIVATION

Strain I-24^T was isolated from a soil sample collected in a natural salt meadow near Bad Endbach, Hessen, Germany (50° 45′ 54.9″ N 8° 26′ 41.2″ E) as a part of an investigation on bacteria with methylotrophic activity. For enrichment, 3 g soil sample was suspended in 30 ml liquid freshwater media [16] supplemented with 0.5% (v/v) methanol and incubated on a shaker at 110 r.p.m. and 25 °C. After several transfers (10%) in new liquid medium, methanol utilizing bacteria enriched cultures were transferred on solid fresh water media containing methanol 0.5% (v/v) and plates were incubated at 28 °C for 5 days. Single colonies with different morphologies appeared on the plates and a white round colony was selected and purified by several rounds of fractioned streaking. The uniformity of the cell morphology was confirmed by microscopic observation (×1000 magnification; Zeiss Axioplan 2).

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Keywords: Bacteroidetes; methanol; salt meadow; *Spirosoma*.
Abbreviations: ANI, average nucleotide identity; dDDH, digital DNA–DNA hybridization; LB, Luria–Bertani; MK-7, menaquinone 7; NA, nutrient agar; R2A, Reasoner's 2A.
The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain I-24^T is MN657228. The whole-genome shotgun BioProject number is PRJNA588685 with the accession number CP045997.
Six supplementary figures and one supplementary table are available with the online version of this article.

Thereafter, the white colony was transferred to Reasoner's 2A (R2A) agar (Oxoid) medium and incubated again under the same conditions. On R2A agar medium, yellow round colonies were observed and designated as I-24^T. Strain I-24^T was routinely cultured in R2A medium at 25 °C and stored in 20% glycerol (v/v) suspension at −80 °C. *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T were used for comparisons of morphological, physiological and biochemical properties with the novel isolated strain I-24^T.

16S rRNA GENE PHYLOGENY

For the phylogenetic analysis, genomic DNA was extracted using the method of Pitcher *et al.* [17]. The 16S rRNA gene was amplified by PCR using the primers EUB9F–EUB1492R as described by Kampmann *et al.* [18]. Sequencing of both strands was done by LGC Genomics, forward and reverse reads were aligned, and a consensus sequence was built with MEGA 7.0 for retrieving a sequence of 1374 bp. The software toolset DECIPHER 2.13.1 [19] was used to prove that the sequence is not chimeric. Next relative species were identified using the EzBioCloud server [20] and the BLAST sequence analysis tool of the National Center for Biotechnology Information [21].

The recovered sequences of related taxa were aligned with the sequence of strain I-24^T using the online tool SINA (version 1.2.11) [22]. The aligned sequences were merged with the pre-aligned 16S rRNA gene database LPTs132 (June 2018) using ARB (version 6.0.6). Phylogenetic trees using the maximum-likelihood, maximum-parsimony and neighbour-joining algorithms were reconstructed using the corresponding ARB tools with 1000 replicates. The tree calculated with the neighbour-joining algorithm was corrected using the Jukes–Cantor correlation model and a termini filter between positions 67 and 1353 (*Escherichia coli* numbering) [23] of the 16S rRNA gene sequences was applied. Maximum-likelihood and maximum-parsimony trees were calculated using the algorithms RAxML and Phylip DNAPARS integrated in the ARB program, respectively.

Sequence similarity analysis of 16S rRNA gene using the EzBioCloud database and the ARB program showed that strain I-24^T has the highest pairwise gene similarity to validly published species *S. agri* KCTC 52727^T (95.9%) [8] and *S. terrae* KCTC 52035^T (95.5%) [9]. Another close relative to strain I-24^T is strain BT328 with a pairwise similarity of 97%. According to Stackebrandt and Ebers [24], strains sharing less than 98.7% pairwise gene similarity are considered different species; therefore indicating that I-24^T may represent a potential novel species of the genus *Spirosoma*. Based on the phylogenetic trees (maximum-likelihood, maximum-parsimony and neighbour-joining) and sequence similarity analysis with EzBioCloud, *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T were selected as reference strains. The phylogenetic trees (Figs 1, S1 and S2, available in the online version of this article) revealed the position of I-24^T in the cluster of the genus *Spirosoma* close to the other species mentioned above.

GENOME FEATURES

From axenic cultures of strains I-24^T grown for 48 h in R2A liquid medium, high molecular weight DNA was isolated using the method of Pitcher *et al.* [17] and sequenced using the Miseq V3 sequencer system (Illumina) at LGC Genomics. The reads were assembled with SPAdes 3.13.1 [25]. Open reading frames and gene annotations were done with the GenDB platform [26]. Digital DNA–DNA hybridization (dDDH) between the genome sequence of strain I-24^T and the genome sequences of the reference strains (PRJNA590616 and PRJNA590610) were determinate using the Genome-to-Genome Distance Calculator (http://ggdc.dsmz.de/distcalc2.php) [27] with default settings. Genome completeness and contamination were estimated with CheckM [28]. Comparative genome analysis such as average nucleotide identity (ANI) scores, as well as the calculation of core and pan genomes and singletons were performed at the online platform EDGAR [29] using default settings. Furthermore, identification of secondary metabolite biosynthesis gene clusters was performed using antiSMASH [30].

The draft genome of strain I-24^T consisted of 10326072 base pairs of which 9153 were predicted as coding sequences. The content of G+C was 47.7 mol%, the completeness value was >99% and the contamination was 1.19%. The 16S rRNA gene sequence retrieved by PCR was identical to the single copy of 16S rRNA gene contained in the draft genome of strain I-24^T. The ANI values of strain I-24^T to *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T were 76.71 and 74.01%, respectively (Fig. S4); the dDDH value of I-24^T with *S. agri* KCTC 52727^T was 20.8% and for *S. terrae* KCTC 52035^T 19%. These values [<96% (ANI) and <70% (dDDH)] were too low to assign the strains to the same genomic species [31]. This statement also applies equally to strain BT328 whose dDDH value was 49.60% and the ANI value was 92.04% (Fig. S3) compared to strain I-24^T. The comparative analysis between the genomes of I-24^T, *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T showed 3731 core genes, 11866 genes in the pangenome and 3443 singleton coding sequences for strain I-24^T (Fig. S4). Calculation of a maximum-likelihood tree with 1504 shared genes of the genome of I-24^T and other *Spirosoma* species genomes also confirmed the position of I-24^T next to *S. agri* KCTC 52727^T, *S. terrae* KCTC 52035^T and strain BT328 (Fig. 2). Secondary metabolite clusters annotated in antiSMASH from I-24^T were ladderane, terpene, polyketide synthase type I and III (T1PKS, T3PKS) and non-ribosomal peptide synthetase (NRPS) (Fig. S5).

PHYSIOLOGY

Gram-staining of strain I-24^T was performed according to Gerhardt *et al.* [32]. Cell morphology of cells grown for 3 days in R2A liquid culture at 25 °C was assessed by light microscopy using a Zeiss Axioplan 2 microscope with ×1000 magnification. The cell motility was evaluated by the microscopic hanging drop technique. Catalase activity was determined by the production of bubbles in 3% v/v H_2O_2 and oxidase activity was tested with oxidase

Fig. 1. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the phylogenetic position of strain I-24^T among related members in the genus *Spirosoma* and other members of the family *Cytophagaceae*. Bootstraps values (based on 1000 replications) greater than 70% are shown at the branch points. Filled black points indicate that the corresponding nodes were also recovered in trees generated with neighbour-joining and maximum-parsimony algorithms. The tree was rooted using type strains of *Sphingobacterium*, *Flammeovirga*, *Flexithrix* and *Adhaeribacter*. Bar, 0.10 substitutions per nucleotide position.

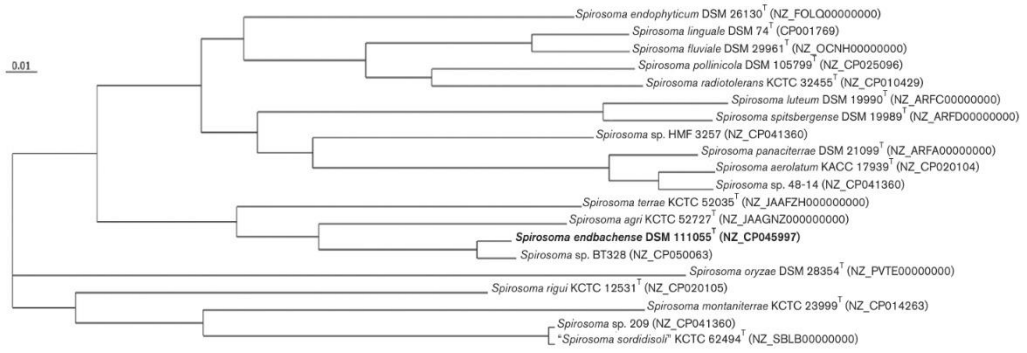

Fig. 2. Maximum-likelihood tree based on 1504 shared genes of the genomes showing the phylogenetic position of strain I-24^T among related members in the genus *Spirosoma*. Bar, 0.01 substitutions per nucleotide position.

test strips (Merck). The presence of flexirubin pigments was assessed by the KOH test proposed by Bernardet *et al.* [33]. Growth was tested on R2A agar, Luria–Bertani agar (LB; Sigma), nutrient agar (NA; Difco) and CASO agar (Roth). Growth at pH 4, 5, 6, 7, 8, 9 was assessed in R2A broth (LabM) adjusted with the following buffers (final concentration 100 mM) citrate (pH 4–5), phosphate (pH 6–8) and Tris-hydrochloride (pH 9). Growth was checked at temperatures of 4, 15, 20, 25, 30, 37 and 40 °C on R2A agar medium. Salt tolerance was examined by observing growth in R2A agar medium containing 1.0, 2.0 and 5.0% (w/v) NaCl after 7 days of incubation. Anaerobic growth of I-24^T was tested for 7 days on R2A agar medium at 25 °C using the Anaerocult A system (Merck). Enzyme activities, assimilation of carbon sources and other biochemical characteristics were determined using API ZIM, API 20 NE and API 50CH strips following the manufacturer instructions (bioMérieux).

Cells of strain I-24^T were Gram-stain-negative, aerobic, non-spore-forming, rod-shaped (0.8–1.2 µm wide and 3–5 µm long), non-motile, catalase-positive and oxidase-positive. After 3 days of growth on R2A agar medium at 25 °C, colonies were yellow-pigmented, circular and slimy. The strain grew well on R2A agar, NA and LB agar medium but not on CASO agar medium. The pH and temperature ranges for growth were pH 6–8 (optimum at pH 7) and 15–25 °C (optimum at 25 °C). No growth was observed below 15 °C and over 25 °C nor at pH below pH 6 and over pH 8.

Strain I-24^T could not grow on R2A agar medium with more than 1% NaCl. Flexirubin-type pigments were absent in strain I-24^T. In API 20NE tests, β-glucosidase activity was positive but arginine dihydrolase, glucose fermentation, indole production, nitrate reduction and urease activity were negative. Test results were positive for glucose, *N*-acetyl-glucosamine and maltose assimilation but negative for other substrates.

In API 50 CH tests, acid was produced from D,L-arabinose, D-ribose, D,L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetyl-glucosamine, arbutin, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose and L-fucose. In the API ZIM kit, strain I-24^T was positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase. The morphological, physiological and biochemical characteristics that could be used to distinguish strain I-24^T from its closest relatives *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T are shown in Table 1.

CHEMOTAXONOMY

Cellular fatty acids, respiratory quinones as well as polar lipid composition of strain I-24^T and the reference strains were analysed using cultures grown in R2A medium at 30 °C for 3 days in shaken flasks. Centrifuged and freeze-dried cultures were sent to the identification service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) for analyses. Cellular fatty acid composition was analysed with conversion to fatty acid methyl ester by saponification, methylation and extraction using the method of Kuykendall *et al.* [34] and a modification of the method of Miller [24]. Subsequently, fatty acid methyl esters were analysed by gas chromatography and identified with the Sherlock Microbial Identification System (MIDI, Microbial ID). Respiratory quinones were extracted using the method described by Tindall *et al.* [35, 36] from freeze-dried cells using hexane and then purified and analysed by HPLC using a reverse-phase column recording absorption spectra. Ubiquinones were read at 270 nm and menaquinones at 326 nm. Polar lipids were recovered using a chloroform–methanol–0.3% NaCl mixture solution at 1:2:0.8 (v/v/v) [37]. The polar lipids were concentrated in chloroform phase adjusting the mixture ratio solution by 1:1:0.9 (v/v/v) and separated by two-dimensional silica gel TLC [first direction, chloroform–methanol–water (65:25:4, v/v/v); second direction, chloroform–methanol–acetic acid–water (80:12:15:4, v/v/v/v)]. Total lipid material and specific functional groups were revealed using spray reagents specific dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate–Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen) and α-naphthol–sulphuric acid (glycolipids) according to the procedure described by Tindall *et al.* [38].

