Peer-reviewed version available at Microorganisms 2018, 6, 14; doi:10.3390/microorganisms6010014

Article

Rice Bacterial Endophytes; 16S-Based Taxonomic Profiling, Isolation and Simplified Endophytic Community from Two Venezuelan Cultivars

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Abstract: Rice is currently the most important food crop in the world and we are only just beginning to study the bacterial associated microbiome. It is of importance to perform screenings of the core rice microbiota and also to develop new plant-microbe models and simplified communities for increasing our understanding about the formation and function of its microbiome. In order to begin to address this aspect, we have performed the isolation of bacterial strains from the endorhizosphere of two rice cultivars from Venezuela. The validation of plant-growth promoting bacterial activities *in vitro* has led us to select and characterize 15 isolates for *in planta* studies such as germination test, endophytism ability and plant growth promotion. Consequently, a set of 10 isolates was selected for the set-up of an endophytic consortium as a simplified model of the natural rice bacterial endomicrobiota. Upon inoculation, the colonization and abundance of each strain within the rice roots was tracked by a culture-independent technique in gnotobiotic conditions in a 30 days period. Four strains belonging to *Pseudomonas*, *Agrobacterium* and *Delftia* genera have shown a promising capacity for colonizing and coexistence in root tissues. On the other hand, a bacterial community taxonomic profiling of the rhizosphere and the endorhizosphere of both cultivars were obtained and are discussed. This study is part of a growing body of research on core crops microbiome and simplified microbiomes, which strengthens the formation process of the endophytic community leading to a better understanding of the rice microbiome.

Keywords: rice; endophyte; sustainable agriculture; plant microbiome; simplified bacterial community; syncom; taxonomic profiling; core plant microbiome

1. Introduction

Rice is the staple food for more than a half of the world population and its production is dependent on chemical fertilizers and pesticides [1] which are in part responsive for global warming and groundwater pollution [2]. To meet the world's demand for rice it is imperative to find environmentally sound ways that supplement the need for fertilizers [3]. The use of microbial inoculants is attractive because they can complement and mitigate the use of the agrochemicals ensuring a healthier environment [2].

Microorganisms play an important role in agricultural systems where they live in close association with plants and can exert different kinds of positive effects on the crop's health and growth [4]. The effects of this microbiota include (i) increased nutrient availability (biofertilization), (ii) the ability to compete with or inhibit/antagonize potential pathogens, or reduce their effects (antagonism), (iii) the ability to chemically stimulate the growth and/or tolerance of the host to abiotic stress (phytostimulation) and (iv) the ability to inactivate or degrade existing toxic substances

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in the soil (detoxification) [5]–[7]. Rhizosphere bacteria which live in the soil that is in intimate contact with the roots and are able to perform one or more of these functions are known as plant-growth promoting rhizobacteria or PGPR [8]. Some rhizospheric bacteria are capable of penetrating the surface of the roots and colonize the internal tissues of the root, a niche also known as endorhizosphere [9]. These bacterial endophytes overcome plant defenses and establish themselves as permanent inhabitants of internal tissues without causing harm to the host plant [10]. It is believed that bacteria colonizing the interior plant tissues could interact closely with the host having less competition for nutrients and living in a more protected environment [11].

Several studies have focused on the isolation and identification of rice bacterial endophytes from different locations and varieties [12]. Moreover, a metagenomic analysis of the rice endophytic microbiome provided clues about its composition and functions for the plant host [13] and the dynamics changes during rice root-associated microbiomes have been described [14]. More recently, an extensive isolation, identification and plant-growth promoting traits determination of rice bacterial endophytes has been performed [15], providing further information on bacterial diversity in the rice endosphere. Although also the composition of the endophytic microbiota of various plants is being studied [10], [16], [17], our knowledge of the endophytic bacterial ecology remains limited and the identification and characterization of novel beneficial endophytes is still needed. In addition, most studies involving PGPR and endophytic bacteria are mostly restricted to monostrain set-ups under laboratory conditions [18], and our understanding of the effect of entire microbial communities to plant growth remains at large unexplored.

The main objective of this study is to provide and to describe additional data regarding the bacterial endophytic diversity of rice, as well as to isolate and characterize promising strains with beneficial traits. In addition, we hypothesize that a simplified endophytic bacterial community can be designed and applied as bioinoculants, which constitutes a reductionist approach that can also facilitate the understanding of the plant-microbiota interaction. We have undertaken the 16S rDNA taxonomic bacterial profiling of the rhizosphere and endorhizosphere of two high-yield rice cultivars, Pionero 2010 FL and DANAC SD20A, extensively grown in Venezuela in 2014. Fifteen putative bacterial endophytes were then isolated from surface-sterilized roots and further studied for *in vitro* and *in planta*. We have performed inoculation of rice seedlings with a simplified community composed by 10 of the isolates and we have tracked them in the course of 30 days in greenhouse cultivation. The results obtained suggest that a group of them was able to significantly colonize together the rice endorhizospheres, indicating possible cooperation and ability to form a stable multispecies community. To our knowledge, this is the first study of its kind performed with Venezuelan rice. We believe this approach can be useful in the development of microbial solutions for a more sustainable agriculture.

2. Materials and Methods

2.1 Sample collection and isolation of bacteria from rhizosphere and endorhizosphere

Three rice plants of cultivars Pionero 2010 FL (88 days after planting) and DANAC SD20A (90 days after planting) were collected in April 2014 from two fields in Acarigua (Portuguesa, Venezuela), packaged in sterile bags and cooled at 4 °C for 4 days until bacterial isolation. Five grams of roots with the adherent soil were gently vortexed for 5 minutes in 20 mL of sterile saline solution (0.85 % NaCl) and the rhizospheric soil suspensions were serially diluted and plated (100 µL) in triplicate on LB agar with cycloheximide (CHX) 50 mg/ml for determining the amount of rhizospheric colony-forming units (RCFU). The same 5 grams of rice roots were then surface sterilized in 70 % ethanol for 1 minute followed by 1.2 % hypochlorite for 15 minutes with agitation and finally washed 6 times with sterile distilled water. The extent of the sterilization was verified by plating the final wash concentrated to 100 µL on LB plates before proceeding maceration. Sterilized roots were then macerated using sterile mortar and pestle in 10 mL of 0.85 % NaCl sterile solution and different serial dilutions were plated in triplicate on LB/CHX plates for determining the

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of putative endophytic colony-forming units (ECFU). The plates were incubated at 30 °C for 2 days. Independent ECFU showing distinct colony morphology were picked and streaked again on LB plates to ensure purity of the culture. The remnants of macerated roots and rhizospheric soil suspensions were then used for DNA extraction.

2.2 Total bacterial diversity of rhizosphere and endorhizosphere

The rhizospheric and endorhizospheric DNA from the two rice cultivars were extracted using Soilmaster DNA Extraction Kit (Epicentre, USA) following the manufacturer's guidance. The quantity and quality of the DNA were assessed with Nanodrop (Thermo Fisher Scientific, USA) and electrophoresis in 0.7 %. agarose gel. The extracted DNA was used as template for the first amplification of the V4 variable region of the 16S rRNA by PCR using primers V4 515F, 802R, 806R tailed with two different GC rich sequences enabling barcoding with a second amplification. Each sample was amplified in triplicate in 20 µL volume reaction containing 8 µL HotMasterMix 5Prime (Quanta Bio, USA), 0,4 µL BSA 20X, 1 µL EvaGreen™ 20X (Biotium, USA), 0.5 µL 515F primer (10 µM modified with unitail 1), 0.25 µL 802R primer (10 µM modified with unitail 2), 0.25 µL 806R primer (10 µM modified with unitail 2), 0.5 µL MitoBlk_515F V4 mithocondrial blocking primer (100 µM,), 0.,5 µL ChloBlk_806R V4 chloroplast blocking primer (100 µM and 2 µL (10-50 ng) of DNA template. The PCR amplifications were performed with CFX 96™ PCR System (Bio-Rad, USA) with 34 cycles of 94 °C for 20 s, 52 °C for 20 s, 65 °C for 40 s and a final extension of 65 °C for 2 min. The primary amplification takes advantage of rice specific V4 blocking mitochondrial and chloroplast primers in order to increase amplification of prokaryotic sequences. The rationale for these blocking PCR reactions is described by [19]. Deionized water was used in the negative controls.

The second PCR amplification (switch PCR) is required to attach the barcodes and was performed using a forward primer with the A adaptor (a sample-specific 10 bp barcode and the tail of the primary PCR primers) and a reverse primer with the P1 adaptor sequence and the reverse tail. The reaction was performed in 25 µL volume containing 10 µL HotMasterMix 5Prime, 1.25 µL EvaGreen™ 20X, 1.5 µl barcoded primer (10 µM), 1 µl of the first PCR product with the following conditions: 8 cycles of 94 °C for 10 s, 60°C for 10 s, 65 °C for 40 s and a final extension of 72 °C for 3 min. The list of oligonucleotides used and its sequences characteristics are shown in supplementary table 1.

We verified the size and the amount of the amplicons by agarose gel electrophoresis and then they were pooled in equimolar amounts. The library was purified by the E-Gel® SizeSelect™ (Invitrogen, USA) and verified the size and the amount with Agilent 2100 Bioanalyzer and a Qubit 1.0 fluorometer Q32857 (Thermo Fisher Scientific).

For sequencing the library was submitted to emulsion PCR on the Ion OneTouch™ 2 system using the Ion PGM™ Template Hi-Q OT2 View (Life Technologies, USA) according to the manufacturer's instructions. Ion sphere particles (ISP) were enriched using the E/S module. Resultant live ISPs were loaded and sequenced on an Ion 316 chip (Life Technologies). This sequencing was done in the Life Science Department of the University of Trieste (Trieste, Italy).

2.3 Plant-growth promoting activities

Eighty-seven putative bacterial endophytes or EUFC were tested for indole-3-acetic acid (IAA) production *in vitro*. The IAA is a plant hormone secreted by plant-associated bacteria that increases the root elongation, root exudates and plant biomass (Etesami et al 2015). The bacterial cultures were grown in LB broth amended with tryptophan (100 µg/mL) at 30 °C for 4 days. The cells were sedimented by centrifugation and the supernatant (2 mL) was mixed with 4 mL of Salkowsky reagent (50 mL, 35 % perchloric acid, 1 mL 0.5 M FeCl3 solution) and incubated in darkness for 30 min. The appearance of a red-pink color indicated IAA production and OD_{530nm} was recorded [20]. The concentration of IAA produced by cultures was measured with a calibration graph of commercial IAA obtained in the range of 10 – 100 mg/mL and plotted in relation to the dry bacterial biomass. Fifteen bacterial isolates positive for the IAA production were chosen for further

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plant-growth promoting tests. Phosphate solubilization was determined by growing bacteria on Pikovskaya agar [21]. The phosphate solubilizing bacteria solubilize inorganic soil phosphorous, making it available to the plant and promoting the plant growth (Sharma et al 2013). The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was determined as described in [22], comparing the growth of bacteria on minimal medium (M9), M9 without N source and M9 with 30 μmol of ACC as sole N source. The ACC deamination lowers the hormone ethylene levels in the plant and promotes its growth (Glick 2015). N-acyl homoserine lactone quorum sensing signal assays were carried out as using *Chromobacterium violaceum* CV026 and *C. violaceum* CV017 as biosensors [23]. Motility assay was performed as described by [24]. The exopolysaccharide (EPS) production was assessed culturing the isolates on yeast extract mannitol medium as described in [25]. The lipolytic activity was determined on 1/6 TSA medium amended with 1 % tributyrin [26] and proteolytic activity on 1/6 TSA medium amended with 2 % of powder milk [27]. The quorum sensing signals, the motility, the EPA production and the enzymatic activities are important traits for endophytic colonization and lifestyle. The production of volatile hydrogen cyanide (HCN) was estimated qualitatively as previously described [28]. HCN is an antifungal agent released by some beneficial bacteria. The antibacterial activity against rice pathogens (*Dickeya zea, Pseudomonas fuscovaginae* and *Xanthomonas oryzae*) was carried out plating the bacterial isolates on a bacterial lawn seeded with the pathogen.