On R2A medium the major fatty acids of I-24^T were summed feature 3 ($C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; 36.07%), $C_{16:1}\omega5c$ (18.34%), $C_{15:0}$ iso (11.65%) and a moderate amount of $C_{16:0}$ (6.34%) (Table 2). A difference between I-24^T and its closest relatives could be observed in the low percentages of $C_{15:0}$ anteiso (3.45%), $C_{13:0}$ iso (0.73%), $C_{14:0}$ iso (0.91%) and summed feature 8 (0.71%), traces of $C_{17:0}$ 2-OH and no detected $C_{19:0}$ anteiso in I-24^T. The major polar lipids of the isolate were phosphatidylethanolamine (also detected in other *Spirosoma* species [8, 9]), six unidentified lipids, three unidentified aminolipids, one glycolipid, five unidentified phospholipids and an unidentified phosphoaminolipid (Fig. S6). It is important to note that glycolipid was found in I-24^T, *S. agri* KCTC 52727^T and *Spirosoma terrae* KCTC 52035^T but not in *S. migulaei* KCTC 52028^T, *S. pulveris* KCTC 42550^T and other species of *Spirosoma*, as well as aminolipid, was detected in I-24^T, *S. agri* KCTC 52727^T, *S. terrae* KCTC 52035^T but not in *S. migulaei* KCTC 52028^T (Table S1). MK-7 was the predominant isoprenoid quinone of strain I-24^T and its relatives which is also the major respiratory quinone in other members of the genus *Spirosoma* [8, 9, 39]. In addition, MK-8 was detected in the closest relatives *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T.

Table 1. Differential characteristics of strain I-24^T and its nearest relatives

Strains: 1, I-24^T; 2, *Spirosoma agri* KCTC 52727^T, 3, *Spirosoma terrae* KCTC 52035^T. +, Positive; –, negative. All data were obtained in this study, except the DNA G+C content for *S. agri* KCTC 52727^T [4] and *S. terrae* KCTC 52035^T [5]. All strains were Gram-stain-negative, non-motile, oxidase-positive and catalase-positive.

Characteristics	1	2	3
Isolation source	Soil	Soil	Soil
Cell shape	Rods	Rods	Rods
Growth on/at/with:			
CASO	–	–	+
Temperature range (°C) (optimum)	15–25 (25)	15–30 (25)	15–37 (25)
pH range (optimum)	6–8 (7)	6–8 (7)	6–8 (7)
NaCl (%)	0–1	0–2	0–2
Methanol	+	–	–
Assimilation of (API 20NE):			
D-Mannose	–	–	+
Potassium gluconate	–	–	+
Acid production from (API 50CH):			
L-Arabinose, L-xylose, D-tagatose	+	–	+
L-Rhamnose, D-tagatose, D-fucose, L-fucose	+	–	–
Raffinose, melezitose	+	+	–
Enzyme activity (API ZYM):			
Trypsin	–	–	+
DNA G+C content (mol%)	47.7	50.5	47.4

PROTOLOGUE

According to the results of our polyphasic approach it was demonstrated that strain I-24^T belongs to the genus *Spirosoma*. Based on its distinctive phenotypic characteristics and its phylogenetic distances from other members of the genus *Spirosoma*, it is suggested that strain I-24^T represents a novel species, for which the name *Spirosoma endbachense* sp. nov. is proposed.

DESCRIPTION OF *SPIROSOMA ENDBACHENSE* SP. NOV.

Spirosoma endbachense (end.bach.en'se. N.L. neut. adj. *endbachense* pertaining to Bad Endbach, a town in Germany, where the strain was isolated).

Cells are Gram-stain-negative, non-motile, aerobic, rod-shaped, non-spore-forming, 0.8–1.2 µm wide and 3–5.0 µm long. Colonies are circular, convex, smooth, slimy and yellow after 3 days of incubation at 25 °C on R2A agar. Growth occurs at 15–25 °C and pH 6–8, with optimal growth 25 °C and pH 7. Cells tolerate 0.5 and 1% NaCl, but not 2% NaCl. Cells are positive for aesculin, β-galactosidase, oxidase and catalase but negative for indole production and nitrate reduction. Positive for *N*-acetyl-glucosamine, D-glucose and maltose assimilation, but none of the other substrates in the API 20 NE kit. In the API 50 CH kit, acid is produced from D,L-arabinose, D-ribose, D,L-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, *N*-acetyl-glucosamine, arbutin, amygdalin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, gentiobiose, turanose, D-lyxose, D-tagatose, D-fucose and L-fucose. In the API ZIM kit, strain I-24^T is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase, but negative for the other substrates. The major cellular fatty acids are summed feature 3 ($C_{16:1}\omega7c/C_{16:1}\omega6c$), $C_{16:1}\omega5c$ and $C_{15:0}$ iso. The predominant respiratory quinone is MK-7. Phosphatidylethanolamine is the main polar lipid. The type strain, I-24^T (DSM 111055^T=KCTC 72613^T), was isolated from a soil sample collected in a natural salt meadow near Bad Endbach, Hessen, Germany (50° 45′ 54.9″ N 8° 26′ 41.2″ E). The DNA G+C content is 47.7 mol% (draft genome data). The GenBank/EMBK/DDBJ accession number of the 16S rRNA gene sequence is MN657228, the whole-genome

Table 2. Major fatty acids composition of strain I-24^T and its close relatives

Strains: 1, I-24^T; 2, *Spirosoma agri* KCTC 52727^T, 3, *Spirosoma terrae* KCTC 52035^T. All data were obtained in the present study. Strains were cultured in R2A liquid medium on a rotary shaker at 25 °C. Values are percentages of total fatty acids.

Fatty acids	1	2	3
$C_{15:0}$ iso	11.65	7.72	12.83
$C_{15:0}$ iso 3-OH	2.79	2.04	2.32
$C_{15:0}$ anteiso	3.45	10.51	8.16
$C_{16:0}$ iso	1.18	0.38	0.50
$C_{16:1}$ $\omega 5c$	18.34	19.76	22.51
$C_{16:0}$	6.34	5.66	4.72
$C_{16:0}$ 3-OH	2.68	2.75	1.92
$C_{17:0}$ iso	1.55	0.69	1.03
$C_{17:0}$ iso 3-OH	9.87	4.02	8.19
$C_{17:0}$ anteiso	0.55	3.56	1.95
Summed feature 3*	36.07	37.89	31.37

*Summed features contain two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 comprises $C_{16:1}$ $\omega 7c$/$C_{16:1}$ $\omega 6c$.

shotgun BioProject number is PRJNA588685 with the accession number CP045997.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References

1. **Rosenberg E**, **DeLong EF**, **Lory S**, **Stackebrandt E**, **Thompson F**. *The Prokaryotes*, 4th ed. Springer Berlin Heidelberg; 2014.
2. **Migula W**. Über ein neues system Der Bakterien, Arbeiten AUS de, Bakteriologischen Institut Der Technischen Hochschule zu Karlsruhe 1894.
3. **Skerman VBD**, **Sneath PHA**, **McGowan V**. Approved lists of bacterial names. *Int J Syst Evol Microbiol* 1980;30:225–420.
4. **Finster KW**, **Herbert RA**, **Lomstein BA**. *Spirosoma spitsbergense* sp. nov. and *Spirosoma luteum* sp. nov., isolated from a high Arctic permafrost soil, and emended description of the genus *Spirosoma*. *Int J Syst Evol Microbiol* 2009;59:839–844.
5. **Ahn JH**, **Weon HY**, **Kim SJ**, **Hong SB**, **Seok SJ** *et al*. *Spirosoma oryzae* sp. nov., isolated from rice soil and emended description of the genus *Spirosoma*. *Int J Syst Evol Microbiol* 2014;64:3230–3234.
6. **Euzéby J**. List of new names and new combinations previously effectively, but not validly, published. *Int J Syst Evol Microbiol* 2018;59:923–925.
7. **Kang H**, **Cha I**, **Kim H**, **Joh K**. *Spirosoma telluris* sp. nov. and *Spirosoma arboris* sp. nov. isolated from soil and tree bark, respectively. *Int J Syst Evol Microbiol* 2020;70:5355–5362.
8. **Li W**, **Lee S-Y**, **Kang I-K**, **Ten LN**, **Jung H-Y**. Spirosoma *agri* sp. nov., isolated from apple orchard soil. *Curr Microbiol* 2018;75:694–700.
9. **Ten LN**, **Okiria J**, **Lee J-J**, **Lee S-Y**, **Park S** *et al*. *Spirosoma terrae* sp. nov., isolated from soil from Jeju Island, Korea. *Curr Microbiol* 2018;75:492–498.
10. **Li W**, **Ten LN**, **Lee SY**, **Lee DH**, **Jung HY**. *Spirosoma jeollabukense* sp. nov., isolated from soil. *Arch Microbiol* 2018;200:431–438.
11. **Manirajan BA**, **Suarez C**, **Ratering S**, **Rusch V**, **Geissler-plaum R** *et al*. *Spirosoma pollinicola* sp. nov., isolated from pollen of common hazel (Corylus avellana L.). *Int J Syst Evol Microbiol* 2018:1–7.
12. **Weilan L**, **Lee JJ**, **Lee SY**, **Park S**, **Ten LN** *et al*. *Spirosoma humi* sp. nov., isolated from soil in South Korea. *Curr Microbiol* 2018;75:328–335.
13. **Li W**, **Ten LN**, **Lee S-Y**, **Kang I-K**, **Jung H-Y**. *Spirosoma horti* sp. nov., isolated from apple orchard soil. *Int J Syst Evol Microbiol* 2018;68:930–935.
14. **Ten LN**, **Elderiny N**, **Lee JJ**, **Lee SY**, **Park S** *et al*. *Spirosoma harenae* sp. nov., a Bacterium Isolated from a Sandy Beach. *Curr Microbiol* 2018;75:179–185.
15. **Li W**, **Lee S-Y**, **Kang I-K**, **Ten LN**, **Jung H-Y**. *Spirosoma pomorum* sp. nov., isolated from apple orchard soil. *J Microbiol* 2018;56:90–96.
16. **Widdel F**, **Bak F**. Gram-Negative Mesophilic Sulfate-Reducing Bacteria. In: *The Prokaryotes*. New York, NY: Springer New York. pp. 3352–3378.
17. **Pitcher DG**, **Saunders NA**, **Owen RJ**. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* 1989;8:151–156.
18. **Kampmann K**, **Ratering S**, **Kramer I**, **Schmidt M**, **Zerr W** *et al*. Unexpected stability of *Bacteroidetes* and *Firmicutes* communities in laboratory biogas reactors fed with different defined substrates. *Appl Environ Microbiol* 2012;78:2106–2119.
19. **Wright ES**, **Yilmaz LS**, **Noguera DR**. DECIPHER, a search-based approach to chimera identification for 16S rRNA sequences. *Appl Environ Microbiol* 2012;78:717–725.
20. **Yoon S-H**, **Ha S-M**, **Kwon S**, **Lim J**, **Kim Y** *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
21. **Altschul SF**, **Gish W**, **Miller W**, **Myers EW**, **Lipman DJ** *et al*. Basic local alignment search tool. *J Mol Biol* 1990;215:403–410.
22. **Pruesse E**, **Peplies J**, **Glöckner FO**. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* 2012;28:1823–1829.
23. **Brosius J**, **Palmer ML**, **Kennedy PJ**, **Noller HF**. Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli. *Proc Natl Acad Sci U S A* 1978;75:4801–4805.
24. **Miller LT**. Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584–586.
25. **Bankevich A**, **Nurk S**, **Antipov D**, **Gurevich AA**, **Dvorkin M** *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
26. **Meyer F**, **Goesmann A**, **McHardy AC**, **Bartels D**, **Bekel T** *et al*. GenDB--an open source genome annotation system for prokaryote genomes. *Nucleic Acids Res* 2003;31:2187–2195.
27. **Meier-Kolthoff JP**, **Auch AF**, **Klenk H-P**, **Göker M**. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 2013;14:60.
28. **Parks DH**, **Imelfort M**, **Skennerton CT**, **Hugenholtz P**, **Tyson GW**. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.

29. **Blom J**, **Albaum SP**, **Doppmeier D**, **Pühler A**, **Vorhölter F-J** *et al*. EDGAR: a software framework for the comparative analysis of prokaryotic genomes. *BMC Bioinformatics* 2009;10:154.
30. **Medema MH**, **Blin K**, **Cimermancic P**, **de Jager V**, **Zakrzewski P** *et al*. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. *Nucleic Acids Res* 2011;39:W339–W346.
31. **Chun J**, **Oren A**, **Ventosa A**, **Christensen H**, **Arahal DR** *et al*. Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *Int J Syst Evol Microbiol* 2018;68:461–466.
32. **Gerhardt P**, **Wood W**, **Murray RGE**, **Krieg NR**. *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology; 1994.
33. **Bernardet J-F**, **Nakagawa Y**, **Holmes B**. Proposed minimal standards for describing new taxa of the family. *Int J Syst Evol Microbiol* 2002;52:1049–1070.
34. **Kuykendall LD**, **Roy MA**, **O'NEILL JJ**, **Devine TE**, **Acids F**. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988;38:358–361.
35. **Tindall BJ**. A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* 1990;13:128–130.
36. **Tindall BJ**. Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* 1990;66:199–202.
37. **Bligh EG**, **Dyer WJ**. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 1959;37:911–917.
38. **Tindall BJ**, **Sikorski J**, **Smibert RA**, **Krieg NR**. Phenotypic Characterization and the Principles of Comparative Systematics. *Methods for General and Molecular Microbiology*, 3rd ed. American Society of Microbiology; 2007. pp. 330–393.
39. **Zhang L**, **Zhou X-Y**, **Su X-J**, **Hu Q**, **Jiang J-D**. *Spirosoma sordidisoli* sp. nov., a propanil-degrading bacterium isolated from a herbicide-contaminated soil. *Antonie van Leeuwenhoek* 2019;112:1523–1532.

Supplement Material

International Journal of Systematic and Evolutionary Microbiology

Spirosoma endbachense sp. nov., isolated from a natural salt meadow

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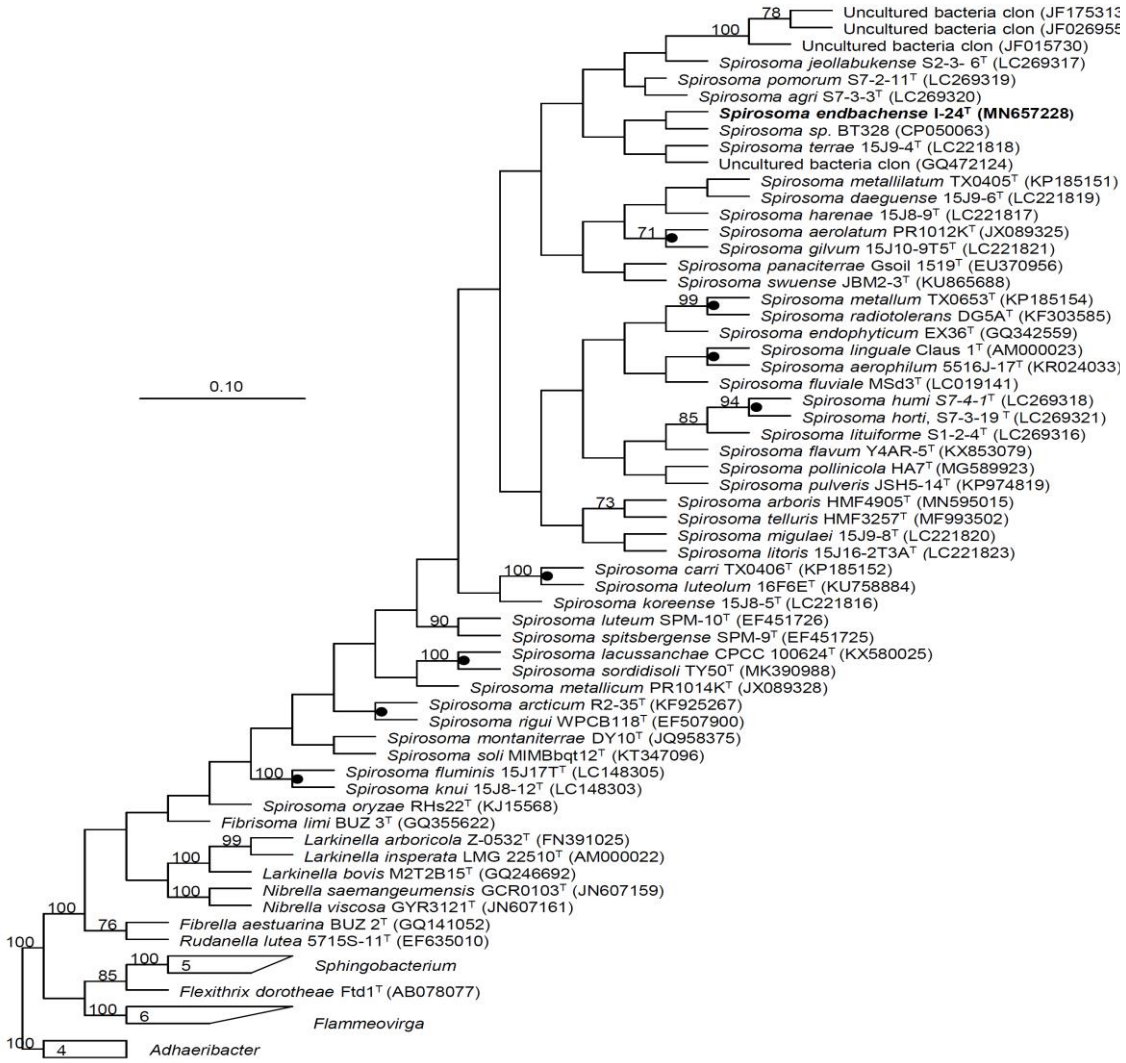


Fig. S1. Maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain I-24^T among related members in the genus *Spirosoma* and other members of the family *Cytophagaceae*. Bootstraps values (based on 1000 replications) greater than 70% are showed at the branch points. Filled black points indicate that the corresponding nodes were also recovered in trees generated with maximum-likelihood and neighbor-joining algorithms. The tree was rooted using type strains of *Sphingobacterium*, *Flammeovirga*, *Flexithrix* and *Adhaeribacter*. Bar: 0.10 substitutions per nucleotide position.

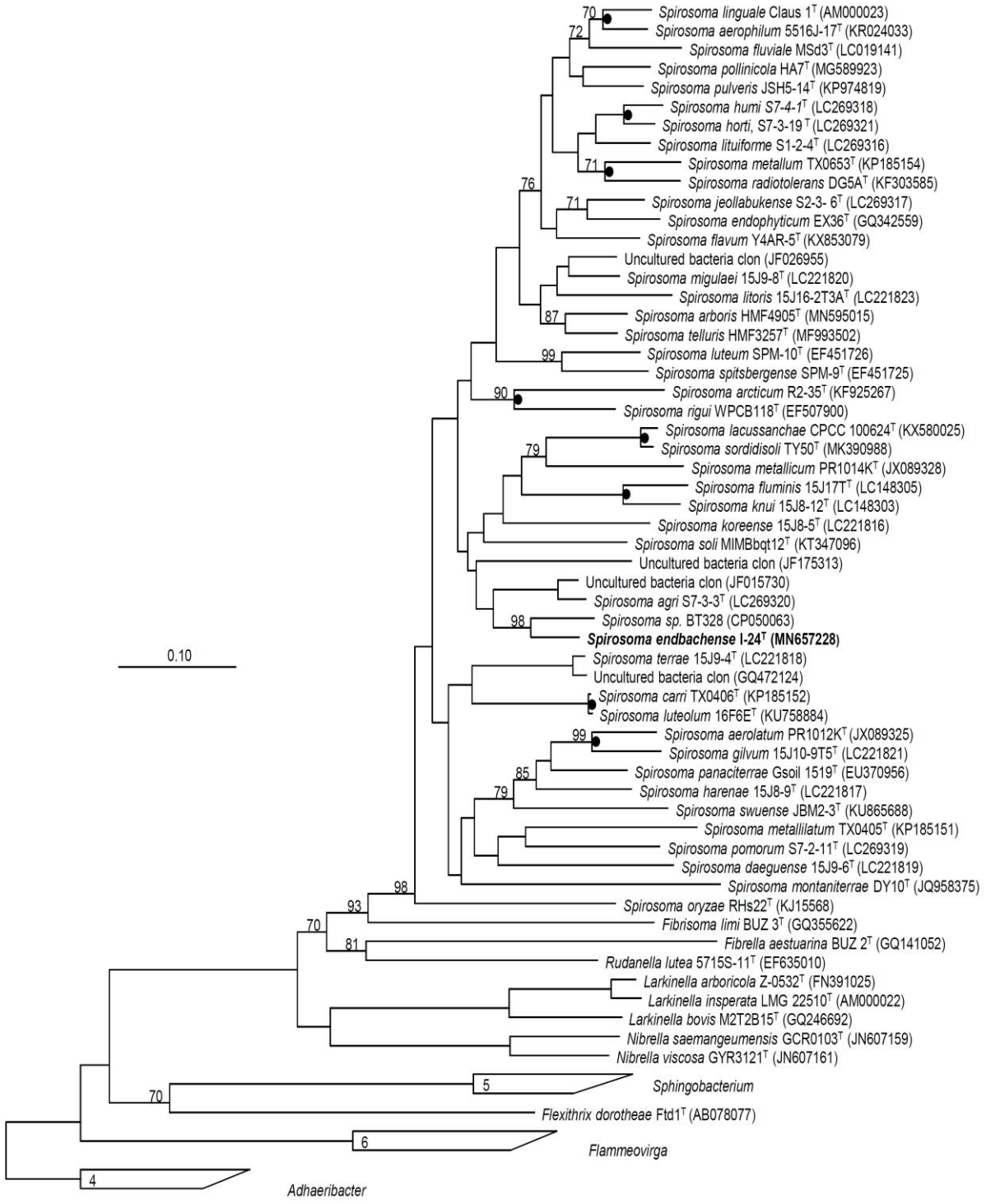


Fig. S2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences (a-termini filter between positions 67 and 1353, *Escherichia coli* numbering), showing the phylogenetic position of strain I-24^T among related members in the genus *Spirosoma* and other members of the family *Cytophagaceae*. Bootstraps values (based on 1000 replications) greater than 70% are showed at the branch points. Filled black points indicate that the corresponding nodes were also recovered in trees generated with maximum-likelihood and maximum-parsimony algorithms. The tree was rooted using type strains of *Sphingobacterium*, *Flammeovirga*, *Flexithrix* and *Adhaeribacter*. Bar: 0.10 substitutions per nucleotide position.

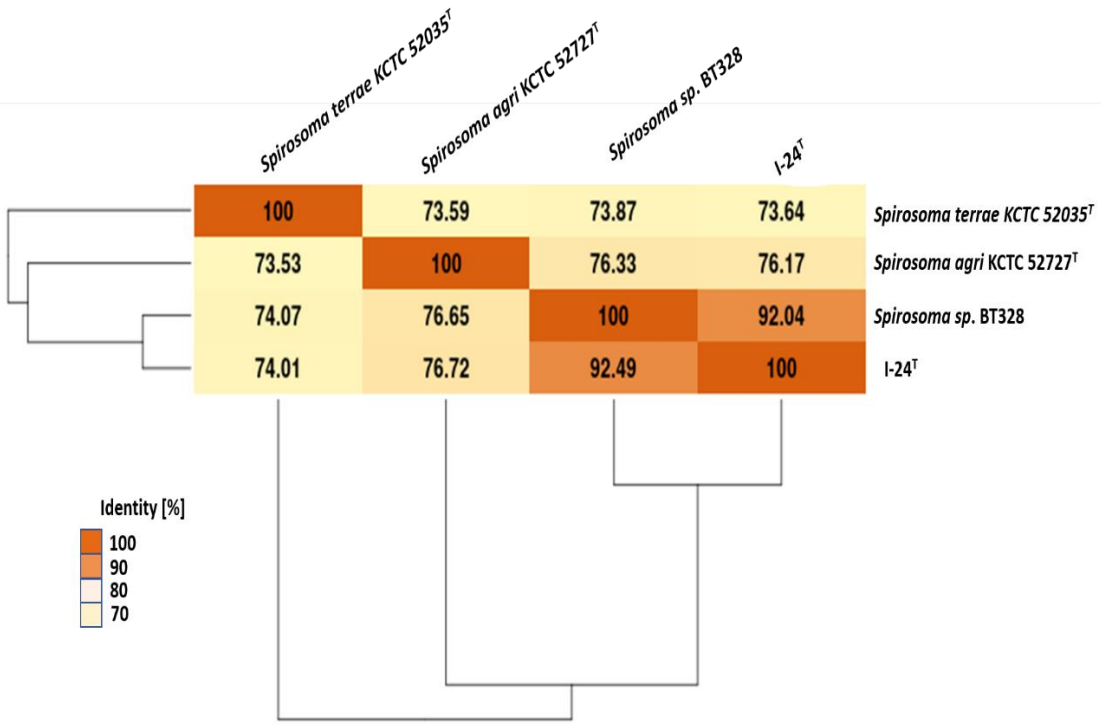
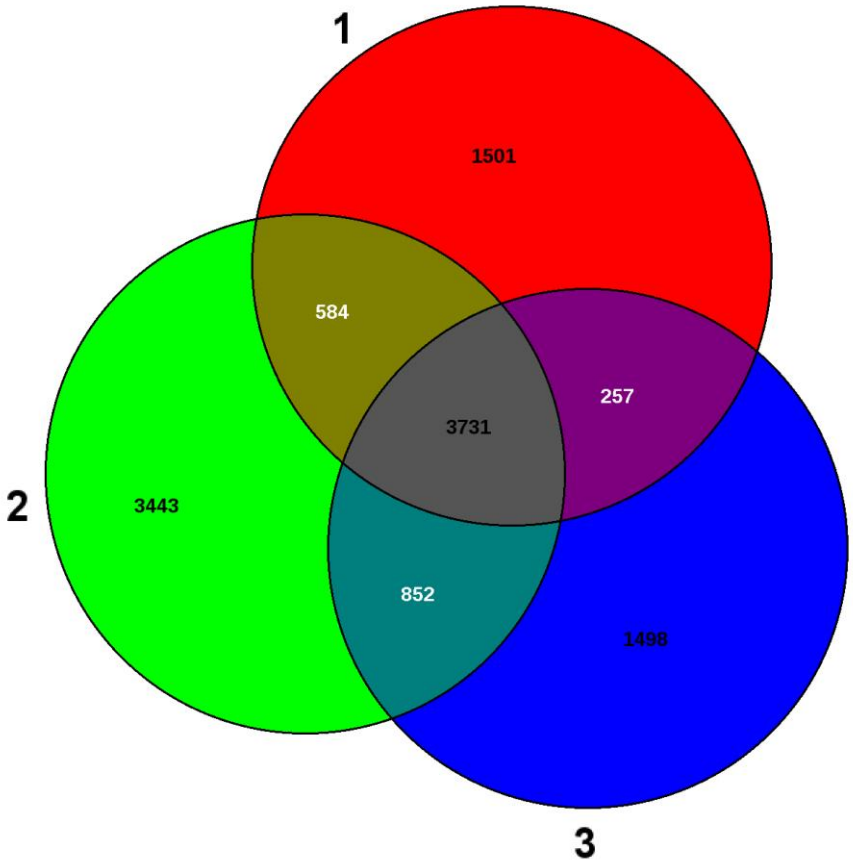


Fig. S3. ANI (Average Nucleotide Identity) mean values matrix heatmap between strain I-24^T, *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T.