2.4 Identification of selected isolates

Bacterial cells from 1 mL of overnight cultures in 2 mL of LB medium were sedimented by centrifugation and resuspended in sterile PSB 0.5 mL. The cells were boiled for 3 minutes, cooled in ice 3 minutes and centrifuged at maximum speed for 5 minutes. The supernatants were used as template in PCR reactions for amplifying 16S rDNA gene with the universal oligonucleotides fD1 and rP2 in 30 cycles of 95 °C 30 seconds, 57 °C 30 seconds and 72 °C 30 seconds with Taq DNA Polymerase (Promega, Madison, WI, USA). The PCR products were purified with EuroGOLD Gel Extraction Kit (EuroClone, Milan, Italy) following manufacturers' instructions and sequenced with universal oligos 515F and 800R (Macrogen, Seoul, Korea) yielding > 1500 bp rDNA sequences. The Basic Local Alignment Tool for nucleotide sequences (BLASTn 2.7.0, NCBI) ran against the rRNA type strains/prokaryotic 16S ribosomal RNA database allowed the identification of the isolates. We considered > 97 % of identity for assigning species. The phylogenetic analysis was performed on the Phylogeny online platform. This software aligned the sequences with MUSCLE (v3.8.31), curated them with Gblocks (v0.91b), reconstructed the phylogenetic tree using the maximum likelihood method implemented in the PhyML program (v3.1/3.0 aLRT) and the tree rendering performed with TreeDyn (v198.3) [29]. The isolates were deposited in the Venezuelan Center for Microorganisms Collection (Institute of Experimental Biology, Central University of Venezuela, Caracas) and the 16S rDNA sequences of the isolates were deposited in GenBank (NCBI).

2.5 Germination test, endophytism and plant-growth promotion assay

In order to track endorhizosphere bacterial colonization after inoculation in gnotobiotic conditions, the generation of rifampicin spontaneous resistant mutant was first achieved for the 15 selected isolates, as previously described [15], [30]. Single colonies of endophytic isolates were grown on 5 mL of LB medium for 24 h at 30 °C and aliquots of 100 uL were then plated on LB agar containing rifampicin (Rif) 100 μg/mL and incubated 48 h at 30 °C. Single rifampicin resistant colonies were re-streaked on LB Rif, stored at – 80 °C and used for *in planta* experiments.

The rice seeds of the Baldo cultivar have a germination rate > 97 % in untreated samples (data not shown) so the effect of the bacterial inoculation on seed germination was measured as the biomass of 4 days old seedlings. The seeds were surface sterilized for 30 minutes with 15 % hypochlorite solution and then rinsed six times with sterile water. Fifty sterilized seeds were germinated in a Petri dish containing 20 mL sterilized water plus 500 μL of an overnight culture of each strain in 1 mL of LB medium, separately. The plates with seeds were kept in the dark at 30 °C

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for 4 days, before determining the wet weight of 10 groups of 5 germinated seeds, randomly chosen and with the water excess uniformly absorbed with clean paper. A control plate with only water (20 mL) and LB (500 µL) was included. Individual seedlings were then transferred to a 50 mL tube containing 35 ml of semisolid (0.25 % agar) ½ Hoagland solution [31] and incubated at 28 °C, 75 % humidity, 16 h/8 h light-dark cycles. The seedlings were watered every two days using $^1/_{10}$ Hoagland solution. After 15 days, the inoculated plant roots were washed abundantly with tap water, dried with paper, separated from the aerial parts (cutting just below the cotyledon) and weighed. The root surface sterilization was performed as explained above and checked by plating the centrifuged sediment of the last wash (30 mL) on LB Rif 100 µg/mL. Then the roots were macerated with sterile pestle and mortar with 3 mL of phosphate buffered saline (PBS) sterile solution and 100 µL of the macerate was plated on LB/Rif plates, incubated at 30 °C for 48 h. The CFU of recovered bacteria were counted and the number of the putative bacterial endophytes was calculated as CFU per gram of root. The aerial parts of the plants were dried at 65 °C for 5 days for determining the plant growth promotion. A control group of plants without bacteria was included. Five rice plants per treatment were harvested and processed. The mean of each treatment was compared to that in control with a two-tailed paired t-test (confidence interval 95%) using Graph Pad Prism version 5.0a.

2.6 Simplified community colonization assay

Ten bacterial strains were cultured for 48 h at room temperature in 10 mL of LB medium and diluted to OD_{600nm} of 2.0. The cells were then sedimented by centrifugation, washed with sterile 10 mL PBS and resuspended in 3 ml PBS. 2 mL of each bacterial/PBS suspension were mixed and finally, 30 mL of PBS were added bringing the final volume to 50 mL. 2 mL of this mixed suspension were used for DNA extraction and the remaining 48 mL were added to 800 mL of semisolid ½ Hoagland solution. A control without bacteria (only with LB broth) was included. One-week-old Baldo rice individual seedlings (sterilized and germinated as described above) were transferred to 40 mL (in Falcon tubes) of this community-containing semisolid Hoagland solution incubated and watered as described above. Three plants from the control and the treatment were recovered at 10, 20 and 30 days after planting, for a total of 18 plants harvested. The roots and aerial parts were separated and weighed. The roots were then sterilized and macerated with liquid nitrogen. The resulting root powder was used for DNA extraction and a 16S rRNA gene library was constructed and sequenced exactly as described in Material and Methods 2.2, for carrying on the amplicon-based taxonomic profiling. The general stepwise procedure is shown in Figure 1.

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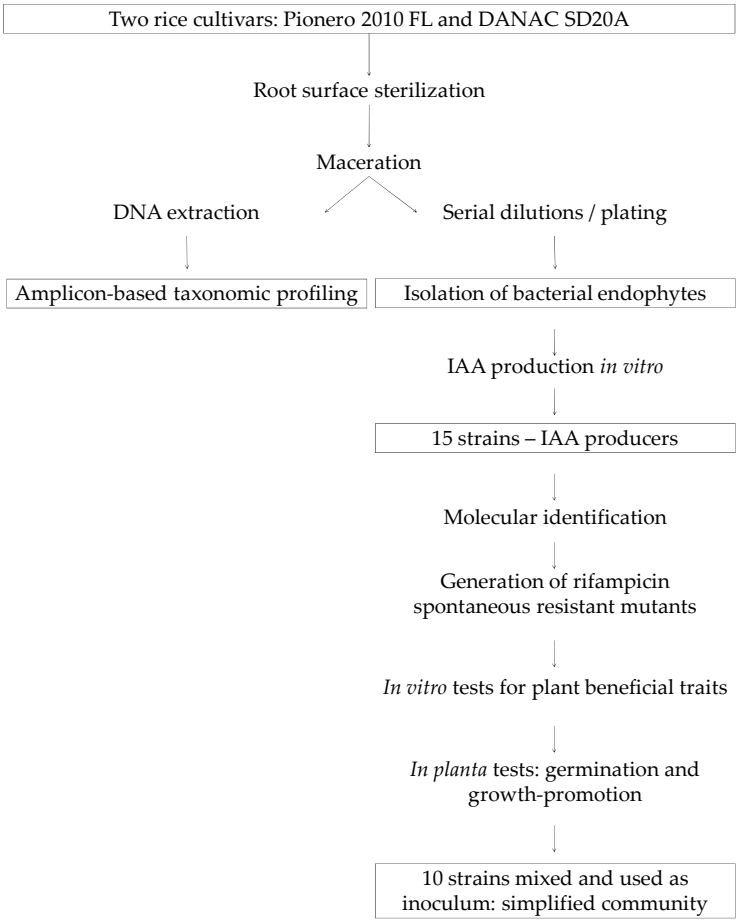

Figure 1. Methods workflow. Stepwise approach for determining the taxonomic profile of the bacterial endophytic microbiota of two rice cultivars and the setup of a simplified community based on *in vitro* and *in planta* performance of the isolates.

2.7 Analyses of sequencing data.

Reads were initially mapped against *O. sativa* mithocondrial (NC_011033) and plastidial genomes (NC_001320). Unmapped reads were further processed. We used CloVR 1.0 RC9 [32] on the Amazon Elastic Compute Cloud (EC2) to run the QIIME workflow 'pick_otus_through_otu_tables.py' [33]. Within the QIIME workflow: (i) we set the minimum and maximum sequence length to 150 and 350 bp, respectively, the maximum homopolymer length to 8 bp and maximum number of ambiguous calls to zero; (ii) just after the quality filter we removed putative chimeras with UCHIME using the default parameters; (iii) clustering was performed using UCLUST with a nucleotide sequence identity threshold within each cluster at 97% and alignment against the Greengenes 16S database with PyNAST; (iv) taxonomy assignment of each OTU-representing sequence through the RDP classifier with a confidence threshold of 0.8; (v) richness and diversity estimators were computed by Mothur (alpha diversity) and UniFrac (beta diversity).

3. Results

3.1 Biodiversity of Venezuelan rice rhizosphere and endorhizosphere communities by culture-independent methods

In order to obtain a picture of the taxonomic diversity of the two Venezuelan rice cultivars, the population of the total rhizospheric and endorhizospheric bacterial community was assessed. It was analyzed in 6 plants that were harvested from two fields, 3 plants belonging to Pionero 2010 FL

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cultivar and the other 3 to DANAC SD20A cultivar. The total DNA from rhizosphere and endorhizosphere was extracted for performing 16S rDNA amplicon library sequencing. We obtained 326496 high-quality bacterial reads of 248 bp length in average. The reads count per sample, as well as those obtained from plant organelles, are shown in Table 1, section A. The relation of the number of reads per OTU detected is shown in the rarefaction curve in Supplementary Figure 1. After the removal of plant-derived, anonymous and singletons OTUs, the high-quality reads were clustered in a total of 341 different OTUs with a taxonomic assignment evaluated with > 97% sequence identity as the cutoff.

Table 1. Sequences characteristics. The number (#) and its corresponding percentage (%) of plant-derived and bacterial-derived 16S reads sequenced, as well as the average length in bp, are listed. A) Results for the 16S-based taxonomic profiling of the two rice cultivars. B) Results for the simplified community assay.

Samples		Plant derived reads		Bacterial derived reads		Average length (bp)
		#	%	#	%	
A) Amplicon-based taxonomic profiling						
Pionero FL 2010	Rhizospheres	320	0.16	175530	99.84	248
	Endorhizospheres	60	0.06	81171	99.94	247
DANAC SD20A	Rhizospheres	81	0.09	49374	99.91	249
	Endorhizospheres	16	0.04	20421	99.96	248
B) Simplified community						
Control endorhizospheres	10 days	94362	53.16	83143	46.84	247
	20 days	111238	95.63	5083	4.37	247
	30 days	153205	98.41	2475	1.59	248
Inoculated endorhizospheres	10 days	3530	2.29	150625	97.71	246
	20 days	100128	57.54	73887	42.46	248
	30 days	300845	91.34	28523	8.66	248

Microbiome analysis by phylum distribution and frequency (expressed as the percentage on the total number of OTUs) is summarized in Figure 2. Representatives of Proteobacteria, the most abundant phylum, were 71 % to 87 % of the total OTUs. Also, the proteobacterial classes were considered: Gammaproteobacteria was most abundant, followed by Betaproteobacteria and Alfaproteobacteria, while representatives of Deltaproteobacteria and Epsilonproteobacteria were not detected in the endorhizospheres. Other abundant phyla were Bacteroidetes, which were nearly equally distributed among the samples. Verrucomicrobia were enriched in the endorhizosphere of Pionero 2010 FL whereas Actinobacteria, Cyanobacteria, Fibrobacteres and Spirochaetes were equally distributed among the samples. Acidobacteria, Chloroflexi, Nitrospirae and Planctomycetes phyla were only detected in the rhizospheres.

As expected, the snapshot of the total bacterial community showed a greater abundance and diversity of bacterial species in the rhizosphere than in the endorhizosphere, as suggested by the richness and diversity estimators shown in Table 2. The rhizosphere of DANAC SD20A cultivar was colonized by a larger bacterial community than that of Pionero 2010 FL.