1. *Spirosoma agri* KCTC 52727^T
2. *Spirosoma I-24*^{T}
3. *Spirosoma terrae* KCTC 52035^T

Fig. S4. Venn diagram depicted the number of coding sequences shared between I-24^T, *S. agri* KCTC 52727^T and *S. terrae* KCTC 52035^T.

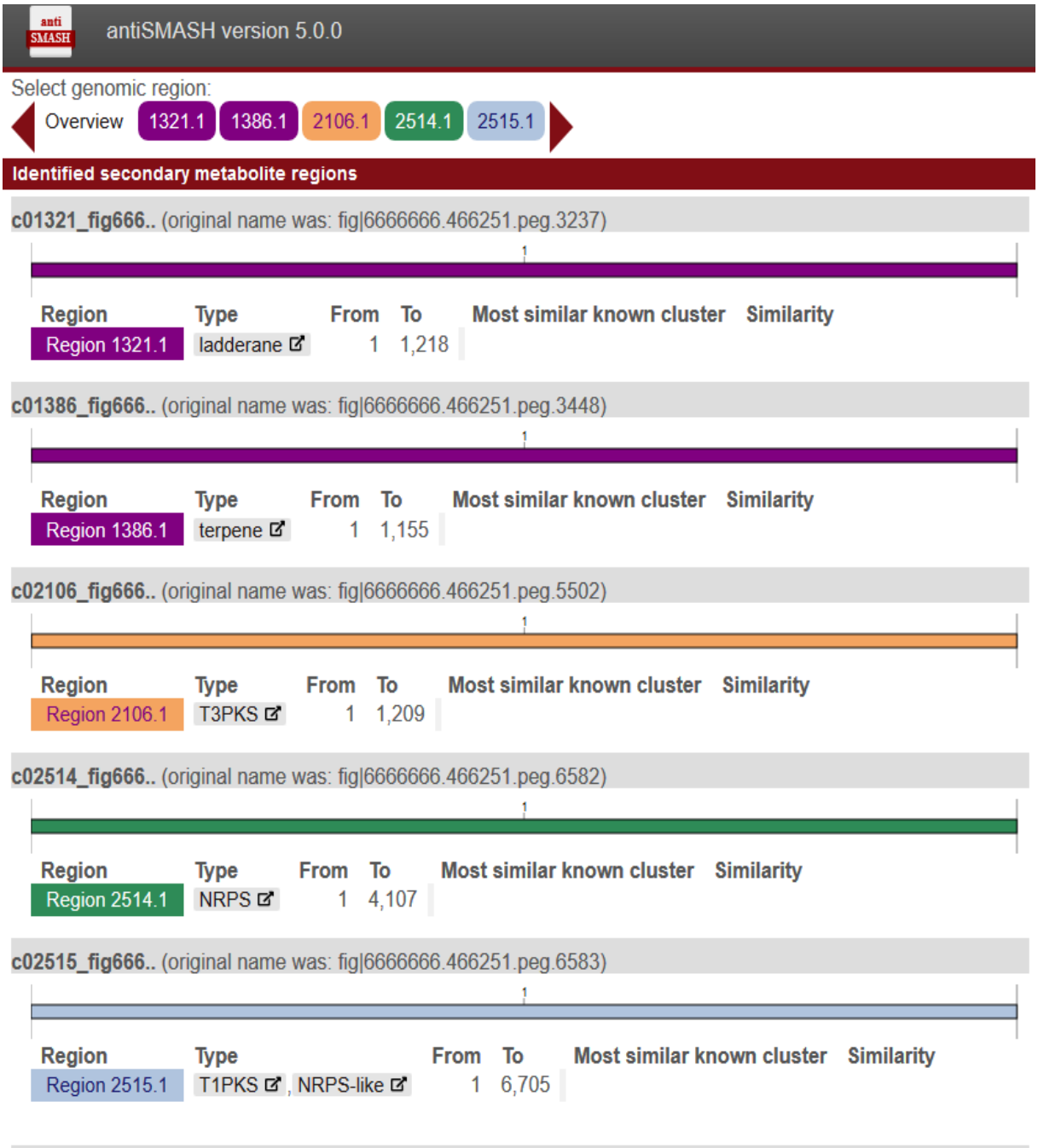
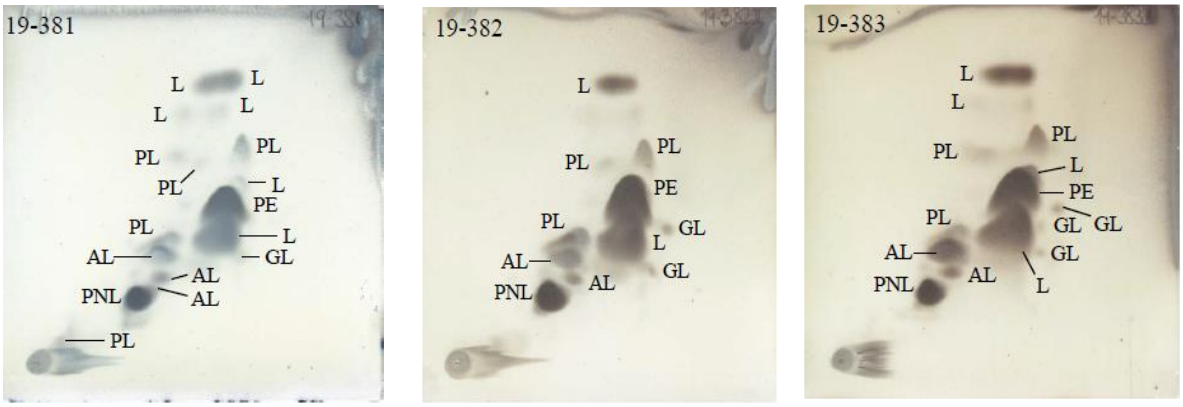


Fig. S5. Predicted gene clusters of secondary metabolites biosynthesis annotated in antiSMASH against strain I-24^T draft genome. Analyses showed the identification of 4 clusters involved in biosynthesis of ladderane, terpene, polyketide synthase type I and III (T1PKS, T3PKS) and non-ribosomal peptide synthetase (NRPS)



A. **B.** **C.**

Fig. S6. Polar lipids profile of strains **A.** I-24^T **B.** *S. agri* KCTC 52727^T and **C.** *S. terrae* KCTC 52035^T separated by two-dimensional silica gel thin layer chromatography. L, unidentified lipid; AL, unidentified aminolipid; PL, unidentified phospholipid; GL, glycolipid; PE, phosphatidylethanolamine; PNL, phosphoaminolipid. Total lipid material and specific functional groups were revealed using spray reagents specific dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate-Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen) and α – naphthol-sulphuric acid (glycolipids).

Table S1. Polar lipids profile of strain I-24^T compared to nearest *Spirosoma* species. L, unidentified lipid; AL, unidentified aminolipid; PL, unidentified phospholipid; GL, glycolipid; PE, phosphatidylethanolamine; PNL, phosphoaminolipid. *Data obtained in this study

	PE	GL	PL	AL	APL	L	Reference
I-24^T	+	+	+	+	+	+	*
Spirosoma agri	+	+	+	+	+	+	*
Spirosoma terrae	+	+	+	+	+	+	*
Spirosoma migulaei	+	-	+	-	+	+	[1]
Spirosoma pulveris	+	-	+	+	+	+	[2]
Spirosoma swuense	+	+	+	+	+	+	[3]
Spirosoma aerophilum	+	-	-	+	+	+	[4]
Spirosoma rigui	+	-	-	+	-	+	[5]
Spirosoma litoris	+	-	+	-	+	+	[6]
Spirosoma koreense	+	-	+	+	+	+	[7]
Spirosoma linguale	+	-	-	+	-	+	[8]

Spirosoma jeollabukense	**-**	**-**	**+**	**+**	**+**	**+**	[9]

REFERENCES

1. **Okiria J, Ten LN, Park S-J, Lee S-Y, Lee DH, *et al.*** *Spirosoma migulaei* sp. nov., isolated from soil. *J Microbiol* 2017;55:927–932.

2. **Joo ES, Lee J-J, Cha S, Jheong W, Seo T, *et al.*** *Spirosoma pulveris* sp. nov., a bacterium isolated from a dust sample collected at Chungnam province, South Korea. *J Microbiol* 2015;53:750–755.

3. **Joo ES, Kim EB, Jeon SH, Srinivasan S, Kim MK**. *Spirosoma swuense* sp. nov., isolated from wet soil. *Int J Syst Evol Microbiol* 2017;67:532–536.

4. **Hong S-B, Seok S-J, Kim J-S, Kwon S-W, Ahn J-H, *et al.*** *Spirosoma aerophilum* sp. nov., isolated from an air sample. *Int J Syst Evol Microbiol* 2016;66:2342–2346.

5. **Baik KS, Kim MS, Park SC, Lee DW, Lee SD, *et al.*** *Spirosoma rigui* sp. nov., isolated from fresh water. *Int J Syst Evol Microbiol* 2007;57:2870–2873.

6. **Okiria J, Ten LN, Lee J-J, Lee S-Y, Cho Y-J, *et al.*** *Spirosoma litoris* sp. nov., a bacterium isolated from beach soil. *Int J Syst Evol Microbiol* 2017;67:4986–4991.

7. **Ten LN, Okiria J, Lee J-J, Lee S-Y, Kang I-K, *et al.*** *Spirosoma koreense* sp. nov., a species of the family Cytophagaceae isolated from beach soil. *Int J Syst Evol Microbiol* 2017;67:5198–5204.

8. **Lail K, Sikorski J, Saunders E, Lapidus A, Glavina Del Rio T, *et al.*** Complete genome sequence of *Spirosoma linguale* type strain (1T). *Stand Genomic Sci* 2010;2:176–184.

9. **Li W, Ten LN, Lee SY, Lee DH, Jung HY**. *Spirosoma jeollabukense* sp. nov., isolated from soil. *Arch Microbiol* 2018;200:431–438.

CHAPTER 4.

Draft Genome Sequences of *Spirosoma agri KCTC 52727* and *Spirosoma terrae KCTC 52035*

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GENOME SEQUENCES

Draft Genome Sequences of *Spirosoma agri* KCTC 52727 and *Spirosoma terrae* KCTC 52035

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ABSTRACT *Spirosoma agri* S7-3-3 (KCTC 52727) and *Spirosoma terrae* 15J9-4 (KCTC 52035) are type strains isolated from an apple orchard and beach soil in South Korea, respectively; their draft genome sequences were assembled and annotated. The draft genome sequences of S7-3-3^T (7,239,915 bp; G+C content, 50.6%) and 15J9-4^T (7,551,610 bp; G+C content, 47.3%) are reported.

Spirosoma is the largest genus in the family *Cytophaga*, class *Bacteroidetes*. Recently described species have been isolated from several environmental habitats, such as air, dust, water, and soil (1–4).

Typical characteristics of the group members are the following: diversity of morphology such as rods, coils, and filaments; Gram-negative staining; colonies yellow to orange pigmented; phosphatidylethanolamine as the major polar lipid; MK7 as the major menaquinone; summed feature 3 ($C_{16:1}$ ω7c/$C_{16:1}$ ω6c) as the major fatty acid; and DNA G+C content range of 47.2 to 57.0 mol% (2, 3, 5–8).

Spirosoma agri S7-3-3^T was described by Li et al. (3) and *Spirosoma terrae* 15J9-4^T by Ten et al. (2), and both were validly published according to the International Code of Nomenclature of Prokaryotes (9). Both strains are Gram-negative, nonmotile, rod-shaped bacteria initially isolated from apple orchard soil in Gyeongsangnam, South Korea, and from soil collected on Jeju Island, South Korea, respectively, using a dilution plating method on R2A agar (Difco). Both grow optimally in R2A medium at 25°C and pH 7.0. According to the 16S rRNA gene similarities, the closest relatives to *S. agri* S7-3-3^T were *Spirosoma rigui* WPCB118^T (94.3%) and *Spirosoma pulveris* JSH5-14^T (93.9%) (3), and those for *Spirosoma terrae* 15J9-4^T were *Spirosoma panaciterrae* Gsoil 1519^T (94.2%) and *Spirosoma luteolum* 16F6E^T (94.1%) (2).

S. agri S7-3-3^T (KCTC 52727) and *S. terrae* 15J9-4^T (KCTC 52035) were purchased from the Korean Collection for Type Cultures (KCTC). The total genomic DNA for each strain was obtained using the method of Pitcher et al. (10) after growing the colony in R2A liquid medium for 48 h at 25°C. Two paired-end libraries were sequenced using 300-bp paired-end chemistry on a MiSeq v3 sequencer system (Illumina) at LGC Genomics (Germany). The sequencing yielded 1,780,390 raw reads for *Spirosoma agri* S7-3-3^T and 1,350,368 for *Spirosoma terrae* 15J9-4^T, and quality control of the reads was assessed with FastQC (11). The reads were assembled with SPAdes v3.13.1 (12) using k-mer values of 21, 33, and 55. Open reading frames (ORFs), gene annotation, and G+C contents were determined using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (13). Genome completeness and contamination were assessed with CheckM v1.0.18 using default parameters (14). Carbohydrate-active enzymes (CAZymes) were annotated with the dbCAN database using model HMMdb v8.0 (E value, $<1e^{-15}$; coverage, >0.35) (15), and secondary metabolite biosynthesis gene clusters were identified using antiSMASH v5.0.0 with default parameters (16). Information about sequencing and annotation results is summarized in Table 1.

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TABLE 1 Sequencing and annotation results for *S. agri* S7-3-3^T and *S. terrae* 15J9-4^T

Strain	Assembly size (bp)	No. of contigs	N_{50} (bp)	No. of predicted coding sequences	No. of:		G+C content (%)
					tRNAs	rRNAs (5S, 16S, 23S)	
S. agri S7-3-3^T	7,239,915	36	4,167,621	5,826	41	3 (1, 1, 1)	50.6
S. terrae 15J9-4^T	7,551,610	62	365,996	6,170	43	4 (1, 2, 1)	47.3

Based on CheckM, the draft genomes were estimated to be ≥99% complete with <1.2% contamination.

Genome annotation revealed genes for nitrate reduction in *S. agri* S7-3-3^T but not in *S. terrae* 15J9-4^T; furthermore, both strains have genes for alkaline phosphatase, cellulase, and amylase activity.

The dbCAN analysis described 355 genes for *S. agri* S7-3-3^T and 314 genes for *S. terrae* 15J9-4^T encoding proteins for carbohydrate binding, carbohydrate esterases, glycoside hydrolases, and glycoside transferases. Additionally, using antiSMASH, gene clusters for the production of ladderane, terpene, polyketide synthase types I and III (T1PKS, T3PKS), and nonribosomal peptide synthetase (NRPS) were annotated. These genomes will contribute to the genomic knowledge of the members of genus *Spirosoma*.

Data availability. The genome sequences of these two strains have been deposited in GenBank; the raw data sets can be found under BioProject accession numbers PRJNA590610 for *S. agri* S7-3-3^T and PRJNA590616 for *S. terrae* 15J9-4^T. The assembled sequences for *S. agri* S7-3-3^T (BioSample accession number SAMN13335970) can be accessed under accession number ASM1074741v1; the assembly version described in this paper is the first version. For *S. terrae* 15J9-4^T (BioSample accession number SAMN13335992), the assembled sequences can be accessed under accession number ASM1043591v1; the assembly version described in this paper is the first version.

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REFERENCES

1. Editorial Board. 2015. Spirosoma, p 1–6. *In* Whitman WB, John Wiley & Sons, Inc. (ed), Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Inc., Hoboken, NJ.
2. Ten LN, Okiria J, Lee J-J, Lee S-Y, Park S, Lee DS, Kang I-K, Kim MK, Jung H-Y. 2018. *Spirosoma terrae* sp. nov., isolated from soil from Jeju Island, Korea. Curr Microbiol 75:492–498. https://doi.org/10.1007/s00284-017-1408-6.
3. Li W, Lee S-Y, Kang I-K, Ten LN, Jung H-Y. 2018. *Spirosoma agri* sp. nov., isolated from apple orchard soil. Curr Microbiol 75:694–700. https://doi.org/10.1007/s00284-018-1434-z.
4. Li W, Ten LN, Lee S-Y, Lee DH, Jung H-Y. 2018. *Spirosoma jeollabukense* sp. nov., isolated from soil. Arch Microbiol 200:431–438. https://doi.org/10.1007/s00203-017-1453-3.
5. Li W, Lee SY, Kang IK, Ten LN, Jung HY. 2018. *Spirosoma pomorum* sp. nov., isolated from apple orchard soil. J Microbiol 56:90–96. https://doi.org/10.1007/s12275-018-7430-y.
6. Li W, Ten LN, Lee S-Y, Kang I-K, Jung H-Y. 2018. *Spirosoma horti* sp. nov., isolated from apple orchard soil. Int J Syst Evol Microbiol 68:930–935. https://doi.org/10.1099/ijsem.0.002614.
7. Zhang L, Zhou X-Y, Su X-J, Hu Q, Jiang J-D. 2019. *Spirosoma sordidisoli* sp. nov., a propanil-degrading bacterium isolated from a herbicide-contaminated soil. Antonie Van Leeuwenhoek 112:1523–1532. https://doi.org/10.1007/s10482-019-01278-4.
8. Weilan L, Lee J-J, Lee S-Y, Park S, Ten LN, Jung H-Y. 2018. *Spirosoma humi* sp. nov., isolated from soil in South Korea. Curr Microbiol 75:328–335. https://doi.org/10.1007/s00284-017-1384-x.
9. International code of nomenclature of prokaryotes. 2019. Int J Syst Evol Microbiol 69:S1–S111. https://doi.org/10.1099/ijsem.0.000778.
10. Pitcher DG, Saunders NA, Owen RJ. 1989. Rapid extraction of bacterial DNA with guanidium thiocyanate. Lett Appl Microbiol 8:151–156. https://doi.org/10.1111/j.1472-765X.1989.tb00262.x.
11. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
13. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
14. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.
15. Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y. 2018. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res 46:W95–W101. https://doi.org/10.1093/nar/gky418.
16. Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R. 2011. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39:W339–W346. https://doi.org/10.1093/nar/gkr466.

CHAPTER 5.

Screening of bacterial strains with methylotrophic activity lanthanum dependent

Summary

The methanol is one of the most abundant volatile organic compounds in the atmosphere (Galbally and Kirstine, 2002) and it is also one of the carbon sources that methylotrophic bacteria oxidize to formaldehyde by means of methanol dehydrogenase (MDH) as their sole carbon and energy source (Kolb, 2009). In gram-negative methylotrophic bacteria, MDH is well characterized, it possesses pyrroloquinoline quinone (PQQ) as a prosthetic group and is encoded by *mxaF-gene.* More recently a lanthanide-dependent methanol dehydrogenase, encoded by the gene *xoxF* has been discovered (Hibi et al., 2011a; Fitriyanto et al., 2011). No study has focused on bioprospection of methanol consuming microorganisms that may use the recently discovered lanthanum-dependent MDH-*xoxF* from saline and alkaline environments which is why this study aimed to isolate lanthanum-dependent methanol utilizing bacteria from saline habitats.

Methodology

Sample collection

Soil samples were collected from natural salt meadows nearby Münzenberg, Hessen, Germany (50°27'46.2"N 8°45'55.1"E) and Bad Endbach, Hessen, Germany (50° 45’ 54.9’’ N 8° 26’ 41.2’’E), branches from a blackthorn wall at graduation buildings where salty spring water slowly trickles down (50° 21’ 48.8’’N 8°44’ 39.6’’E) as well a water sample from a fountain with salty spring water (50° 22’ 2.23’’N 8°44’ 36.8’’E) in Bad Nauheim, Hessen, Germany were collected.

Isolation and identification of methanol utilizers

The samples of soils and water were used as inoculum for enrichment cultures in modified liquid freshwater media (Widdel and Bak, 1992) containing 1.0 g NaCl, 0.4 g MgCl x $6H_2O$, 0.15 g $CaCl_2$ x $2H_2O$, 0.5 g KCl, 0.2 g KH_2PO_4 , 0.25 g NH_4 Cl, 1.4 g Na_2SO_4 , 1.0 ml trace element solution containing no lanthanum (SL10), 50 ml Phosphate buffer 0.4 M pH 7.0, 1.0 ml vitamin B12-solution, 1.0 ml 5-vitamin solution, 1.0 ml thiamine solution, 1.0 ml riboflavine solution in 1.0 liter deionized water, the pH was adjusted to 6.5; additionally 30 µM lanthanum and methanol

5.0% V/V were added to the liquid medium. The enrichment cultures were shaken with 110 rpm and incubated at 28°C for one month. After several transfers in new medium the enriched bacteria were isolated on freshwater agar medium containing 30 µM lanthanum chloride and methanol 5% V/V. Different morphologies of colonies were obtained and various cell morphologies have been observed microscopically. For isolated strains the 16S RNA gene was amplified using the primer EUB 9f according to the method of Kampmann et al., 2012; PCR products were purified using the PCR purification kit (Qiagen) prior to sequencing by LGC genomics and the taxonomic affiliations were identified using EzBioCloud server (Yoon et al., 2017).

Growth test with new isolates

A test to evaluate the growth of the identified isolates on fresh water solid media with methanol in presence and absence of lanthanum was performed. For those isolates with good growth in presence of lanthanum on agar medium also liquid medium was tested and growth was evidenced by high turbidity and microscopic controls. Growth curves in liquid freshwater media with methanol 5% v/v and with/without addition of 30 µM $LaCl_3$ were carried out. Optical density (OD) measurements were performed in a TECAN infinity 200 fluorescent spectrophotometer using a Greiner 48-well plate. Isolates were cultured for 5 days with orbital shaking amplitude 4 mm, eight OD measurements were taken at 600 nm (bandwidth 9) with 5 flashes at 25 °C.

Results

From collected samples, thirty-one strains were isolated, and their 16S RNA gene was sequenced for taxonomic affiliation. The next relatives of the isolated strains and the morphological and colony description are given in Table 1 and Table S1.

Strain N°	**Source**	**Blast Bacteria 16S rRNA gene**	**% Similarity**	**Next Relatives (EZ-Biocloud)**
1	Salt meadow Bad Endbach	*Ancylobacter defluvii*	99.33	*Starkeya novella* 99.07% *Starkeya koreensis* 98.53%

				Ancylobacter rudongensis 98.4%
2	Salt meadow Bad Endbach	*Ancylobacter defluvii*	99.47	*Ancylobacter oerskovii* 98.32% *Ancylobacter rudongensis* 98% *Ancylobacter dichloromethanicus* 97.9%
3	Salt meadow Bad Endbach	*Paenibacillus glucanolyticus*	99.72	*Paenibacillus lautus* 98.21% *Paenibacillus qingshengii* 97.66% *Paenibacillus lactis* 96.96%
4	Salt meadow Bad Endbach	*Rhizobium nepotum*	99.79	*Rhizobium radiobacter* 99.04% *Rhizobium skierniewicense* 98.72% *Rhizobium rubi* 98.29%
5	Salt meadow Bad Endbach	*Ancylobacter defluvii*	99.47	*Ancylobacter oerskovii* 98.32% *Ancylobacter rudongensis* 98% *Ancylobacter dichloromethanicus* 97.9%
6	Salt meadow Münzenberg	*Variovorax boronicumulans*	99.29	*Variovorax paradoxus* 99.15% *Variovorax gossypii* 99.01% *Variovorax guangxiensis* 99.01%
7	Salt meadow Münzenberg	*Paenibacillus validus*	100	*Paenibacillus xylanisolvens* 97.38% *Paenibacillus mucilaginosus* 96.97% *Paenibacillus filicis 96.97%*
8	Graduation Building Bad Nauheim	*Ancylobacter rudongensis*	99.89	*Ancylobacter dichloromethanicus 99.47% Ancylobacter aquaticus 99.36% Ancylobacter vacuolatus 99.26%*
9	Graduation Building Bad Nauheim	*Jiella aquimaris*	98.67	*Aurantimonas coralicida* 96.45% *Aurantimonas endophytica* 96.45% *Aureimonas glaciistagni* 96.45%
10	Graduation building Bad Nauheim	*Tistrella bauzanensis*	99.33	*Tistrella mobilis* 97.33% *FM209132_s uncultured bacteria clon* 89.97% *Aliidongia dinghuensis* 89.86%
11	Water Fountain Bad Nauheim	*Paracoccus homiensis*	99.73	*Paracoccus zeaxanthinifaciens* 99.33% *Paracoccus beibuensis* 98.38% *Pararhodobacter aggregans* 98.12%