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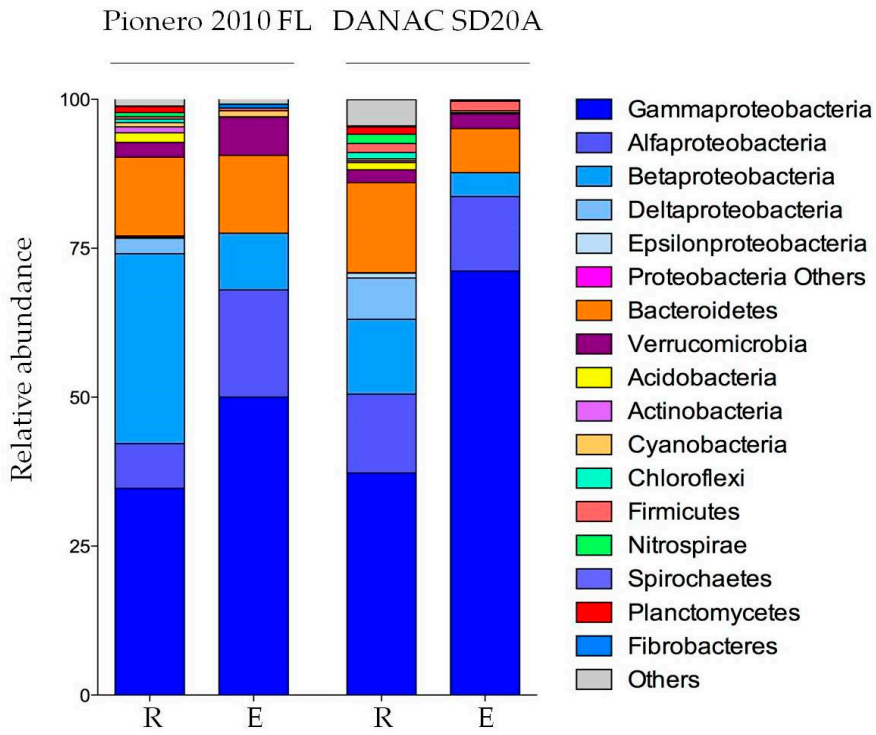

Figure 2. Frequency distribution of the bacterial phyla in the rhizosphere (R) and endorhizosphere (E) of the sampled rice roots. Bar graphs of the taxonomic annotation of bacterial reads among the distribution of the most abundant phyla. The classes of Proteobacteria phylum are also shown in shades of blue.

Table 2. Richness and diversity estimators. The number of observed sequences (S_{obs}) and estimated richness (Chao, ACE), diversity (Simpson, Shannon and Effective Number of Species ENS) for Pionero FL2010 and SD20A rice cultivars microbiota, using 97 % 16S rRNA gene sequence similarity cutoffs, are listed. R, rhizosphere; E, endorhizosphere.

		Richness estimator			Diversity estimator		
		S_{obs}	*Chao*	*ACE*	*Simpson*	*Shannon*	*ENS*
Pionero FL2010	**R**	1497	1549.6	1586.8	0.078	4.28	72
	E	794	825.5	855.2	0.089	3.74	42
SD20A	**R**	1620	1663.4	1706.7	0.014	5.52	250
	E	562	635.6	651.2	0.148	3.06	21

341 OTUs in total were binned to a taxonomical category and their distribution within the samples is summarized in Figure 3 and the complete list is in Supplementary Table 2. The Pionero 2010 FL cultivar microbiota was composed of 73 and 52 OTUs exclusively detected in the rhizosphere and in the endorhizosphere, respectively. 51 OTUs on the other hand were detected in both compartments (Figure 2A). Among the species detected in both compartments, which corresponded to the 86.46 % of the reads, *Cellvibrio* sp., *Pseudomonas pseudoalcaligenes*, *Opitutus* sp., *Agrobacterium* sp., *Pedobacter* sp. and *Variovorax* sp., were significantly enriched in the endorhizosphere. The bacteria *Microvirgula aerodenitrificans* and *Caulobacter* sp. were the most abundant bacteria found exclusively in the endorhizosphere. The DANAC SD20A microbiota was composed of 135 and 51 OTUs exclusively detected in the rhizosphere and in the endorhizosphere, respectively, and 63 OTUs that were detected in both compartments (Figure 2B). Among the species

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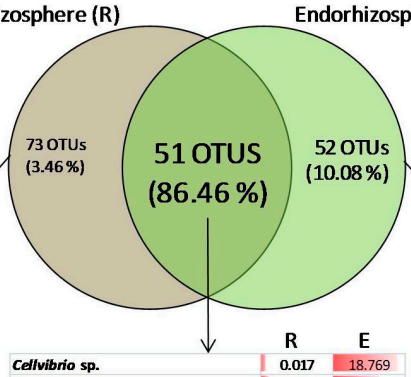
304 detected in both compartments which corresponded to the 80.42 % of the reads, the following genera
305 were highly enriched in the endorhizosphere: *Cellvibrio* sp., *Caulobacter* sp., *Rhodoferax* sp., *P.*
306 *pseudoalcaligenes*, *Opitutus* sp., *Agrobacterium* sp., *Asticcacaulis* sp. and *Shewanella* sp. The bacteria
307 *Azospirillum massiliensis*, *Acintobacter lwoffii* and *Citrobacter* sp., were the most abundant in the 51
308 OTUs group detected exclusively in the endorhizosphere.

309

A

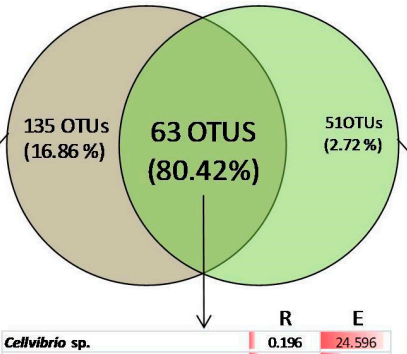


	R
Kaistobacter sp.	0.42
Pseudomonas stutzeri	0.30
Marinobacter sp.	0.27
Thiobacillus sp.	0.26
Thermomonas sp.	0.22
Rhodococcus fascians	0.20
Geobacter sp.	0.19
GOUTA19 sp.	0.12
Anaeromyxobacter sp.	0.10
Candidatus Koribacter sp.	0.10
Parasegitibacter luojiensis	0.06
Arenimonas sp.	0.06
Sulfuricurvum kujiense	0.05
Methyloversatilis sp.	0.05
Nitrospira sp.	0.05
Ancylobacter sp.	0.04
Rhodobacter sp.	0.04
Arthrobacter psychrolactophilus	0.04
Syntrophobacter sp.	0.04
Candidatus Nitrososphaera SCA1170	0.03
Others 53 OTUs	0.79

	R	E
Cellvibrio sp.	0.017	18.769
Pseudomonas pseudoalcaligenes	0.765	12.969
Opitutus sp.	0.041	4.134
Agrobacterium sp.	0.228	3.128
Pedobacter sp.	0.048	2.861
Variovorax sp.	0.027	2.113
Devosia sp.	0.307	1.914
Pseudomonas sp.	8.351	0.176
Limnobacter sp.	7.175	0.334
Methylotenera mobilis	3.652	0.191
Flavobacterium sp.	2.123	1.886
Pseudomonas veronii	1.774	0.146
Flavisolibacter sp.	0.535	0.000
Acidovorax sp.	0.325	0.210
Mycoplana sp.	0.289	1.131
Rheinheimera sp.	0.271	0.915
Pseudomonas Other	0.150	0.641
Chryseobacterium sp.	0.029	1.390
Fluvicola sp.	0.027	0.711
Shewanella sp.	0.021	1.787
Others 31 OTUS	1.910	2.984

	E
Microvirgula aerodenitrificans	3.20
Caulobacter sp.	1.56
Pleomorphomonas sp.	0.72
Asticcacaulis sp.	0.65
Sphingobium sp.	0.53
Azospirillum massiliensis	0.41
Lutibacterium sp.	0.31
Agrobacterium undicola	0.30
Azospirillum sp.	0.26
Prosthecobacter debontii	0.24
Rhodoferax sp.	0.15
Flavobacterium gelidilacus	0.12
Vibrio sp.	0.08
Roseivivax sp.	0.09
Ochrobactrum sp.	0.08
Phenylobacterium sp.	0.08
Lysobacter sp.	0.07
Pseudoxanthomonas sp.	0.08
Winogradskyella sp.	0.07
Crocinitomix sp.	0.07
Others 32 OTUs	0.88

B



	R
Halothiobacillus sp.	3.16
Methylophaga sp.	2.39
Arenimonas sp.	1.78
Thiobacillus sp.	1.73
Limnobacter sp.	1.49
Kaistobacter sp.	0.55
Anaeromyxobacter sp.	0.44
Sulfuricurvum kujiense	0.39
Flavisolibacter sp.	0.33
Gallionella sp.	0.32
GOUTA19 sp.	0.28
4-29 sp.	0.27
Geobacter sp.	0.23
LCP-6 sp.	0.17
Acidovorax sp.	0.16
Aquaspirillum putridiconchylium	0.14
Cloacibacterium sp.	0.14
Parasegitibacter luojiensis	0.14
Syntrophobacter sp.	0.13
Methylotenera mobilis	0.13
Others 115 OTUs	2.496

	R	E
Cellvibrio sp.	0.196	24.596
Pseudomonas Other	1.414	6.657
Caulobacter sp.	0.006	4.317
Rhodoferax sp.	0.037	3.946
Pseudomonas pseudoalcaligenes	0.461	3.310
Pseudomonas veronii	1.808	1.371
Opitutus sp.	0.146	2.861
Flavobacterium sp.	0.183	2.735
Agrobacterium sp.	0.361	2.209
Asticcacaulis sp.	0.033	2.234
Mycoplana sp.	1.128	1.072
Flavobacterium gelidilacus	0.322	1.706
Pseudomonas sp.	1.050	0.582
Algoriphagus terrigena	0.371	1.036
Shewanella sp.	0.011	1.344
Fluvicola sp.	0.056	1.002
Flavobacterium succinicans	0.097	0.848
Staphylococcus sp.	0.265	0.645
Rheinheimera sp.	0.603	0.304
Enhydrobacter sp.	0.036	0.842
Others 43 OTUs	3.814	4.407

	E
Azospirillum massiliensis	0.56
Acinetobacter lwoffii	0.48
Citrobacter sp.	0.34
Propionivibrio sp.	0.13
Pleomorphomonas sp.	0.12
Fusobacterium sp.	0.11
Yersinia sp.	0.10
Aquimarina sp.	0.09
Prevotella melaninogenica	0.08
Sphingomonas wittichii	0.05
Achromobacter sp.	0.04
Cohnella sp.	0.04
Sediminibacterium sp.	0.04
Ancylobacter sp.	0.03
Veillonella dispar	0.03
Bulleidia moorei	0.03
Antarctobacter sp.	0.02
Janthinobacterium sp.	0.02
Sphingobacterium sp.	0.02
Actinotalea sp.	0.02
Others 31 OTUs	0.332

310

Figure 3. Microbiota composition of the two rice cultivars. A total of 341 OTUs were identified by 16S rRNA sequencing profiling , using a 97 % of similarity against the database. 326426 high-quality reads were obtained, 256701 from Pionero 2010 FL (A) and 69795 from DANAC SD20A (B) cultivar. The values in the Venn diagrams indicate the number of OTUs found exclusively in the rhizosphere (R), in the endosphere (E) or those found in both compartments, and the number in parenthesis indicates the relative abundance of those OTUs. The 20 most abundant species detected in each compartment and their abundance are shown (%). The length of the color bars represents the value in the cell.

3.2 Isolation of culturable bacteria from rhizosphere and endorhizosphere

The adherent soil of 5 grams of roots (i.e. the rhizospheric soil) was serially diluted and plated in triplicate on LB/CHX plates. The estimated average number of culturable bacteria recovered was 5.5 x 10^7 CFU per gram of rhizospheric soil. On the other hand, the 5 grams of roots yielded from 1420 to 361120, with an average of 121076 CFU per gram of sterilized-macerated roots. In order to perform the plant-growth promoting tests, 87 putative endophytic bacterial isolates were chosen based on color and colony morphology differences.

3.3 Production of indoleacetic acid (IAA)

We decided to test the 87 putative bacterial endophytic isolates for the production of IAA, the main auxin in plants and an important phenotype linked to plant growth promotion. Thirty-five of the isolates were positive for IAA production, 17 from Pionero 2010 FL and 18 isolates from DANAC SD20A. The IAA production ranged from 0.153 to 4.86 µg/mg and 15 representative isolates (Supplementary Figure 2) were chosen for further characterization, namely: E1101, E1103, E1108, E1201, E1205, E1308, E2102, E2105, E2202, E2205, E2309, E2315, E2321, E2330 and E2330.

3.4 Molecular identification

In order to identify and classify the 15 bacterial isolates which produced IAA, they were subjected to 16S rDNA amplification and sequencing. The sequence comparison against the ribosomal type strains database revealed that 2 isolates belong to the Firmicutes phylum (*Bacillus amyloliquefaciens* E1101 and *B. altitudinis* E2315) and 13 to Proteobacteria. Of these, 1 belongs to α-Proteobacteria (*Agrobacterium* sp. E2321), 1 to β-Proteobacteria (*Delftia lacustris* E2330) and 11 to ϒ-Proteobacteria (*Serratia glossinae* E2105,*S. glossinae* E2309; *Aeromonas veronii* E2102, *A. hydrophila* E2202;*A. veronii* E2205, *Pseudomonas gessardii* E1201, *P. pseudoalcaligenes* E1103;, *P. chengduensis* E1108, *P. Pseudoalcaligenes* E1205, *P. gessardi E1308i*, *P. Jessenii* E2333). The results are summarized in Table 3. The 16S sequences were then used for determining the phylogenetic relationships through a cladogram as shown in Figure 4A.

Table 3. Molecular identification of the putative bacterial endophytes isolated from the two rice cultivars. The 16S rRNA gene were sequenced and compared to the rRNA type prokaryotic strains database. The accession number to the NCBI (A), the accession number to the Venezuelan Center for Microorganisms Collection (B), the closest type strain (C) and the corresponding reference sequence (D) are listed.

Rice cultivar	Bacterial isolate	Accession NCBI [A]	Accession CVCM [B]	Closest type strain [C]	Reference sequence [D]	Identity (%)
Pionero 2010 FL	**E1101**	KY867521	CVCM2317	*Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42	NR_075005.1	99
	E1103	KY867522	CVCM2318	*Pseudomonas pseudoalcaligenes* strain Stanier_63	NR_037000.19	99
	E1108	KY867523	CVCM2319	*Pseudomonas chengduensis* strain MBR	NR_125523.1	99
	E1201	KY867525	CVCM2322	*Pseudomonas gessardii* strain CIP 105469	NR_024928.1	98
	E1205	KY867526	CVCM2324	*Pseudomonas pseudoalcaligenes* strain Stanier_63	NR_037000.19	99
	E1308	KY867527	CVCM2326	*Pseudomonas gessardii* strain CIP105469	NR_024928.1	99
DANAC SD20A	**E2102**	KY867528	CVCM2328	*Aeromonas veronii* bv. *veronii* strain ATCC 35624	NR_118947.1	99
	E2105	KY867529	CVCM2329	*Serratia glossinae* strain C1	NR_116808.1	99
	E2202	KY867530	-	*Aeromonas hydrophila* strain ATCC 7966	NR_074841.1	99
	E2205	KY867531	CVCM2330	*Aeromonas veronii* bv. *veronii* strain ATCC 35624	NR_118947.1	99
	E2309	KY867532	CVCM2331	*Serratia glossinae* strain C1	NR_116808.1	99
	E2315	KY867533	CVCM2334	*Bacillus altitudinis* strain 41KF2b	NR_042337.1	99
	E2321	KY867534	CVCM2335	*Agrobacterium vitis* strain K309	NR_036780.1	97
	E2330	KY867535	-	*Delftia lacustris* strain 332	NR_116495.1	99
	E2333	KY867536	CVCM2338	*Pseudomonas jessenii* strain CIP 105274	NR_024918.1	99

3.5 in vitro assays of plant beneficial traits

It was of interest to determine whether the 15 IAA-producing putative rice bacterial endophytes possessed other important plant beneficial traits such as nitrogen fixation, phosphate solubilization, ACC deaminase activity, HCN production and antibacterial activities. Other relevant traits for endophytic lifestyle like quorum sensing acyl-homoserine lactone (AHL) production, quorum quenching activity, exopolysaccharide (EPS) production, motility and secretion of enzymes were also assayed. The results of these assays are summarized in Figure 4B.

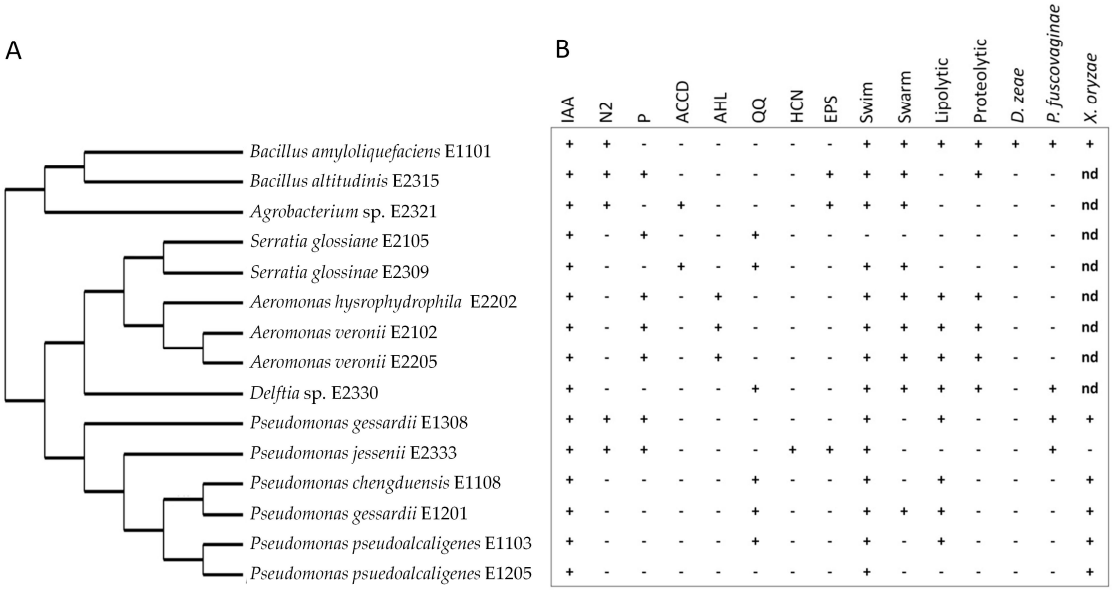


Figure 4. Putative endophytic bacteria isolated from surface-sterilized rice roots. A) The bacterial isolates were putatively identified by 16S sequencing and the rDNA sequences (average length 1518 bp) were used for constructing the cladogram. B) Plant-growth promoting activities and antibacterial activities detected in *in vitro* tests (IAA, indole acetic acid production; N2, nitrogen fixation; P, phosphorous solubilization; ACCD, ACC deaminase activity; AHL, acyl homoserine lactone production; QQ, quorum quencher activity.;HCN, hydrogen cyanide production; EPS, exopolysaccharide production; Swim and swarming and motility; Lipolytic and proteolytic activity; antibacterial activity against *Dickeya zea, Pseudomonas fuscovaginae and Xanthomonas oryzae*. The assays were performed in biological triplicates.

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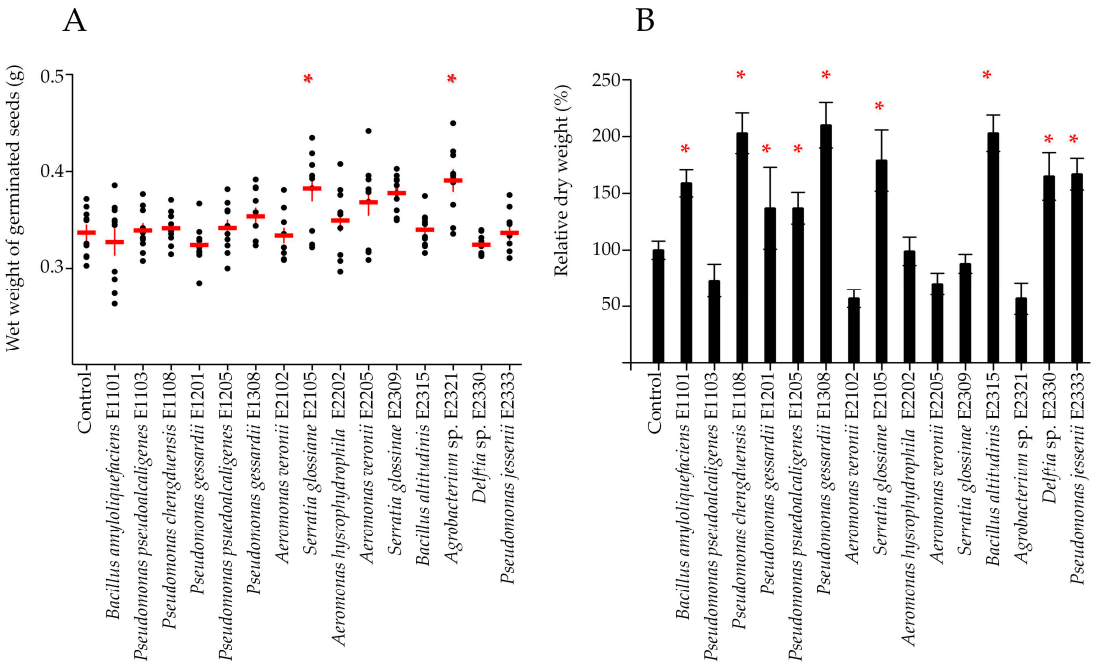
368 *3.6 Germination test, endophytism assay, and plant-growth promotion*

369 The 15 isolates were *in planta* assayed for germination, endophytic colonization, and plant
370 growth promotion. For these experiments, we created spontaneous rifampicin resistant mutant
371 derivatives in order to select them after their recovery from colonized plant tissues. Only 2 strains
372 significantly increased the germination rate of the seeds; *Agrobacterium* sp. E2315-germinated seeds
373 were 7.6 % higher on average than control seeds and *Serratia glossinae* E2309 with a 7.3 %
374 germination increase (Figure 5A).
375



376

377 **Figure 5. Plant growth promotion by single-strain inoculation.** A) Germination rate. The wet
378 weight of 4 days old germinated seeds was determine. Each dot represents the average weight of 5
379 germinated seeds in the dispersion graph. The average and standard deviation are shown as red
380 lines. B) Plant growing rate. The dry weight of the aerial parts (stems and leaves) was determined.
381 The averages are shown relative to the control (arbitrarily 100) with its standard deviation. The
382 values were obtained from 5 different inoculated plants cultivated during 15 days. The red asterisks
383 indicate statistical significance ($p<0.05$).

384 Of the 15 isolates tested, only 1 could be recovered after inoculation from the endorhizosphere,
385 this was *Pseudomonas fluorescens* E1308. The CFU of this strain ranged from 170 to 44000 CFU per
386 gram of surface-sterilized roots. This isolate was also the best promoter of plant growth since the
387 plants displayed an increase of 110 % of the aerial parts dry weight when compared to the control
388 plants ($p < 0.05$) (Figure 5B). Also, other 8 strains showed a statistically significant positive effect on
389 plant growth promotion, namely *P. mendocina* E1108 (103 %), *Rhizobium* sp. E2315 (103 %), *Serratia*
390 *fonticola* E2105 (79 %), *P. jessenii* E2333 (67 %), *Delftia tsuruhatensis* E2330 (65 %), *Bacillus*
391 *amyloliquefaciens* E1101 (59 %), *P. pseudoalcaligenes* E1205 (37 %) and *Pseudomonas* sp. E1201 (37 %).
392
393

394 *3.7 Simplified community inoculation, colonization, and plant growth promotion*

395 It was of interest to perform *in planta* studies with a bacterial consortium in order to determine
396 possible bacterial inter-species community effects on host colonization. We decided to use a bacterial

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consortium of 10 out of the 15 bacterial isolates, namely: *P. chengduensis* E1108, *P. pseudoalcaligenes* E1205, *P. gessardii* E1308, *A. veronii* E2102, *A. veronii* E2205, *S. glossinae* E2309, *B. altitudinis* E2315, *Agrobacterium* sp. E2321, *D. lacustris* E2330 and *P. jessenii* E2333. An amount of bacterial suspension equivalent to OD_{600nm} of 2.0 of each culture was used for the mixed bacterial inoculum. This inoculum was included in the semisolid Hoagland solution where plants were grown. After 30 days, there was a significant increase of 15 % ($p < 0.05$) in the wet weight of the inoculated plants compared to control non-inoculated, both in the roots and in aerial parts (Figure 6).

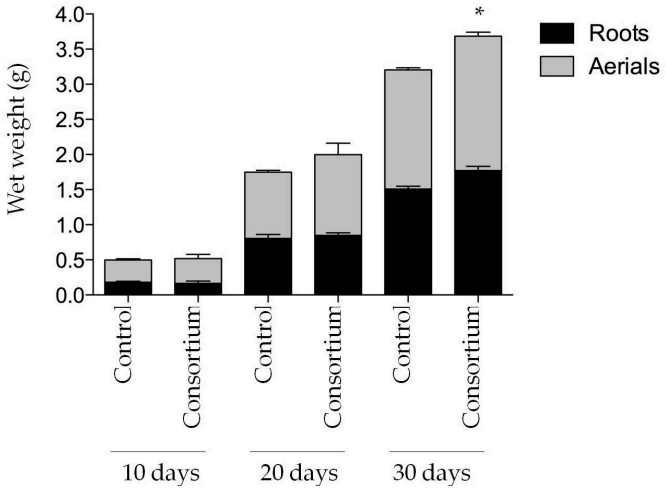

Figure 6. Effect of the bacterial consortium in plant growth. One-week old rice seedlings were inoculated with a mixture of 10 bacterial strains and grown in controlled conditions for 30 days. Each ten days, 3 plants were harvested, cut in the two parts shown, and weighted. A control without bacterial inoculation was included. The asterisk indicates statistic significance ($p<0.05$).