12	Salt meadow Münzenberg	*Azospirillum oryzae*	99.73	*Azospirillum zeae* 99.47% *Azospirillum humicireducens* 98.28% *Azospirillum largimobile* 98.25%
13	Salt meadow Münzenberg	*Pseudomonas hunanensis*	99.88	*Pseudomonas plecoglossicida* 99.76% *Pseudomonas monteilii* 99.64% *Pseudomonas taiwanensis* 99.51%
14	Salt meadow Münzenberg	*Idonella dechloratans*	99.25	*Ideonella paludis* 98% *Ideonella azotifigens* 97.99% *Rubrivivax gelatinosus* 97.75%
15	Graduation building Bad Nauheim	*Aurantimonas coralicida*	99.05	*Aurantimonas litoralis* 99.05% *Aurantimonas manganoxydans* 98.78% *Aureimonas frigidaquae* 97.42%
16	Salt meadow Bad Endbach	*Hyphomicrobium facile subsp. facile*	99.58	*Hyphomicrobium facile subsp. Tolerans 99.58% Hyphomicrobium facile subsp. Ureaphilum 99.58% Hyphomicrobium methylovorum 98.31%*
18	Salt meadow Bad Endbach	*Variovorax boronicumulans*	98.86	*Variovorax paradoxus* 98.74% *Variovorax gossypii* 98.61% *Variovorax ginsengisoli* 98.61%
20	Salt meadow Bad Endbach	*Hyphomicrobium faciles subs. facile*	99.58	*Hyphomicrobium facile subsp. Tolerans 99.58% Hyphomicrobium facile subsp. Ureaphilum 99.58% Hyphomicrobium methylovorum 98.31%*
21	Salt meadow Bad Endbach	*Microbacterium natoriense*	99,9	*Microbacterium aerolatum* 98.69% *Microbacterium ginsengiterrae* 98.64% *Microbacterium foliorum* 98.54%
22	Salt meadow Bad Endbach	*Variovorax boronicumulans*	99.05	*Variovorax paradoxus* 98.74% *Variovorax gossypii* 98.61% *Variovorax ginsengisoli* 98.61%
23	Salt meadow Bad Endbach	*Nocardiodes simplex*	98.60	*Nocardioides caeni* 98.6% *Nocardioides nitrophenolicus* 98.6%

				Nocardioides daeguensis 98.38%
24	Salt meadow Bad Endbach	*Spirosoma* *enbachense*	95	*Spirosoma pulveris* 94.52% *Spirosoma aerophilum* 94.3% *Spirosoma swuense* 94.29%
25	Water fountain Bad Nauheim	*Novosphingobium* *indicum*	100	*Novosphingobium panipatense* 97.38% *Novosphingobium mathurense* 97.38% *Novosphingobium barchaimii* 97.17%
26	Salt meadow Münzenberg	*Methyllophilus* *rhizosphaerae*	99.57	*Methylophilus quaylei* 99.36% *Methylophilus luteus* 98.94% *Methylophilus flavus* 98.72%
27	Salt meadow Münzenberg	*Sphyngopyxis* *taejonensis*	100	*Sphingopyxis chilensis* 98.2% *Sphingopyxis ginsengisoli* 98.2% *Sphingopyxis italic* 98.10 %
28	Salt meadow Münzenberg	*Pseudomonas* *putida*	100	*Pseudomonas hunanensis* 99.79% *Pseudomonas monteilii* 99.79% *Pseudomonas taiwanensis* 99.69%
29	Salt meadow Münzenberg	*Pseudomonas* *hunanensis*	100	*Pseudomonas hunanensis* 99.79% *Pseudomonas monteilii* 99.79% *Pseudomonas taiwanensis* 99.69%
30	Salt meadow Münzenberg	*Pseudomonas* *putida*	100	*Pseudomonas hunanensis* 99.79% *Pseudomonas monteilii* 99.79% *Pseudomonas taiwanensis* 99.69%
31	Salt meadow Münzenberg	*Pseudomonas* *putida*	100	*Pseudomonas hunanensis* 99.79% *Pseudomonas monteilii* 99.79% *Pseudomonas taiwanensis* 99.69%

Table 1. Bacterial isolates obtained from different salty habitats and their next relatives based on the 16S rRNA sequence.

As showed in table 1. most of the colonies were isolated from salt meadows and the genera with high prevalence in the samples were *Ancylobacter*, *Pseudomonas*, *Variovorax*, *Paenibacillus* and *Hyphomicrobium*, moreover the most predominant morphology was rod-shaped (**Table S.1**).

After taxonomic identification, the isolates were grown in FWM liquid medium with methanol and lanthanum addition. For strain 14 (99.25% similarity to *Idonella dechlorotans*) and strain 15 (99.05% similarity to *Aurantimonas coralicida*) a high level of turbidity (measured as optical density) and cell numbers (observed microscopically) were determined. A growth curve on methanol for these two strains was determined in presence and absence of La^{3+} as depicted in Fig.1

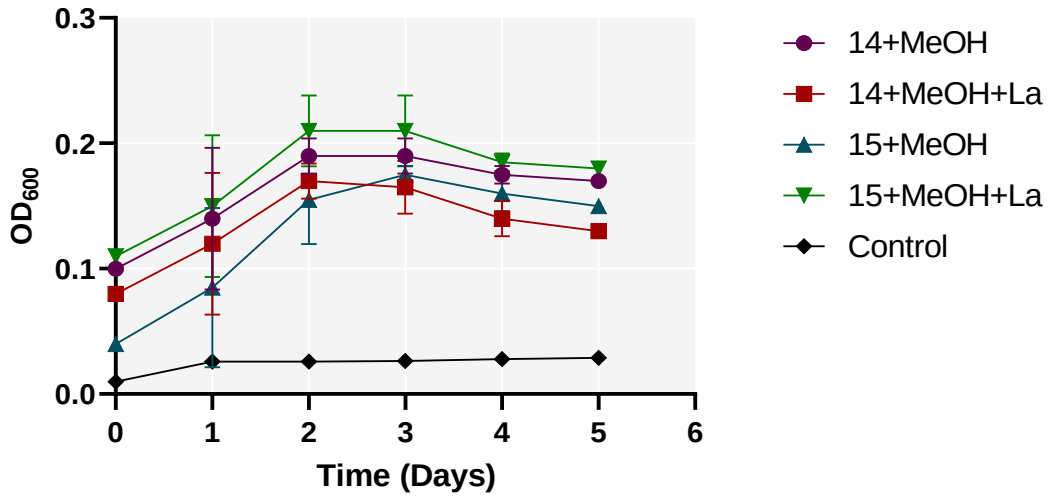

Figure 1. Growth of strains 14 and 15 in freshwater media with La^{3+} ($LaCl_3$ 30 µM) and MeOH (Methanol 5%v/v). Control is medium without bacteria addition. Values represent the average of eight replicate growth experiments with corresponding standard deviations, the optical density (OD 600) was used as growth parameter.

As shown in the Fig. 1, no remarkable difference in growth of both strains on methanol with or without lanthanum was observed. Therefore, lanthanum did not allow better growth on methanol. With strain 15 growth was slightly stimulated in the presence of lanthanum although it was not significant different to growth in absence of lanthanum.

DISCUSSION

Different samples from naturally salty environments within Hesse, Germany that included soils, water and branches impregnated with saline water were used for this study. These samples were used as inoculum for enrichment cultures in freshwater medium with methanol in presence of lanthanum. After three subsequent transfers in liquid medium the enriched bacteria were plated on FWM

agar medium. A great variety of colonies and bacterial cell morphologies checked by microscopy were obtained. DNA was extracted from thirty-one pure colonies and based on the 16S rRNA gene sequence a taxonomic affiliation revealed that 83.0% of the isolates belong to the phylum *Proteobacteria*, 7.0% to *Firmicutes*, 7.0% to *Actinobacteria* and 3.0% to *Bacteroidetes*. Several isolates were affiliated to genera *Ancylobacter, Pseudomonas*, *Variovorax, Paenibacillus* and *Hyphomicrobium.* For genus *Ancylobacter* methanol consumption was demonstrated and the presence of *mxaF*-gene that encodes a methanol dehydrogenase dependent of calcium as showed for *Ancylobacter aquaticus* (Lau et al., 2013) and *Ancylobacter sonchi* (Agafonova et al., 2017). However also the presence of *xoxF*-gene a methanol dehydrogenase dependent of lanthanum has been reported for *Ancylobacter* sp. FA202 and *Ancylobacter* sp. 501b. (Huang et al., 2018). *Pseudomonas* was considered a non-methylotrophic model organism but recently a lanthanide-dependent PQQ-alcohol dehydrogenase (*PedH*) was discovered, which is a homologue of the *xox*F-protein which has enzymatic activity on aldehydes, aromatic primary and secondary alcohols and is dependent of lanthanides such as La^{3+}, Ce^{3+}, Pr^{3+}, Sm^{3+}(Wehrmann et al., 2017). *Variovorax* has been described as a genus of versatile metabolism with abilities as plant growth promoters (S.-L. Sun et al., 2018); a metagenomic study to identify the diversity, abundance and activity of methylotrophs revealed a great abundance of *Variovorax* in the methanol-enriched pea rhizosphere community (Macey et al., 2020); *Paenibacillus* species can be plant associated and have a good ability to fix nitrogen (Hong et al., 2009), the capacity to use methanol in this genus has been also described (Madhaiyan et al., 2016). The genus *Hyphomicrobium* is known as facultative methylotrophs which can be found in water or soil (Martineau et al., 2013), methanol dehydrogenase genes such as *mxaF* are used as molecular markers for identification of new species of *Hyphomicrobium* (Fesefeldt and Gliesche, 1997) but also *xoxF*-gene has been detected (Huang et al., 2018).

To evaluate the need of lanthanum for methanol consumption, lanthanum was added to freshwater medium and growth of strain 14 (99.25% similarity to *Idonella dechlorotans)* and strain 15 (99.05% similarity to *Aurantimonas coralicida*) was determined. Growth was very similar in presence and absence of lanthanum (Fig. 1). Similar results have been reported for growth of *Methylobacterium extorquens* in studies with methanol with and without lanthanum (Good et al., 2019; Vu et al., 2016). Also growth of *Methylosinus trichosporium* in medium with methanol and

cerium was not reported to be different than with methanol (Ul Haque et al., 2015). Moreover OD values obtained for strain 14 and 15 are lower (OD_{600} 0.1-0.2) in comparison to the studies mentioned before where values were around OD_{600} 0.4-1). The low yield exhibited for both strains may indicate other specific methylotrophy metabolic modules including the serine cycle, the ethylmalonyl-CoA-pathway EMCP, the H4MPT-linked pathway and not the presence of methanol dehydrogenases encoded by *mxaF* and *xoxF* genes as described by Chistoserdova, (2011) for which other factors may affect the yield growth.

This study revealed a great variety of different bacterial genera able to grow on methanol. No clear indication for growth using the lanthanum-dependent methanol dehydrogenase was found. A better identification of lanthanum-dependent methylotrophic bacteria in enrichment cultures could be the detection of *xoxF*-gene through molecular probes in realtime PCR assays or dot plot analysis. Well adapted methylotrophic bacteria with PGPR activity potential could help the plants to cope with salt stress.

REFERENCES

Agafonova, N. V., Kaparullina, E. N., Trotsenko, Y. A., & Doronina, N. V. (2017). *Ancylobacter sonchi* sp. nov., a novel methylotrophic bacterium from roots of *Sonchus arvensis L*. International Journal of Systematic and Evolutionary Microbiology, 67(11), 4552–4558. https://doi.org/10.1099/ijsem.0.002330

Chistoserdova, L. (2011). Modularity of methylotrophy, revisited. Environmental Microbiology, 13(10), 2603–2622. https://doi.org/10.1111/j.1462-2920.2011.02464.x

Fesefeldt, A., & Gliesche, C. G. (1997). Identification of *Hyphomicrobium* spp. using PCR-amplified fragments of the mxaF gene as a molecular marker. Systematic and Applied Microbiology, 20(3), 387–396. https://doi.org/10.1016/S0723-2020(97)80007-0

Fitriyanto, N. A., Fushimi, M., Matsunaga, M., Pertiwiningrum, A., Iwama, T., & Kawai, K. (2011). Molecular structure and gene analysis of Ce^{3+}-induced methanol dehydrogenase of *Bradyrhizobium* sp. MAFF211645. Journal of Bioscience and Bioengineering, 111(6), 613–617. https://doi.org/10.1016/j.jbiosc.2011.01.015

Galbally, I. E., & Kirstine, W. (2002). The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry, 43, 195–229. https://doi.org/10.1023/A:1020684815474

Good, N. M., Moore, R. S., Suriano, C. J., & Martinez-Gomez, N. C. (2019). Contrasting in vitro and in vivo methanol oxidation activities of lanthanide-dependent alcohol dehydrogenases XoxF1 and ExaF from *Methylobacterium extorquens* AM1. Scientific Reports, 9(1), 1–12. https://doi.org/10.1038/s41598-019-41043-1

Hibi, Y., Asai, K., Arafuka, H., Hamajima, M., Iwama, T., & Kawai, K. (2011). Molecular structure of La^{3+}-induced methanol dehydrogenase-like protein in *Methylobacterium radiotolerans*. Journal of Bioscience and Bioengineering, 111(5), 547–549. https://doi.org/10.1016/j.jbiosc.2010.12.017

Hong, Y. Y., Ma, Y. C., Zhou, Y. G., Gao, F., Liu, H. C., & Chen, S. F. (2009). *Paenibacillus sonchi* sp. nov., a nitrogen-fixing species isolated from the rhizosphere of *Sonchus oleraceus*. International Journal of Systematic and Evolutionary Microbiology, 59(11), 2656–2661. https://doi.org/10.1099/ijs.0.009308-0