A cultivation-independent tracking, using 16S rDNA amplicon sequencing, was carried out in order to obtain insight into the colonization ability of the 10-strain simplified community over time. The numbers of reads obtained, bacterial- and plant-derived, are shown in Table 1 section B. Regarding the total bacterial endophytic abundance, it was noted that the uninoculated plants were systematically lower in bacterial populations at each time point compared to that in inoculated plants (Supplementary figure 2)

The composition of the cell mix (the pooled bacterial cultures that were then used as inoculum) varied from 36 reads (*P. chengduensis* E1108) to 13145 reads (*S. glossinae* E2309) in a total of 45246 reads, as shown in Figure 7A. In order to track the abundance of each strain of the bacterial consortium within the plants, their 16S sequences were used against the total 16S rDNA library sequenced. This was also performed for the control plants in order to determine if any seed-borne bacterial endophyte was taxonomically close enough to the strains used in the consortium, which could lead to false positives. The abundance of the simplified bacterial community was tracked in control and inoculated plants and it is represented as relative abundances in Figure 7B.

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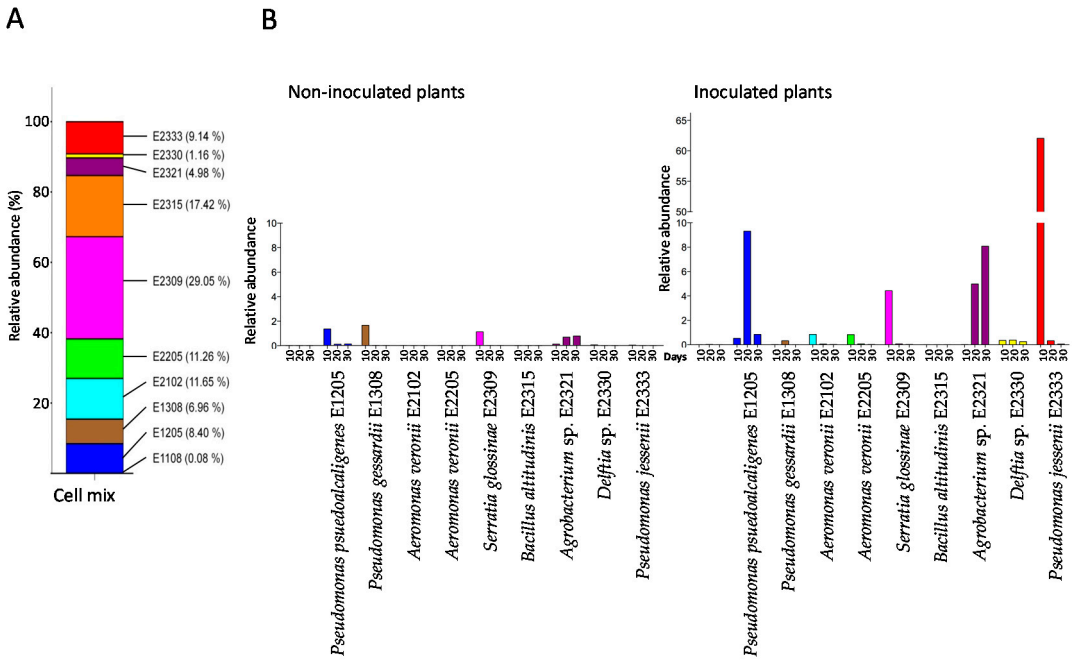

Figure 7. Composition of the 10-strains simplified community and its abundance during 30 days growth of rice seedlings. A) The cell mix represents the 10 species mixed and used as inoculum. The relative abundance of each strain is shown in brackets. The total number of reads was n = 45246. B) The relative abundance of each consortium strain was tracked at 10, 20 and 30 days after the inoculation of the rice seedlings. The results for non-inoculated and inoculated plants are shown in the colored bars. The total number of reads was n = 111291.

The abundance and identity of the reads suggested that taxonomically related strains to *P. pseudoalcaligenes* E1205, *P. gessardii* E1308, *S. glossinae* E2309 and *Agrobacterium* sp. E2321 were present in the control plants in low abundance. In the inoculated plants, at least 8 out of 10 bacterial strains were detected within the plant roots. Only 4 strains were however detected after 30 days of cultivation, namely: *P. pseudoalcaligenes* E1205, *Agrobacterium* sp. E2321, *D. lacustris* E2330 and *P. jessenii* E2333. This dataset suggested that these strains were capable to colonize together the rice roots.

4. Discussion

It is of great importance to study the microbiota diversity and functionality on the main agricultural crops [34], as well as to develop models for the study of plant-microbe interaction through simplified microbiota [35]. In this study, (i) we have performed a survey on the total bacterial endophytic community in *Oryza sativa* cv. Pionero FL 2010 and *O. sativa* cv. DANAC SD20A, (ii) we have carried out the isolation and partial characterization of 15 putative bacterial endophytes, and (iii) we have narrowed a 4-strains simplified microbiota as a starting point for a working model for bacteria-bacteria and bacteria-plant interactions in rice, towards a future efficient bioinoculant formulation possibly based on a mixed inoculum.

4.1 Amplicon-based taxonomic profiling.

Profiling the bacterial communities allowed us to determine that the rhizospheres of the sampled plants were more diverse than the endorhizospheres, an observation widely documented [14], [36], [37]. The use of blocking primers was successful since > 99.9 % of the endorhizospheric reads belonged to bacteria. Proteobacteria were by far the most predominant group in both

compartments of both rice varieties, and this is in agreement with several previous studies [14], [15], [38], [39]. However, members of Deltaproteobacteria and Epsilonproteobacteria class were not detected in the endorhizospheres analyzed here; this is in contrast to what has been reported in a previous report of rice microbiome in Italy [15] and Philippines [38]. We further compared the OTUs abundance differentially distributed between the rhizosphere and the endorhizosphere of each rice cultivar. We identified members of *Cellvibrio* genus as being highly predominant inhabitants in both endorhizospheres. The members of this genus are known as obligates aerobic cellulolytic bacteria and other complex carbohydrates degraders [40] which are believed to be key activities necessary for the colonization of the plant endosphere. *Cellvibrio* spp. have been reported as members of the rice endosphere [15], however with a lower abundance (between 0.01 and < 1 %) than in our study. Some *Cellvibrio* species are nitrogen-fixing bacteria, especially the *Cellvibrio diazotrophicus* [41]. Other species enriched in both endospheres were *P. pseudoalcaligenes*, *Agrobacterium* sp. and *Opitutus* sp. Endophytic *P. pseudoalcaligenes* and *Agrobacterium* sp. have been previously reported in rice [42], [43] and they have also been frequently isolated from different plant types and tissues [44]–[47]. *Opitutus* sp. has been reported as an inhabitant of anoxic rice paddy soils [48] and as a rice endophyte [15], moreover, members of Verrucomicrobiae in the rice endosphere have also been reported by [38]. Interesting *Opitutus* sp. is obligate anaerobic with a fermentative metabolism that utilizes rice plant-derived carbons [36]. The presence of anaerobic microbes within the plant, an environment which is O_2-rich, seems paradoxical and was also reported by [14].

In the Pionero FL 2010 cultivar, *Pedobacter*, *Variovorax* and *Devosia* genus were enriched in the endorhizosphere with respect to the rhizosphere. *Pedobacter* sp. has been previously isolated from rice paddy soil [49]. *Variovorax* sp. is a versatile PGP bacterium able to colonize the plant endosphere [50] including rice [51]. *Devosia* sp. is a soil bacterium from the Rhizobiales family, nodule-forming and nitrogen fixing [52]. Bacteria belonging to these three genera have been detected in the rice endosphere of rice grown in Italy [15].

Two bacterial species counted for half of the total bacterial population in the endosphere of Pionero FL 2010. First, *Microvirgula aerodenitrificans*, the most abundant one, is an aerobic denitrifier [53] and has been reported previously as a rice endorhizosphere inhabitant [15]. Secondly *Caulobacter* sp., which has also been reported to be associated rice in two other parts of the world [54][55][15] and to have PGP properties [44]. In the endorhizosphere of the DANAC SD20A cultivar, strains belonging to the *Azospirillum*, *Acinetobacter* and *Citrobacter* genera were dominant. *Azospirillum* and *Acinetobacter* are diazotrophic plant-growth promoting bacteria that can modulate the phytohormone balance [56], [57]. To our knowledge, there is just one report of the isolation of *Citrobacter* as rice endophyte [58], although the rice metagenomic study most likely revealed loci which belong to *Citrobacter* sp. [38]. Apart from *Cellvibrio*, *P. pseudoalcaligenes,* and *Opitupus* sp., the endosphere of the DANAC SDS20A cultivar was highly enriched by *Rhodoferax* sp., a nitrate reducer bacterium [59].

It is important to mention that this analysis was subjected to the intrinsic bias of the amplification and sequencing techniques, as well as the data processing [34], thus some taxa could not be appropriately represented in our study. On the other hand, the number of plants sampled (three for each cultivar) would not reflect the real bacterial endophytic microbiota of each cultivar. Nevertheless, the taxonomic range of putative endophytic microbiota of rice has been extended with this work, making an important contribution to the rice microbiome research, improving the progress towards the elucidation of the rice core microbiota.

4.2 Isolation of putative endophytic bacteria, determination of its PGP traits, and plant colonization.

Beneficial endophytic bacteria play important roles that positively affect directly or indirectly plant growth and development [60]. In this study, we selected 15 putative bacterial endophytes isolated from Venezuelan rice because they were IAA producers. IAA is the main auxin in plants, controlling the roots architecture, thereby improving nutrient acquisition [61]–[63]. Our estimations

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of the produced IAA are related to milligrams of dry bacterial biomass, instead of milliliters of culture, since we think it could be more useful for future comparisons.

Two *Bacillus* strains (Firmicutes phylum), *B amyloliquefaciens* E1101 and *B. altitudinis* E2315, were identified among our isolates. Although these two strains did not affect the germination rate of the surface-sterilized rice seeds, they positively influenced the plant growth however our inoculation experiments did not reveal them as endophytes. *Bacillus* spp. are widely used commercially as biofertilizer and biocontrol agents in agriculture due to their spore-forming ability and stability in their formulations. In our work, *B. amyloliquefaciens* has shown the most potent antibacterial activity, antagonizing or inhibiting the growth of 14 bacterial species (data not shown). *B. amyloliquefaciens* is known to produce surfactins and an array of secondary metabolites and is considered a model for unraveling plant-microbe interactions and biocontrol [71]. It is interesting to note that in our taxonomic profiling, *Bacilli* abundance was extremely low in the four compartments analyzed, with a maximum abundance of 0.016 % of the total reads. It cannot be excluded that the isolation procedure favored the growth of non-abundant *Bacillus* spp. or alternatively that the PCR for 16S-based taxonomic profiling was not so efficient for this bacterial group.

The other 13 isolates belong to Proteobacteria, the most abundant phylum in the taxonomic analysis. The alfaproteobacteria *Agrobacterium* sp. E2321 had the most positive impact on the germination rate, but this did not translate into a plant growth promotion. This strain displayed a number of PGP traits *in vitro,* however, was not able to perform beneficial effects *in planta*; this contradiction was discussed by [64] when they found similar discordance when analyzed the effect of rhizobacteria on the growth of barley under salt stress. These results would suggest that the current *in vitro* PGP screening methods may need to be re-evaluated. The isolate *Serratia glossinae* E2309 was the only bacterial inoculum that increased the germination rate and also plants growth. Others *Serratia* spp. have been previously reported as PGP strains [65]–[67] and could, therefore, be a good candidate to further study. However, the other *S. glossinae* isolated (E2105), did not promote the plant growth. Interestingly our two *S. glossinae* isolates displayed a different profile of *in vitro* activities thus despite being to the same species, probably there are differences between the two isolates which affect the PGP performance. In our taxonomic profiling, *Serratia* spp. were not detected in the endorhizospheres of DANAC SD20A cultivar but were detected in low abundance in the rhizosphere of Pionero 2010 FL. This discrepancy could be explained by cultivation and or PCR amplification bias.

Other isolates such as *Delftia sp.*E2330 and another *Pseudomonas* spp. did not affect the germination rate but promoted the plant growth. *Delftia sp.*has been isolated from the rhizosphere of rice and is considered as a PGP bacterium [68]. In our taxonomic survey, *Delftia spp.* were present in low abundance in both compartments of DANAC SD20A cultivar. Our isolate *Delftia sp.* E2330 showed the strongest quorum quenching activity *in vitro*. Since *Delftia* sp. VM4 was reported to possess AHL-acylase activity [69], we speculate that our isolate could also possess this enzyme activity as quorum sensing interference. *Pseudomonas* spp., are very abundant members of the rice endorhizospheres [38], [55], [70], [71], however, only *P. aeruginosa* E1103 displayed some PGP traits in the conditions that we have tested. Maybe *P. aeruginosa* could be included in the category of *Pseudomonas*_OTHER or *Pseudomonas* sp. in our total community determination. The *Aeromonas* spp. isolates did not show PGP activity or improved germination; *Aeromonas* isolates have however been reported to have PGP activity in and rice [72], [73]. Cultivation media and/or the genotype of the host could be influencing this.

Of the 15 isolates re-inoculated, only *P. fluorescens* E1308 could be re-isolated from the endosphere of the 5 plants harvested. The other strains could be in low abundance not enough for cultivation. The recovery of rifampicin spontaneous mutants is an approach used elsewhere with this aim and has shown to be a valid way and stable approach for the detection [15], [30].

We should mention some limitations of our methods and analysis. For instance, endophytic strains were isolated from two rice cultivars genotypically different from the one used in the *in planta* experiments hence it is possible that plant genotype influences endosphere colonization/microbiota,

as stated by [35], [39], [74]–[76]. On the other hand, the number of sampled plants per treatment (5 plants) could be insufficient for the objectives.

4.3 Seedling inoculation with a simplified bacterial community.

Microorganisms do not act as individuals but rather act as a dynamically changing microbial community, where cells interact and communicate with one another. This communication influences bacterial behavior significantly affecting the phenotypes of the microbial community [77]. It is therefore of importance to developing new model systems for incorporating communities of microorganisms in plant microbiota research [35]. The use of traceable simplified ecosystems reduces the complexity of naturally complex microbiota and its investigation increase our knowledge regarding factors that shape and influence microbial communities. We, therefore, performed rice inoculations with a 10 strain simplified community in order to assess its potential for host colonization and possible differences compared to single strain inoculations. We did not use strains which possessed strong *in vitro* antibacterial activity. Assessing colonization via 16S rDNA gene community profiling showed that 8 strains were detected in the endorhizosphere. Within this group, *P. pseudoalcaligenes* E1205, *Agrobacterium* sp. E2321, *Delftia* sp. E2330 and *P. jessenii* E2333 remained in the endorhizospheres after 30 days of plant growth. The isolate *P. fluorescens* E1308, the only one recovered from surface-sterilized inoculated rice plants in the single-strain *in planta* tests, was surprisingly not detected when co-inoculated with the 9 other strains. The bacterial community can be influencing the endophytic colonization of this strain or the host plant favored the colonization of other strains. The design of simplified microbial communities has been recently considered as a priority for harnessing the plant microbiome in sustainable agriculture [35] and this approach has been addressed in *Arabidopsis* [78] and in maize [79]. In this work, we initiated PGP and colonization studies of a simplified community of 10 bacterial strains and initial results encourage further studies of synergistic, signaling and cooperative behavior of a multispecies consortium as well as the role of the plant genotype.

Acknowledgments: We wish to thank Dr. Alejandro Pieters from the IVIC for logistic support and to Dr. Rosa Alvarez from the Instituto de Investigaciones Agricolas – Portuguesa, Venezuela, for the support during sampling. The graphic abstract icons were obtained from Noun Project (htt://thenounproject.com). FMB is the recipient of an ICGEB Arturo Falaschi fellowship.

Author Contributions: F.MB. and V.V. conceived and designed the experiments and wrote the paper; F.MB. performed the experiments; F.G. performed the 16S rRNA library construction and sequencing and A.P. analyzed the data. E.M. provided logistical and negotiation support for the undertaking of this binational work.

Conflicts of Interest: The authors declare no conflict of interest.

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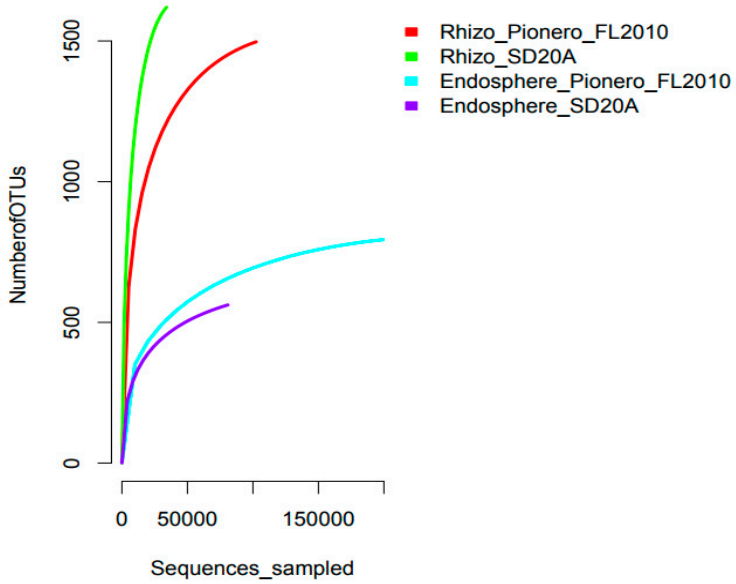
Supplementary Materials:



Supplementary Figure 1. Rarefaction curve. Representation of the observed number of OTUs as a function of sequences sampled.

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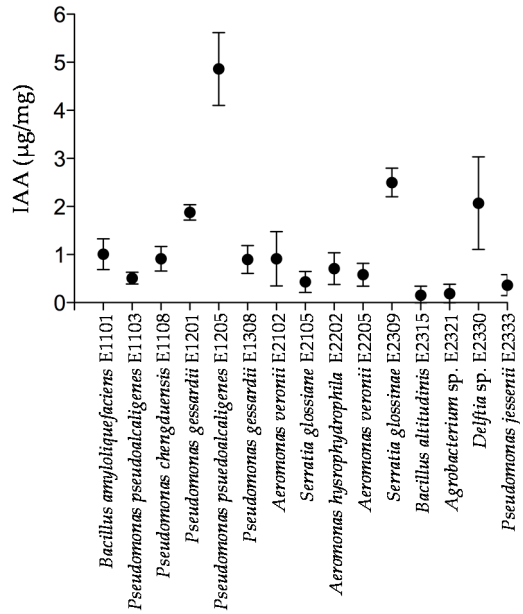

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793 **Supplementary figure 2. Production of indole acetic acid (IAA) by the putative endophyte isolates.** The 5
794 days old supernatant of each bacterial culture was spectrophotometrically analyzed after the Salvkoski
795 reaction for the presence of IAA and the parallel construction of a calibration curve. Each dot represent de
796 average reading of three replicates and the vertical bars the standard deviation. The values correspond to
797 micrograms of IAA by milligram of dry bacterial biomass.

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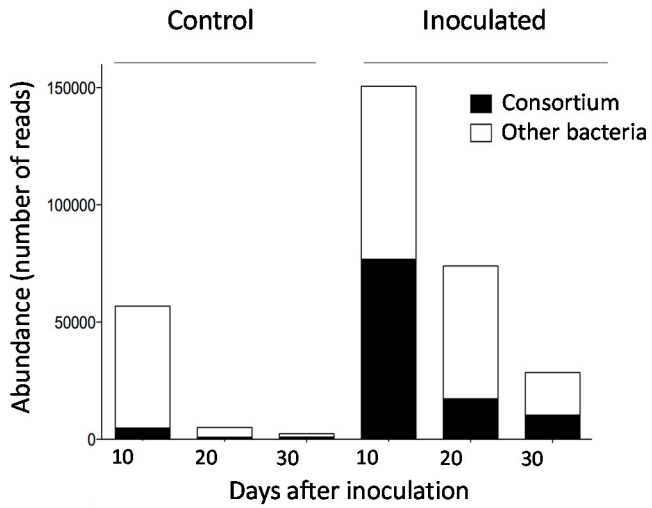

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801 **Supplementary Figure 3.** Abundance of total bacterial reads (natural and inoculated) in the simplified
802 community experiment. The total bacterial reads is plotted for every group of samples and differentiated
803 among those sequences matched with the 10 strains used as the inoculum (consortium) and those with no
804 match with the consortium (other bacteria). The total numbers of reads were: for control plants 10 days
805 n=177505; 20 days n=116321; 30 days n=155680. For inoculated plants 10 days n=154155; 20 days n=174015; 30
806 days n=329368 reads. Control plants refer to non-inoculated plants.

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807 **Supplementary table 1. Oligonucleotides used.** In red, UNITAIL 1. In green, UNITAIL 2. A C3
808 phosphoramidite spacer was incorporated in the 3′-end (/3SpC3/) of the blocking primers. The 10 bp barcodes
809 are underlined.

Name	Sequence 5′ – 3′	Reference
First PCR round		
V4 515F	CAGGACCAGGGTACGGTGGTGCCAGCMGCCGCGGTAA	80
802R	CGCAGAGAGGCTCCGTGTACNVGGGTATCTAATCC	81
806R	CGCAGAGAGGCTCCGTGGACTACHVGGGTWTCTAAT	80
MitoBlk_515F	TCCCCATGCTTTCGCACCCCA**/3SpC3/**	This work
ChloBlk_806R	GTCTCTAATCCCATTTGCTCC**/3SpC3/**	This work
Second PCR round		
ION_UNI1_A_1	CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTAAGGTAAC**CAGGACCAGGGTACGGTG	This work
ION_UN1I_A_2	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TAAGGAGAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_3	CCATCTCATCCCTGCGTGTCTCCGACTCAG**AAGAGGATTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_4	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TACCAAGATC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_5	CCATCTCATCCCTGCGTGTCTCCGACTCAG**CAGAAGGAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_6	CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTGCAAGTTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_7	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TTCGTGATTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_8	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TTCCGATAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_9	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TGAGCGGAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_10	CCATCTCATCCCTGCGTGTCTCCGACTCAG**CTGACCGAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_11	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCCTCGAATC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_12	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TAGGTGGTTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_13	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCTAACGGAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_14	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TTGGAGTGTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_15	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCTAGAGGTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_16	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCTGGATGAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_17	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCTATTCGTC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_18	CCATCTCATCCCTGCGTGTCTCCGACTCAG**AGGCAATTGC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_19	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TTAGTCGGAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_20	CCATCTCATCCCTGCGTGTCTCCGACTCAG**CAGATCCATC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_21	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TCGCAATTAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_22	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TTCGAGACGC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_23	CCATCTCATCCCTGCGTGTCTCCGACTCAG**TGCCACGAAC**CAGGACCAGGGTACGGTG	This work
ION_UNI1_A_24	CCATCTCATCCCTGCGTGTCTCCGACTCAG**AACCTCATTC**CAGGACCAGGGTACGGTG	This work
ION_UNI_trP1 Rev	CCTCTCTATGGGCAGTCGGTGATCGCAGAGAGGCTCCGTG	This work
For 16S sequencing		
fD1	AGAGTTTGATCCTGGCTCAG	Universal
rP2	ACGGCTACCTTGTTACGACTT	Universal
518F	CCAGCAGCCGCGGTAATACG	Universal
800R	TACCAGGGTATCTAATCC	Universal

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811 **(**[80], [81]**)**

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820 **Supplementary table 2. List of bacterial genera identified (from 1 to 341)** 7 PAGES TABLE..!