Huang, J., Yu, Z., & Chistoserdova, L. (2018). Lanthanide-dependent methanol dehydrogenases of XoxF4 and XoxF5 clades are differentially distributed among methylotrophic bacteria and they reveal different biochemical properties. Frontiers in Microbiology, 9(JUN), 1366. https://doi.org/10.3389/fmicb.2018.01366

Kampmann, K., Ratering, S., Kramer, I., Schmidt, M., Zerr, W., & Schnell, S. (2012). Unexpected stability of *Bacteroidetes* and *Firmicutes* communities in laboratory biogas reactors fed with different defined substrates. Applied and Environmental Microbiology, 78(7), 2106–2119. https://doi.org/10.1128/AEM.06394-11

Kolb, S. (2009). Aerobic methanol-oxidizing Bacteria in soil. FEMS Microbiology Letters, 300(1), 1–10. https://doi.org/10.1111/j.1574-6968.2009.01681.x

Lau, E., Fisher, M. C., Steudler, P. A., & Cavanaugh, C. M. (2013). The Methanol Dehydrogenase Gene, mxaF, as a Functional and Phylogenetic Marker for Proteobacterial Methanotrophs in Natural Environments. PLoS ONE, 8(2), 56993. https://doi.org/10.1371/journal.pone.0056993

Macey, M. C., Pratscher, J., Crombie, A. T., & Murrell, J. C. (2020). Impact of plants on the diversity and activity of methylotrophs in soil. Microbiome, 8(1), 1–17. https://doi.org/10.1186/s40168-020-00801-4

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., Pragatheswari, D., Duraipandiyan, V., Al-Dhabi, N. A., & Santhanakrishnan, P. (2016). *Paenibacillus methanolicus* sp. Nov., a xylanolytic, methanol-utilizing bacterium isolated from the phyllosphere of bamboo (*Pseudosasa japonica*). International Journal of Systematic and Evolutionary Microbiology, 66(11), 4362–4366. https://doi.org/10.1099/ijsem.0.001356

Martineau, C., Villeneuve, C., Mauffrey, F., & Villemur, R. (2013). Hyphomicrobium nitrativorans sp. nov., isolated from the biofilm of a methanol-fed denitrification system treating seawater at the Montreal Biodome. International Journal of Systematic and Evolutionary Microbiology, 63(PART10), 3777–3781. https://doi.org/10.1099/ijs.0.048124-0

Sun, S.-L., Yang, W.-L., Fang, W.-W., Zhao, Y.-X., Guo, L., & Dai, Y.-J. (2018). The Plant Growth-Promoting Rhizobacterium *Variovorax boronicumulans* CGMCC 4969 Regulates the Level of Indole-3-Acetic Acid Synthesized from Indole-3-Acetonitrile . Applied and Environmental Microbiology, 84(16), 298–316. https://doi.org/10.1128/aem.00298-18

Ul Haque, M. F., Kalidass, B., Bandow, N., Turpin, E. A., DiSpirito, A. A., & Semrau, J. D. (2015). Cerium regulates expression of alternative methanol dehydrogenases in *Methylosinus trichosporium* OB3b. Applied and Environmental Microbiology, 81(21), 7546–7552. https://doi.org/10.1128/AEM.02542-15

Vu, H. N., Subuyuj, G. A., Vijayakumar, S., Good, N. M., Martinez-Gomez, N. C., & Skovran, E. (2016). Lanthanide-dependent regulation of methanol oxidation systems in *Methylobacterium extorquens* AM1 and their contribution to methanol growth. Journal of Bacteriology, 198(8), 1250–1259. https://doi.org/10.1128/JB.00937-15

Wehrmann, M., Billard, P., Martin-Meriadec, A., Zegeye, A., & Klebensberger, J. (2017). Functional role of lanthanides in enzymatic activity and transcriptional regulation of pyrroloquinoline quinone-dependent alcohol dehydrogenases in *Pseudomonas putida* KT2440. MBio, 8(3), 570–587. https://doi.org/10.1128/mBio.00570-17

Widdel, F., & Bak, F. (1992). Gram-Negative Mesophilic Sulfate-Reducing Bacteria. In The Prokaryotes (pp. 3352–3378). Springer New York. https://doi.org/10.1007/978-1-4757-2191-1_21

Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H., & Chun, J. (2017). Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. International Journal of Systematic and Evolutionary Microbiology, 67(5), 1613–1617. https://doi.org/10.1099/ijsem.0.001755

Supplementary Data - Screening of bacterial strains with methylotrophic activity lanthanum dependent

Table S1. Bacterial isolates obtained from different salty habitats with their morphologies and colony shapes

Strain N°	Source	Blast Bacteria 16S	Colony shape and color	Morphology
1	Salt meadow Bad Endbach	*Ancylobacter defluvii*	Round cream	Rod-shaped
2	Salt meadow Bad Endbach	*Ancylobacter defluvii*	Round White	Rod-shaped
3	Salt meadow Bad Endbach	*Paenibacillus glucanolyticus*	Irregular yellow	Rod-shaped
4	Salt meadow Bad Endbach	*Rhizobium nepotum*	Round transparent	Rod-shaped
5	Salt meadow Bad Endbach	*Ancylobacter defluvii*	Round white	Rod-shaped
6	Salt meadow Münzenberg	*Variovorax boronicumulans*	Round red	Rod-shaped
7	Salt meadow Münzenberg	*Paenibacillus validus*	Irregular white	long curved rod
8	Graduation Building Bad Nauheim	*Ancylobacter rudongensis*	Round cream	Rod-shaped
9	Graduation Building Bad Nauheim	*Jiella aquimaris*	Round cream	Rod-shaped
10	Graduation building Bad Nauheim	*Tistrella bauzanensis*	Round white	Rod-shaped
11	Water Fountain Bad Nauheim	*Paracoccus homiensis*	Round transparent	Coccus
12	Salt meadow Münzenberg	*Azospirillum oryzae*	Round cream	Vibrioid

13	Salt meadow Münzenberg	*Pseudomonas hunanensis*	Round green	Rod-shaped
14	Salt meadow Münzenberg	*Idonella dechloratans*	Round transparent	Rod-shaped
15	Graduation building Bad Nauheim	*Aurantimonas coralicida*	Round yellow	Rod-shaped
16	Salt meadow Bad Endbach	*Hyphomicrobium facile subsp. facile*	Round white	Rod-shaped with prosteca
18	Salt meadow Bad Endbach	*Variovorax boronicumulans*	Round white	Rod-shaped
20	Salt meadow Bad Endbach	*Hyphomicrobium faciles subs. facile*	Round white	Rod-shaped with prosteca
21	Salt meadow Bad Endbach	*Microbacterium natoriense*	Round cream	Vibrioid
22	Salt meadow Bad Endbach	*Variovorax boronicumulans*	Round blue	Rod-shaped
23	Salt meadow Bad Endbach	*Nocardiodes simplex*	Irregular transparent	Rod-shaped
24	Salt meadow Bad Endbach	*Spirosoma enbachense*	Round cream	Rod-shaped
25	Water fountain Bad Nauheim	*Novosphingobium indicum*	Round blue	Long curved rod
26	Salt meadow Münzenberg	*Methyllophilus rhizosphaerae*	Irregular cream	Rod-shaped
27	Salt meadow Münzenberg	*Sphyngopyxis taejonensis*	Round cream	Rod-shaped
28	Salt meadow Münzenberg	*Pseudomonas putida*	Round transparent	Rod-shaped
29	Salt meadow Münzenberg	*Pseudomonas hunanensis*	Round yellow	Rod-shaped
30	Salt meadow Münzenberg	*Pseudomonas putida*	Round cream	Rod-shaped
31	Salt meadow Münzenberg	*Pseudomonas putida*	Round white	Rod-shaped

CHAPTER 6.

GENERAL DISCUSSION

GENERAL DISCUSSION

Recent studies have shown that rare metals play an important role to a specialized group of bacteria involved in the global carbon cycle. Rare metals such as lanthanum and cesium are required for the activity of a widespread methanol dehydrogenase (MDH) enzyme encoded by the *xoxF*-gene which is used for methanol utilization as source of carbon and energy by some bacteria. Genes encoding for methanol dehydrogenase have been detected in rhizosphere of pea plants, rice, cereals and grasses (Butterfield et al., 2016; Knief et al., 2012; Tsurumaru et al., 2015) and some of them affiliated to *xoxF*, however the role of this gene in plant root-bacteria interaction is unclear. *Hartmannibacter diazotrophicus* E19^T was isolated from a saline soil and exhibited abilities as plant growth promoter including ACC-deaminase production, nitrogen fixation, phosphorus solubilization (Suarez et al., 2015) but also methylotrophic activity as methanol consumer in presence of lanthanum associated to the presence of *xoxF*-gene was evidenced (Lv et al., 2017).

Methylotrophic activity assays

In order to reassess the methylotrophic activity of *H. diazotrophicus* E19^T in liquid culture, a growth curve in a mineral medium with methanol 5% v/v and 30 µM lanthanum was determined and methylotrophic growth via lanthanum-dependent MDH was confirmed. The suitable concentration of lanthanum for optimal growth of *H. diazotrophicus* E19^T was determined in growth studies with the pure culture, however, no significant differences were founded for lanthanum concentrations evaluated (Chapter 2). The effect of lanthanum on growth with methanol of *Methylacidiphilum fumariolicum SolV* was described by Pol et al., (2014) to be essential, however in contrast to the study of Pol et al., (2014) in this study no differences in the growth yield were found when concentrations of lanthanum varied.

Greenhouse trials

To evaluate the plant response under salt stress to inoculation of *H. diazotrophicus* E19^T and lanthanum supplementation, a pot experiment with barley plants in greenhouse was carried out (Chapter 2). The trials of the experiment comprised

barley seeds inoculated with strain E19^T sowed in saline soil with/without lanthanum, barley seeds inoculated with strain E19^T dead biomass sowed in saline soil with/without lanthanum, barley seeds in saline soil with lanthanum and the control with barley seeds in saline soil. Growth of plants was monitored after thirty days, when shoots and roots were harvested, and the fresh and dry weights were determined. Furthermore, plant samples from roots and rhizosphere soil were taken to explore changes in microbial diversity upon the treatments and to look into colonization of roots with E19^T.

The barley plants biomass of shoots and roots revealed no effect or differences between plants inoculated with *H. diazotrophicus* E19^T with and without lanthanum addition however better biomass was obtained in comparison to the treatments with dead biomass and the non-inoculated control. The results demonstrated that strain E19^T can promote growth of barley plants in salty conditions which is in agreement with the study of Suarez et al., (2015). These authors postulated a mechanism of *H. diazotrophicus* E19^T to reduce salt stress based on the lowering of ethylene levels in the plant by bacterial production of ACC deaminase.

Unexpectedly with lanthanum addition to soil without bacterial inoculation the plant obtained as good biomass values as the treatments with seed inoculation. The effect of lanthanum to increase plant tolerance under salty conditions was discussed to origin from mechanisms such as up-regulation of the antioxidant capacity in the chloroplast through ascorbate-glutathione cycle, the rising of cytoplasmic calcium levels which block the reactive oxygen species (ROS) production and the enhancement of chlorophyll and carotenoids contents (Huang et al., 2018; Li et al., 2007; Liu et al., 2016).

The inoculation of bacteria with plant promotion activity may also change the composition of rhizosphere communities thus indirectly affecting plant growth (Gadhave et al., 2018; Wang et al., 2018; Zhang et al., 2019; Zhang et al., 2019). In the current study, the indicators for microbial diversity were evaluated comparing effects of *H. diazotrophicus* inoculation with those of lanthanum supplementation. In roots and rhizosphere soil alpha diversity indices showed a significant increase in species richness in treatments of plants with *H. diazotrophicus* and *H. diazotrophicus* with lanthanum compared to treatments with death biomass and lanthanum alone. According to nonmetric multidimensional scaling (NMDS) most similar communities were found in treatments of *H.*

diazotrophicus and *H. diazotrophicus* with La; most different was the community in control soil (Chapter 2).

The quantification of *H. diazotrophicus* E19^T colonization in rhizosphere soil and root environments was explored through qPCR using a specifically designed primer set targeting the 16S RNA gene sequence. High DNA copy numbers of strain E19^T were detected in barley roots (5.3×10^7 DNA copy number g^{-1} root dry weight) 30 days after seed inoculation. In rhizosphere soil strain E19^T DNA copy number were 3.2×10^4 DNA copy number g^{-1} soil dry weight which indicates the good bacterial competence for the plant root colonization but also that the rhizosphere is a suitable environment for the bacteria.