	Genus	R Pionero	R SD20A	E Pionero	E SD20A
1	g__s__	0.018	0.000	0.000	0.000
2	g__4-29_s__	0.009	0.057	0.000	0.000
3	g__A17_s__	0.003	0.000	0.000	0.000
4	g__Abiotrophia_s__	0.000	0.001	0.000	0.000
5	g__Achromobacter_s__	0.003	0.000	0.084	0.009
6	g__Acidovorax_Other	0.002	0.000	0.003	0.000
7	g__Acidovorax_s__	0.256	0.033	0.165	0.000
8	g__Acidovorax_s__delafieldii	0.004	0.000	0.002	0.000
9	g__Acidovorax_s__facilis	0.000	0.000	0.001	0.000
10	g__Acinetobacter_s__	0.036	0.002	0.122	0.087
11	g__Acinetobacter_s__johnsonii	0.000	0.002	0.000	0.000
12	g__Acinetobacter_s__lwoffii	0.004	0.000	0.030	0.103
13	g__Acinetobacter_s__rhizosphaerae	0.015	0.000	0.000	0.000
14	g__Actinobacillus_Other	0.001	0.003	0.000	0.000
15	g__Actinotalea_s__	0.000	0.000	0.000	0.005
16	g__Adhaeribacter_s__	0.002	0.026	0.000	0.000
17	g__Aeromonas_Other	0.000	0.000	0.000	0.000
18	g__Aeromonas_s__caviae	0.001	0.000	0.011	0.002
19	g__Aggregatibacter_s__	0.000	0.002	0.000	0.031
20	g__Agrobacterium_Other	0.000	0.000	0.002	0.000
21	g__Agrobacterium_s__	0.179	0.077	2.459	0.472
22	g__Agrobacterium_s__undicola	0.000	0.000	0.239	0.000
23	g__Agrobacterium_s__vitis	0.000	0.000	0.001	0.000
24	g__Alcanivorax_s__	0.000	0.001	0.000	0.000
25	g__Algoriphagus_s__terrigena	0.000	0.079	0.000	0.221
26	g__Amaricoccus_s__	0.002	0.000	0.000	0.003
27	g__Aminobacter_s__	0.000	0.007	0.000	0.000
28	g__Amorphomonas_s__oryzae	0.000	0.001	0.000	0.000
29	g__Anaerococcus_s__	0.000	0.004	0.000	0.000
30	g__Anaerolinea_s__	0.005	0.006	0.000	0.000
31	g__Anaeromyxobacter_s__	0.080	0.094	0.000	0.000
32	g__Anaerovorax_s__	0.000	0.000	0.000	0.001
33	g__Ancylobacter_s__	0.033	0.000	0.000	0.007
34	g__Antarctobacter_s__	0.000	0.000	0.000	0.005
35	g__Aquaspirillum_s__putridiconchylium	0.000	0.031	0.000	0.000
36	g__Aquicella_s__	0.005	0.002	0.000	0.000
37	g__Aquimarina_s__	0.000	0.000	0.000	0.020
38	g__Aquimonas_s__	0.000	0.001	0.000	0.000
39	g__Arenimonas_s__	0.047	0.381	0.000	0.000
40	g__Arthrobacter_Other	0.005	0.000	0.000	0.000
41	g__Arthrobacter_s__psychrolactophilus	0.029	0.000	0.000	0.000
42	g__Arthronema_s__	0.000	0.004	0.000	0.000
43	g__Aspromonas_s__composti	0.000	0.001	0.000	0.000
44	g__Asticcacaulis_Other	0.000	0.000	0.001	0.000
45	g__Asticcacaulis_s__	0.000	0.007	0.515	0.477
46	g__Azospira_s__	0.000	0.001	0.000	0.000
47	g__Azospirillum_s__	0.000	0.001	0.205	0.008
48	g__Azospirillum_s__massiliensis	0.000	0.000	0.326	0.119

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49	g__Bacillus_Other	0.000	0.000	0.018	0.000
50	g__Bacillus_s__	0.002	0.001	0.000	0.001
51	g__Bacillus_s__cereus	0.009	0.001	0.000	0.000
52	g__Bacteroides_s__	0.000	0.004	0.000	0.000
53	g__Bdellovibrio_s__	0.012	0.005	0.000	0.000
54	g__Bdellovibrio_s__bacteriovorus	0.000	0.001	0.000	0.000
55	g__Blastomonas_s__	0.014	0.002	0.000	0.000
56	g__Blvii28_s__	0.000	0.001	0.000	0.000
57	g__Bosea_s__genosp.	0.000	0.000	0.045	0.002
58	g__Bradyrhizobium_s__	0.001	0.000	0.001	0.000
59	g__Brevibacillus_s__	0.000	0.000	0.015	0.000
60	g__Brevibacterium_s__aureum	0.002	0.000	0.000	0.001
61	g__Brevundimonas_Other	0.000	0.000	0.000	0.000
62	g__Brevundimonas_s__diminuta	0.000	0.004	0.047	0.000
63	g__Bulleidia_s__moorei	0.000	0.000	0.000	0.006
64	g__Burkholderia_s__	0.000	0.000	0.000	0.000
65	g__Candidatus Endobugula_s__	0.000	0.000	0.000	0.003
66	g__Candidatus Koribacter_s__	0.076	0.022	0.000	0.000
67	g__Candidatus Nitrososphaera_s__SCA1170	0.027	0.005	0.000	0.000
68	g__Candidatus Rhabdochlamydia_s__	0.001	0.000	0.030	0.000
69	g__Candidatus Solibacter_s__	0.026	0.010	0.000	0.000
70	g__Candidatus Xiphinematobacter_s__	0.020	0.001	0.050	0.000
71	g__Capnocytophaga_s__	0.000	0.000	0.000	0.003
72	g__Capnocytophaga_s__ochracea	0.000	0.001	0.000	0.000
73	g__Catonella_s__	0.000	0.000	0.000	0.002
74	g__Caulobacter_Other	0.000	0.000	0.033	0.000
75	g__Caulobacter_s__	0.000	0.001	1.224	0.923
76	g__Cellulomonas_s__	0.000	0.000	0.000	0.002
77	g__Cellvibrio_s__	0.013	0.042	14.755	5.257
78	g__Chelativorans_s__	0.001	0.000	0.000	0.000
79	g__Chryseobacterium_s__	0.023	0.002	1.093	0.000
80	g__Citrobacter_s__	0.000	0.000	0.000	0.072
81	g__Cloacibacterium_s__	0.075	0.031	0.013	0.000
82	g__Clostridium_Other	0.000	0.001	0.000	0.000
83	g__Clostridium_s__	0.000	0.002	0.000	0.000
84	g__Clostridium_s__acetobutylicum	0.000	0.002	0.000	0.000
85	g__Clostridium_s__butyricum	0.004	0.000	0.000	0.000
86	g__Clostridium_s__hungatei	0.000	0.002	0.000	0.000
87	g__Clostridium_s__intestinale	0.005	0.000	0.000	0.000
88	g__Coccinimonas_s__marina	0.014	0.000	0.000	0.000
89	g__Cohnella_s__	0.000	0.000	0.000	0.009
90	g__Comamonas_s__	0.024	0.000	0.000	0.000
91	g__Constrictibacter_s__antarcticus	0.000	0.002	0.000	0.000
92	g__Coprococcus_s__	0.000	0.000	0.000	0.002
93	g__Corynebacterium_s__	0.021	0.003	0.002	0.037
94	g__Corynebacterium_s__kroppenstedtii	0.001	0.001	0.000	0.000
95	g__Crenothrix_s__	0.000	0.001	0.000	0.000
96	g__Crocinitomix_s__	0.000	0.000	0.052	0.000
97	g__Cryocola_s__	0.000	0.000	0.010	0.000
98	g__Cylindrospermopsis_s__	0.000	0.002	0.000	0.000

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99	g__Cytophaga_s__	0.023	0.000	0.000	0.000
100	g__DA101_s__	0.010	0.000	0.000	0.000
101	g__DCE29_s__	0.003	0.000	0.000	0.000
102	g__Dechloromonas_s__	0.026	0.000	0.000	0.000
103	g__Defluviitalea_s__saccharophila	0.012	0.000	0.000	0.000
104	g__Delftia_s__	0.002	0.013	0.000	0.010
105	g__Demequina_s__	0.000	0.000	0.000	0.001
106	g__Desulfobacca_s__	0.000	0.017	0.000	0.000
107	g__Desulfobulbus_s__	0.013	0.012	0.000	0.000
108	g__Desulfococcus_s__	0.000	0.010	0.000	0.000
109	g__Desulfomicrobium_s__	0.000	0.001	0.000	0.000
110	g__Desulfomonile_s__	0.000	0.004	0.000	0.000
111	g__Desulforhabdus_s__amnigena	0.001	0.000	0.000	0.000
112	g__Desulfotalea_s__	0.000	0.002	0.000	0.000
113	g__Desulfovibrio_s__	0.002	0.006	0.000	0.000
114	g__Desulfovibrio_s__mexicanus	0.000	0.002	0.000	0.000
115	g__Desulfovibrio_s__putealis	0.010	0.002	0.000	0.000
116	g__Desulfovirga_s__adipica	0.004	0.006	0.000	0.000
117	g__Devosia_s__	0.241	0.057	1.504	0.117
118	g__Dok59_s__	0.007	0.005	0.000	0.000
119	g__Dokdonella_s__	0.011	0.002	0.009	0.000
120	g__Dyadobacter_s__	0.162	0.002	0.012	0.000
121	g__Eikenella_s__	0.000	0.001	0.000	0.008
122	g__Endozoicomonas_s__montiporae	0.000	0.000	0.001	0.000
123	g__Enhydrobacter_s__	0.018	0.008	0.070	0.180
124	g__Enterobacter_s__	0.000	0.000	0.001	0.000
125	g__Epulopiscium_s__	0.012	0.000	0.000	0.000
126	g__Erythrobacter_Other	0.001	0.000	0.000	0.000
127	g__Erythrobacter_s__	0.058	0.000	0.000	0.000
128	g__Escherichia_s__coli	0.026	0.000	0.033	0.005
129	g__Euptelea_s__polyandra	0.000	0.000	0.000	0.000
130	g__Exiguobacterium_s__	0.005	0.000	0.031	0.000
131	g__Fimbriimonas_s__	0.010	0.003	0.000	0.000
132	g__Flavisolibacter_s__	0.421	0.070	0.000	0.000
133	g__Flavobacterium_Other	0.002	0.000	0.026	0.000
134	g__Flavobacterium_s__	1.669	0.039	1.483	0.585
135	g__Flavobacterium_s__frigidarium	0.000	0.000	0.000	0.001
136	g__Flavobacterium_s__gelidilacus	0.000	0.069	0.097	0.365
137	g__Flavobacterium_s__succinicans	0.012	0.021	0.179	0.181
138	g__Flectobacillus_s__	0.000	0.001	0.000	0.000
139	g__Fluviicola_s__	0.021	0.012	0.559	0.214
140	g__Francisella_s__	0.001	0.000	0.000	0.000
141	g__Fritschea_s__eriococci	0.001	0.000	0.000	0.000
142	g__Fusibacter_s__	0.010	0.003	0.000	0.000
143	g__Fusobacterium_s__	0.000	0.000	0.005	0.024
144	g__Gallionella_s__	0.020	0.067	0.000	0.000
145	g__Gemmatimonas_s__	0.000	0.002	0.000	0.000
146	g__Geobacter_s__	0.146	0.050	0.000	0.000
147	g__GOUTA19_s__	0.096	0.059	0.000	0.000
148	g__Granulicatella_s__	0.000	0.003	0.000	0.002

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149	g__Haemophilus_s__parainfluenzae	0.002	0.016	0.000	0.002
150	g__Halomonas_s__	0.000	0.024	0.000	0.053
151	g__Halothiobacillus_s__	0.000	0.675	0.000	0.000
152	g__Herbaspirillum_s__	0.000	0.000	0.024	0.001
153	g__HTCC_s__	0.000	0.012	0.000	0.000
154	g__Hydrogenophaga_s__	0.085	0.033	0.012	0.009
155	g__Hylemonella_s__	0.002	0.000	0.000	0.000
156	g__Hymenobacter_s__	0.002	0.000	0.000	0.000
157	g__Hyphomicrobium_Other	0.000	0.001	0.000	0.000
158	g__Hyphomicrobium_s__	0.017	0.008	0.000	0.000
159	g__Hyphomonas_s__	0.000	0.003	0.000	0.000
160	g__Iamia_s__	0.000	0.002	0.000	0.000
161	g__Janthinobacterium_s__	0.007	0.000	0.003	0.005
162	g__Janthinobacterium_s__lividum	0.024	0.000	0.030	0.000
163	g__K82_s__	0.000	0.001	0.000	0.000
164	g__Kaistia_s__	0.000	0.002	0.000	0.002
165	g__Kaistobacter_s__	0.327	0.117	0.000	0.000
166	g__Klebsiella_s__	0.000	0.002	0.021	0.000
167	g__Kocuria_s__rhizophila	0.000	0.000	0.000	0.002
168	g__Lacibacter_s__cauensis	0.104	0.041	0.000	0.018
169	g__Lactobacillus_s__zeae	0.000	0.000	0.006	0.000
170	g__LCP-6_s__	0.015	0.036	0.000	0.000
171	g__Leadbetterella_s__	0.018	0.028	0.000	0.000
172	g__Leptolyngbya_s__	0.000	0.003	0.000	0.000
173	g__Leptonema_s__	0.000	0.002	0.000	0.000
174	g__Leptospira_s__	0.004	0.003	0.000	0.000
175	g__Leptotrichia_s__	0.000	0.000	0.010	0.000
176	g__Leuconostoc_s__	0.000	0.000	0.000	0.001
177	g__Limnobacter_s__	5.641	0.319	0.263	0.000
178	g__Limnohabitans_s__	0.001	0.001	0.006	0.000
179	g__Loktanella_s__	0.000	0.000	0.010	0.002
180	g__Luteimonas_s__	0.012	0.003	0.000	0.000
181	g__Luteolibacter_s__	0.006	0.002	0.168	0.001
182	g__Lutibacterium_s__	0.000	0.000	0.241	0.000
183	g__Lutimonas_s__	0.000	0.001	0.000	0.007
184	g__Lysobacter_s__	0.003	0.005	0.058	0.011
185	g__Magnetospirillum_s__	0.000	0.000	0.000	0.003
186	g__Maribacter_s__	0.001	0.000	0.000	0.000
187	g__Marinobacter_s__	0.215	0.000	0.000	0.000
188	g__Marinobacter_s__bryozoorum	0.001	0.000	0.000	0.000
189	g__Massilia_s__haematophila	0.001	0.000	0.000	0.000
190	g__Mesorhizobium_s__	0.000	0.004	0.000	0.000
191	g__Methylibium_s__	0.006	0.005	0.000	0.000
192	g__Methylobacterium_s__	0.006	0.001	0.000	0.000
193	g__Methylomicrobium_s__	0.000	0.002	0.000	0.000
194	g__Methylomicrobium_s__agile	0.000	0.011	0.000	0.000
195	g__Methylophaga_s__	0.000	0.511	0.000	0.000
196	g__Methylotenera_s__mobilis	2.872	0.028	0.150	0.000
197	g__Methyloversatilis_s__	0.039	0.053	0.002	0.006
198	g__Methylovorus_s__glucosotrophus	0.000	0.000	0.047	0.000