The quantification of bacterial cell number in root samples of plants grown in pots by qPCR are in similar values reported for studies with DNA quantification of other PGPR species; plants of *Brassica oleracea* were inoculated with *Enterobacter radicincitans* resulting in concentrations of 10^8 bacterial cells g^{-1} root fresh weight (Ruppel et al., 2006), in the same way, quantification of *Azospirillum lipoferum* CRT1 inoculated in maize plants in roots was 10^4–10^6 DNA copy number g^{-1} of root dry weight (Couillerot et al., 2010). On the other hand, the presence of lanthanum not increased the cell density of strain E19^T, the treatments with seed inoculation of *H. diazotrophicus* and *H. diazotrophicus* with lanthanum exhibited similar densities for the inoculated bacteria on roots, while in rhizosphere soil the number of DNA copies were higher in the treatment of strain E19^T without lanthanum supplementation. The differences in cell numbers of *H. diazotrophicus* in roots and rhizosphere soil might be due to the fact that during root growth and cell-plant division with pectin biosynthesis the methanol is directly available for *H. diazotrophicus* in the root and on its surface whereas in rhizosphere the exudate methanol is consumed by a great diversity of bacterial groups. The high substrate competition in the rhizosphere (Sy et al., 2005) may explain the lower cell numbers of *H. diazotrophicus* in rhizosphere. For the treatment with *H. diazotrophicus* and lanthanum supplementation the growth response of *H. diazotrophicus* may mostly be conditioned to substrate and lanthanum sensing, the presence of a "lanthanide-switch" which is described as transcriptional response can at low concentrations of lanthanum upregulate the *xoxF* activity conditioning the uptake of methanol and downregulate other metabolic routes for consumption of other carbon sources. A study carried out by Wehrmann et al., (2020) described the growth of

Pseudomonas putida containing *PedH*-MDH lanthanum dependent, a homologue of *xoxF*-MDH in presence of 10 µM lanthanum and other carbon sources; a downregulation of metabolic proteins expression was observed when growth occurred with glucose and lanthanum.

The quantification of *H. diazotrophicus* E19^T in roots also was performed using a designed primer set targeting the *xoxF*-gene sequence and 4.8 x 10^6 *xoxF* copies numbers g^{-1} root dry weight were obtained, which is similar to those DNA copy numbers retrieved with the specific 16S RNA gene assay (Chapter 2). These results confirm the colonization of *H. diazotrophicus* in roots and on the root surface since the primer was designed specifically for *xoxF*-gene sequence of *H. diazotrophicus*. The designed *xoxF* primer will open the path to study the expression of *xoxF* gene on RNA samples for future research focused on bacteria plant interactions.

Bioprospection

For this study, halotolerant methanol-oxidizing and lanthanum-dependent bacteria from salt-affected environmental samples (soil, water, graduation building) have been enriched in a mineral medium supplemented with methanol 5% v/v and 30 µM lanthanum, 31 isolates were recovered and by 16S RNA gene sequencing the taxonomic affiliation revealed most of them belonging to phylums *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, and some of them also have been reported with genes associated to methylotrophic activity dependent of rare metals (Chapter 5, Table 1). The isolates 14 and 15 exhibited good growth in liquid medium with methanol and lanthanum. Therefor a growth curve in liquid medium with methanol with/without lanthanum supplementation was determined which revealed that lanthanum not caused significant difference in the growth of both strains. However methylotrophic bacteria have high metabolic flexibility, different carbon assimilation pathways have been reported for energy conservation required in growth process including serine cycle, RuMP cycle, Benson cycle where lanthanides are not involved (Khmelenina et al., 2019). Moreover, from the enrichment and screening from soil samples (Chapter 5, Table 1), the bacterium 24 designated I-24 had a 95.0% 16S rRNA gene similarity to the next relative. Since a 16S rRNA gene identity value < 98.7% represents a new species a polyphasic approach was carried out for detailed characterization and identification of isolate I-24. The polyphasic study revealed that isolate I-24 was aerobic, non-motile, rod-shaped, catalase-positive, oxidase-positive and grew optimally at pH

7 and 25 °C. Based on 16S rRNA gene sequence close relatives are *Spirosoma agri* and *Spirosoma terrae* and isolate I-24 was grouped into the family *Spirosomaceae*, genus *Spirosoma* and with the proposed species name *Spirosoma endbachense* accepted. The draft genome sequence of *Spirosoma endbachense* has a size of 10,326,072 bp with a G+C of 47.7% and the completeness value was >99%. Secondary metabolite clusters annotated for *Spirosoma endbachense* were ladderane, terpene, polyketide synthase type I and III (T1PKS, T3PKS) and non-ribosomal peptide synthetase (NRPS) (Chapter 3).

For close the relatives *Spirosoma agri* S7-3-3 (KCTC 52727) and *Spirosoma terrae* 15J9-4 (KCTC 52035) a genome sequencing was not available. For detailed taxonomic characterization whole genome sequencing techniques are suggested to be used along with a polyphasic study to provide more information about identification, phylogenetic analysis, metabolic pathways, antimicrobial resistance and enable comparative genomic. Therefor the total genomes from both strains and *S. endbachense* were sequenced using a MiSeqv3 sequencer system (Illumina), assembled and annotated. The draft genome sequence of *Spirosoma agri* has a size of 7,239,915 bp with a G+C content of 50.6% while for *Spirosoma terrae* the size is of 7,551,610 bp with a G+C content of 47.3% (Chapter 4). Both genomes are ≥ 99% complete. Both species have genes for alkaline phosphatase, cellulose and amylase activity and many other carbohydrate-active enzymes. Furthermore gene clusters for the production of ladderane, terpen, polyketide synthase, non-ribolsomal peptide synthesis and secondary metabolite biosynthesis were annotated.

No reports have so far focused on the attempt to characterize methanol utilizing bacteria containing the recently discovered *xoxF*-MDH from saline and alkaline environments. Therefor in an isolation campaign methylotrophic bacteria from saline environments have been enriched in the presence of lanthanum to possibly retrieve halotolerant bacteria which consume methanol via *xox*F-MDH. These isolates are potential new candidates for plant growth promotion under salt stress due to their adaptation to natural salt environments. Other methylotrophic bacteria have been reported to mitigate salt stress effects on plants like the halotolerant *Actinobacterium Nocardioides sp. NIMMe6* which was reported to enhance the growth of wheat plants under salt stress (Meena et al., 2020). For the methanol-

utilising *Bacillus methylotrophicus* M4-1 enhanced uptake of Mg^{2+}, K^{+} was reported and the ability to reduce Na^{+} content in leaves of winter wheat under salt stress (Ji et al., 2020). For the latter bacteria however pathways or genes involved in C1 compounds have not been elucidated.

The results obtained in this study contribute to understanding the interaction of plant growth-promoting microorganisms with barley plants under salt stress, in addition, contribute to the knowledge of methylotrophic bacteria with a lanthanum-dependent MDH in saline environments and complement the study of the newly isolated and described *Spirosoma endbachense* and closely related *Spirosoma* species *S. agri* and *S. terrae* in a comparative genome study.

REFERENCES

Butterfield, C. N., Li, Z., Andeer, P. F., Spaulding, S., Thomas, B. C., Singh, A., Hettich, R. L., Suttle, K. B., Probst, A. J., Tringe, S. G., Northen, T., Pan, C., & Banfield, J. F. (2016). Proteogenomic analyses indicate bacterial methylotrophy and archaeal heterotrophy are prevalent below the grass root zone. PeerJ, 4, e2687. https://doi.org/10.7717/peerj.2687

Couillerot, O., Bouffaud, M. L., Baudoin, E., Muller, D., Caballero-Mellado, J., & Moënne-Loccoz, Y. (2010). Development of a real-time PCR method to quantify the PGPR strain *Azospirillum lipoferum* CRT1 on maize seedlings. Soil Biology and Biochemistry, 42(12), 2298–2305. https://doi.org/10.1016/j.soilbio.2010.09.003

Gadhave, K. R., Devlin, P. F., Ebertz, A., Ross, A., & Gange, A. C. (2018). Soil Inoculation with *Bacillus* spp. Modifies Root Endophytic Bacterial Diversity, Evenness, and Community Composition in a Context-Specific Manner. Microbial Ecology, 76(3), 741–750. https://doi.org/10.1007/s00248-018-1160-x

Huang, J., Yu, Z., & Chistoserdova, L. (2018). Lanthanide-dependent methanol dehydrogenases of XoxF4 and XoxF5 clades are differentially distributed among methylotrophic bacteria and they reveal different biochemical properties. Frontiers in Microbiology, 9(JUN), 1366. https://doi.org/10.3389/fmicb.2018.01366

Ji, C., Wang, X., Tian, H., Hao, L., Wang, C., Zhou, Y., Xu, R., Song, X., Liu, Y., Du, J., & Liu, X. (2020). Effects of *Bacillus methylotrophicus* M4-1 on physiological and biochemical traits of wheat under salinity stress. Journal of Applied Microbiology, 129(3), 695–711. https://doi.org/10.1111/jam.14644

Khmelenina, V. N., But, S. Y., Rozova, O. N., & Trotsenko , Y. A. (2019). Metabolic Features of Aerobic Methanotrophs: News and Views . In Methylotrophs and

Methylotroph Communities. Caister Academic Press. https://doi.org/10.21775/9781912530045.04

Knief, C., Delmotte, N., Chaffron, S., Stark, M., Innerebner, G., Wassmann, R., Von Mering, C., & Vorholt, J. A. (2012). Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME Journal, 6(7), 1378–1390. https://doi.org/10.1038/ismej.2011.192

Li, J. Y., Jiang, A. L., Chen, H. Y., Wang, Y., & Zhang, W. (2007). Lanthanum prevents salt stress-induced programmed cell death in rice root tip cells by controlling early induction events. Journal of Integrative Plant Biology, 49(7), 1024–1031. https://doi.org/10.1111/j.1672-9072.2007.00458.x

Liu, R. Q., Xu, X. J., Wang, S., & Shan, C. J. (2016). Lanthanum improves salt tolerance of maize seedlings. Photosynthetica, 54(1), 148–151. https://doi.org/10.1007/s11099-015-0157-7

Lv, H., Masuda, S., Fujitani, Y., Sahin, N., & Tani, A. (2017). *Oharaeibacter diazotrophicus* gen. nov., sp. nov., a diazotrophic and facultatively methylotrophic bacterium, isolated from rice rhizosphere. International Journal of Systematic and Evolutionary Microbiology, 67(3), 576–582. https://doi.org/10.1099/ijsem.0.001660

Meena, K. K., Bitla, U. M., Sorty, A. M., Singh, D. P., Gupta, V. K., Wakchaure, G. C., & Kumar, S. (2020). Mitigation of Salinity Stress in Wheat Seedlings Due to the Application of Phytohormone-Rich Culture Filtrate Extract of Methylotrophic Actinobacterium *Nocardioides* sp. NIMMe6. Frontiers in Microbiology, 11, 2091. https://doi.org/10.3389/fmicb.2020.02091

Pol, A., Barends, T. R. M., Dietl, A., Khadem, A. F., Eygensteyn, J., Jetten, M. S. M., & Op den Camp, H. J. M. (2014). Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environmental Microbiology, 16(1), 255–264. https://doi.org/10.1111/1462-2920.12249

Ruppel, S., Rühlmann, J., & Merbach, W. (2006). Quantification and localization of bacteria in plant tissues using quantitative real-time PCR and online emission fingerprinting. Plant and Soil, 286(1–2), 21–35. https://doi.org/10.1007/s11104-006-9023-5

Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A. M. Z., Geissler-Plaum, R., & Schnell, S. (2015). Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare L.*) under salt stress. Applied Soil Ecology, 95, 23–30. https://doi.org/10.1016/j.apsoil.2015.04.017

Sy, A., Timmers, A. C. J., Knief, C., & Vorholt, J. a. (2005). Methylotrophic metabolism is advantageous for Methylobacterium extorquens during colonization of *Medicago truncatula* under competitive conditions. Applied and Environmental Microbiology, 71(11), 7245–7252. https://doi.org/10.1128/AEM.71.11.7245

Tsurumaru, H., Okubo, T., Okazaki, K., Hashimoto, M., Kakizaki, K., Hanzawa, E., Takahashi, H., Asanome, N., Tanaka, F., Sekiyama, Y., Ikeda, S., &

Minamisawa, K. (2015). Metagenomic Analysis of the Bacterial Community Associated with the Taproot of Sugar Beet. Microbes and Environments, 30(1), 63–69. https://doi.org/10.1264/jsme2.ME14109

Wang, J., Li, Q., Xu, S., Zhao, W., Lei, Y., Song, C., & Huang, Z. (2018). Traits-based integration of multi-species inoculants facilitates shifts of indigenous soil bacterial community. Frontiers in Microbiology, 9(JUL), 1–13. https://doi.org/10.3389/fmicb.2018.01692

Wehrmann, M., Toussaint, M., Pfannstiel, J., Billard, P., & Klebensberger, J. (2020). The cellular response to lanthanum is substrate specific and reveals a novel route for glycerol metabolism in *Pseudomonas putida* kt2440. MBio, 11(2), 2020. https://doi.org/10.1128/mBio.00516-20

Zhang, L.-N., Wang, D.-C., Hu, Q., Dai, X.-Q., Xie, Y.-S., Li, Q., Liu, H.-M., & Guo, J.-H. (2019). Consortium of Plant Growth-Promoting Rhizobacteria Strains Suppresses Sweet Pepper Disease by Altering the Rhizosphere Microbiota. Frontiers in Microbiology, 10(July), 1–10. https://doi.org/10.3389/fmicb.2019.01668

Zhang, Y., Gao, X., Shen, Z., Zhu, C., Jiao, Z., Li, R., & Shen, Q. (2019). Pre-colonization of PGPR triggers rhizosphere microbiota succession associated with crop yield enhancement. Plant and Soil, 439(1–2), 553–567. https://doi.org/10.1007/s11104-019-04055-4

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