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199	g__Micrococcus_s__	0.000	0.000	0.000	0.001
200	g__Microvirgula_s__aerodenitrificans	0.000	0.001	2.514	0.000
201	g__Muricola_s__jejuensis	0.000	0.000	0.013	0.000
202	g__Mycoplana_s__	0.227	0.241	0.889	0.229
203	g__Mycoplasma_s__	0.000	0.001	0.000	0.000
204	g__Myxococcus_s__	0.000	0.006	0.000	0.000
205	g__Nautella_s__	0.000	0.000	0.005	0.000
206	g__Neisseria_s__	0.017	0.004	0.000	0.011
207	g__Neisseria_s__oralis	0.000	0.001	0.000	0.000
208	g__Neisseria_s__subflava	0.000	0.015	0.000	0.007
209	g__Nevskia_s__ramosa	0.069	0.012	0.261	0.026
210	g__Niabella_s__	0.002	0.000	0.000	0.000
211	g__Niastella_s__	0.001	0.000	0.000	0.000
212	g__Nitrosomonas_s__nitrosa	0.000	0.018	0.000	0.000
213	g__Nitrosopumilus_s__	0.000	0.002	0.000	0.000
214	g__Nitrospira_s__	0.039	0.006	0.000	0.000
215	g__Novosphingobium_s__	0.066	0.007	0.166	0.019
216	g__Novosphingobium_s__capsulatum	0.006	0.000	0.000	0.000
217	g__Oceanibaculum_s__indicum	0.000	0.130	0.000	0.013
218	g__Ochrobactrum_s__	0.000	0.000	0.064	0.000
219	g__Octadecabacter_s__	0.002	0.002	0.016	0.000
220	g__Octadecabacter_s__antarcticus	0.000	0.000	0.014	0.000
221	g__Opitutus_s__	0.032	0.031	3.250	0.611
222	g__Oribacterium_s__	0.000	0.000	0.000	0.001
223	g__Paenibacillus_s__	0.000	0.000	0.000	0.002
224	g__Paludibacter_s__	0.000	0.008	0.000	0.000
225	g__Pantoea_Other	0.002	0.000	0.000	0.000
226	g__Paracoccus_s__	0.000	0.000	0.000	0.003
227	g__Paracoccus_s__marcusii	0.025	0.013	0.000	0.005
228	g__Parapedobacter_Other	0.001	0.000	0.000	0.000
229	g__Parapedobacter_s__	0.010	0.000	0.000	0.000
230	g__Parasegitibacter_s__luojiensis	0.051	0.030	0.000	0.000
231	g__Pedobacter_s__	0.038	0.001	2.249	0.043
232	g__Pedobacter_s__terricola	0.000	0.000	0.023	0.000
233	g__Pedomicrobium_s__	0.000	0.005	0.000	0.000
234	g__Pedosphaera_s__	0.001	0.000	0.000	0.000
235	g__Peptostreptococcus_s__	0.000	0.000	0.002	0.005
236	g__Peredibacter_s__starrii	0.001	0.004	0.000	0.000
237	g__Phaeobacter_Other	0.000	0.001	0.000	0.000
238	g__Phaeobacter_s__	0.000	0.002	0.001	0.002
239	g__Phaeospirillum_s__fulvum	0.000	0.003	0.000	0.000
240	g__Phenylobacterium_s__	0.002	0.017	0.060	0.014
241	g__Phormidium_s__	0.000	0.001	0.047	0.008
242	g__Phycicoccus_s__	0.002	0.000	0.000	0.000
243	g__Pigmentiphaga_s__	0.003	0.000	0.000	0.000
244	g__Pirellula_s__	0.011	0.017	0.000	0.000
245	g__Planctomyces_s__	0.122	0.010	0.000	0.012
246	g__Planctomycete_s__LF1	0.001	0.000	0.000	0.000
247	g__Planifilum_s__	0.000	0.001	0.000	0.000
248	g__Planktothrix_s__	0.000	0.007	0.000	0.000

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249	g__Pleomorphomonas_Other	0.000	0.000	0.000	0.002
250	g__Pleomorphomonas_s__	0.000	0.000	0.568	0.027
251	g__Pleomorphomonas_s__oryzae	0.000	0.000	0.001	0.000
252	g__Plesiocystis_s__	0.000	0.002	0.000	0.000
253	g__Polaribacter_s__	0.000	0.000	0.010	0.000
254	g__Polaromonas_s__	0.000	0.001	0.000	0.000
255	g__Porphyromonas_s__	0.000	0.003	0.000	0.012
256	g__Prevotella_s__melaninogenica	0.000	0.000	0.000	0.017
257	g__Propionivibrio_s__	0.002	0.000	0.000	0.028
258	g__Prosthecobacter_s__	0.000	0.004	0.000	0.000
259	g__Prosthecobacter_s__debontii	0.003	0.000	0.188	0.000
260	g__PSB-M-3_s__	0.000	0.002	0.000	0.000
261	g__Pseudoalteromonas_s__	0.000	0.000	0.000	0.005
262	g__Pseudomonas_Other	0.118	0.302	0.504	1.423
263	g__Pseudomonas_s__	6.566	0.224	0.139	0.124
264	g__Pseudomonas_s__alcaligenes	0.003	0.000	0.000	0.000
265	g__Pseudomonas_s__mendocina	0.001	0.000	0.006	0.000
266	g__Pseudomonas_s__nitroreducens	0.000	0.002	0.000	0.000
267	g__Pseudomonas_s__pseudoalcaligenes	0.602	0.099	10.196	0.707
268	g__Pseudomonas_s__stutzeri	0.237	0.013	0.002	0.024
269	g__Pseudomonas_s__umsongensis	0.000	0.002	0.002	0.006
270	g__Pseudomonas_s__veronii	1.395	0.386	0.115	0.293
271	g__Pseudomonas_s__viridiflava	0.013	0.000	0.190	0.000
272	g__Pseudonocardia_s__	0.015	0.000	0.000	0.000
273	g__Pseudoxanthomonas_s__	0.000	0.000	0.061	0.000
274	g__Pseudoxanthomonas_s__mexicana	0.041	0.006	0.196	0.013
275	g__Ralstonia_s__	0.000	0.000	0.036	0.000
276	g__Rheinheimera_s__	0.213	0.129	0.719	0.065
277	g__Rhodanobacter_s__lindaniclasticus	0.000	0.003	0.000	0.000
278	g__Rhodobacter_s__	0.032	0.013	0.000	0.006
279	g__Rhodococcus_s__fascians	0.160	0.000	0.000	0.000
280	g__Rhodoferax_s__	0.001	0.008	0.116	0.843
281	g__Rhodoplanes_s__	0.210	0.117	0.009	0.044
282	g__Rhodoplanes_s__elegans	0.000	0.001	0.000	0.000
283	g__Roseivivax_s__	0.000	0.000	0.067	0.000
284	g__Roseobacter_s__denitrificans	0.000	0.000	0.040	0.000
285	g__Roseomonas_s__	0.000	0.004	0.000	0.000
286	g__Rothia_s__aeria	0.000	0.001	0.000	0.000
287	g__Rothia_s__dentocariosa	0.000	0.000	0.000	0.002
288	g__Rothia_s__mucilaginosa	0.000	0.002	0.000	0.000
289	g__Rubrivivax_s__	0.010	0.003	0.000	0.000
290	g__Sandaracinobacter_s__sibiricus	0.008	0.017	0.000	0.000
291	g__Sediminibacterium_s__	0.013	0.000	0.000	0.008
292	g__Sediminicola_s__	0.004	0.000	0.000	0.000
293	g__Serratia_s__marcescens	0.003	0.000	0.000	0.000
294	g__Shewanella_Other	0.001	0.000	0.000	0.000
295	g__Shewanella_s__	0.016	0.002	1.405	0.287
296	g__Silanimonas_s__mangrovi	0.000	0.020	0.000	0.000
297	g__Sinorhizobium_s__	0.011	0.000	0.000	0.000
298	g__Sphingobacterium_s__	0.008	0.000	0.000	0.005

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Peer-reviewed version available at Microorganisms 2018, 6, 14; doi:10.3390/microorganisms6010014

299	g__Sphingobacterium_s__multivorum	0.000	0.000	0.030	0.000
300	g__Sphingobium_s__	0.000	0.017	0.415	0.017
301	g__Sphingobium_s__xenophagum	0.012	0.002	0.090	0.055
302	g__Sphingomonas_Other	0.002	0.001	0.000	0.000
303	g__Sphingomonas_s__	0.006	0.016	0.025	0.000
304	g__Sphingomonas_s__azotifigens	0.016	0.000	0.240	0.003
305	g__Sphingomonas_s__wittichii	0.000	0.000	0.000	0.010
306	g__Sphingomonas_s__yabuuchiae	0.007	0.000	0.000	0.000
307	g__Sphingopyxis_s__	0.008	0.000	0.000	0.003
308	g__Sphingopyxis_s__alaskensis	0.134	0.081	0.056	0.027
309	g__Sphingosinicella_s__microcystinivorans	0.000	0.002	0.000	0.000
310	g__Staphylococcus_Other	0.000	0.000	0.000	0.001
311	g__Staphylococcus_s__	0.030	0.057	0.081	0.138
312	g__Staphylococcus_s__epidermidis	0.000	0.000	0.001	0.000
313	g__Stenotrophomonas_s__	0.031	0.000	0.051	0.003
314	g__Steroidobacter_s__	0.009	0.019	0.000	0.000
315	g__Streptococcus_s__	0.015	0.055	0.029	0.117
316	g__Streptococcus_s__infantis	0.000	0.000	0.001	0.000
317	g__Sulfuricurvum_s__kujiense	0.042	0.084	0.000	0.000
318	g__Sulfuritalea_s__	0.021	0.001	0.000	0.000
319	g__Synechococcus_s__	0.020	0.004	0.107	0.023
320	g__Syntrophobacter_s__	0.028	0.028	0.000	0.000
321	g__Syntrophomonas_s__	0.000	0.002	0.000	0.000
322	g__Tatlockia_s__	0.003	0.000	0.000	0.000
323	g__Tepidimonas_s__	0.000	0.010	0.042	0.000
324	g__Thermomonas_s__	0.173	0.018	0.000	0.000
325	g__Thiobacillus_s__	0.206	0.369	0.000	0.000
326	g__Thiomonas_s__	0.000	0.001	0.000	0.000
327	g__Tolumonas_s__	0.005	0.002	0.000	0.000
328	g__Treponema_s__	0.000	0.001	0.000	0.000
329	g__Turneriella_s__	0.000	0.001	0.000	0.000
330	g__Ulvibacter_s__	0.000	0.000	0.040	0.000
331	g__Variovorax_s__	0.021	0.017	1.661	0.000
332	g__Veillonella_s__dispar	0.000	0.000	0.000	0.007
333	g__Vibrio_Other	0.000	0.001	0.006	0.001
334	g__Vibrio_s__	0.002	0.009	0.066	0.003
335	g__Vogesella_s__	0.004	0.000	0.000	0.000
336	g__WAL_1855D_s__	0.000	0.001	0.000	0.000
337	g__Winogradskyella_s__	0.003	0.000	0.053	0.000
338	g__Winogradskyella_s__thalassocola	0.000	0.013	0.000	0.024
339	g__Xanthobacter_s__	0.000	0.000	0.000	0.005
340	g__Yersinia_s__	0.000	0.000	0.000	0.021
341	Other_Other	0.066	0.010	0.000	0.003

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