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Isolation and Identification of Bacterial Endophytes from Grasses along the Oregon Coast

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Abstract

Bacterial endophytes have been shown to improve abiotic and biotic stress responses in plants. Plants growing under harsh conditions along the Oregon Coast could contain bacterial endophytes that improve persistence and growth of grasses in this environment. Thirty-four plants consisting of eight different species were collected along the Oregon coast from four different sites. Bacterial endophytes were isolated from root crown, stem and leaf tissues. A portion of the 16S rRNA ITS regions of each isolate was amplified, sequenced, and used to perform a BLAST search against the nucleotide database collection at National Center for Biotechnology Information. One-hundred and thirty-three different bacterial isolates, ninety-four of which were unique, representing thirty-six different taxonomic groups were found. Over 50% of the total bacteria isolates were in just five taxonomic groups. *Pseudomonads* were the most predominant bacteria isolated, making up 20.3% of the total isolates, followed by *Curtobacterium* and *Microbacterium*, each at 8.2%, *Bacillus* at 7.5% and *Xanthomomas* at 6%. Forty-seven percent (17 of 36) of the taxonomic groups contained only a single isolate. Fourteen bacterial isolates from five taxonomic groups, nine of which were from the genus *Pseudomonas*, were found to have 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, an enzyme associated with improving plant growth under stress. These newly discovered bacterial endophytes will be a valuable biological resource to develop approaches to increase the yield and adaptability of grasses and other crops grown in diverse environments and to meet the challenges associated with an unpredictable climate.

Keywords

Endophyte, Grasses, ACC Deaminase, Bacteria, Abiotic Stress, Salt Stress

1. Introduction

Global freshwater resources are rapidly decreasing, which in many regions of the world endangers food production; roughly 70% of global freshwater consumption is by agriculture [1]. Over the past few decades there has been a significant increase in salinization of arable land [2] [3] [4]. As land becomes more limited for conventional agriculture, plants grown on marginal soils will be exposed to higher levels of mineralization and soil salinity. In a variety of plant species, transgenic lines have shown the ability to significantly increase yields and growth potential under salt stress [5] [6]. However, the negative public perception associated with transgenic approaches necessitates the development of novel approaches to find solutions to this increasing problem of diminishing water resources. Furthermore, climate change has contributed to decreased available water, which has had a negative impact on agriculture throughout the US and worldwide [7] [8]. Innovative approaches will be necessary to improve water-stress tolerance in crops and will be critical for food security. The discovery and development of novel beneficial endophytes have the potential to improve the adaptability of grasses and other crop species for production in less than ideal environments with limited water resources.

Symbiotic microorganisms are essential for almost all living organisms from insects to animals to plants. Over the last decade there has been extensive interest in the identification, isolation and elucidation of the role of these microorganisms in the rhizosphere, roots and the phyllosphere have on plant health and growth [9] [10]. The best-studied and characterized bacterial symbionts in plants are *Rhizobia*. These bacteria interact with the plant's root tissue to cause the development and formation of nodules, where they convert nitrogen to a plant-usable form [11] [12] [13]. Bacteria have been found in almost every environment and species of plant [8] [14] [15] [16] [17]. Endophytic bacteria are found in most organs of the plant including roots, stems, leaves, seeds, fruits, tubers, ovules, as well as inside nodules [18] [19] [20]. There is a diverse range of bacterial species living in the soil associated with the roots or inside plants, as endophytes that improve the growth potential and tolerance to a wide range of biotic and abiotic stresses of the host plant [8] [9] [10] [15] [16] [20] [21] [22] [23].

Rhizospheric and endophytic bacterial species utilize a variety of mechanisms to enhance the growth of plants in different environments or in response to biotic and abiotic stresses [8] [9] [10] [16] [24] [25]. In addition to *Rhizobium*, there are a wide range of bacteria associated with plant roots that fix nitrogen [26] [27] [28] [29] [30]. Plant-associated bacteria have been shown to improve nutrient uptake and availability. For example, bacteria in the genera *Pseudomonas*, *Bacillus*, and *Rhizobium* are some of the best solubilizers of inorganic or organic phosphate, thus increasing the availability and uptake of phosphorus, a major macronutrient essential for plant growth [31]. Many of these growth enhancing endophytes can affect plant physiological processes by producing plant hormones such as indole acetic acid, gibberellins and cytokinins, which have the

potential to enhance plant growth [32]-[37]. Another plant hormone, ethylene, is often produced in response to abiotic and biotic stress [38] [39]. Bacteria producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks down the precursor to ethylene, lowers stress ethylene levels in the plant [40]. This improves the plant's growth potential when plants are exposed to a variety of abiotic stresses [25]. The presence of growth-promoting endophytes can also improve a plant's ability to adapt and grow in soils contaminated with organic compounds and heavy metals [41]-[49]. These bacteria can alleviate the toxic effects of these compounds through decomposition, by eliminating organic pollutants in soil, by their volatilization from the plant, or by the enhanced uptake and sequestration of heavy metals from soil. These bacteria coupled with specific plant species that are able to uptake metals from the soil can provide improved approaches for phytoremediation of polluted or toxic soils. Endophytic bacteria have also been shown to be effective biological control agents against different pathogens or organisms. They utilize a range of mechanisms, including competition or exclusion, production of inhibitory compounds, and induction of systemic resistance for disease suppression in host plants [22] [50]-[56]. In addition to the production of antimicrobial compounds, bacterial endophytes also provide a valuable resource for a wide range of natural products [56] [57] [58] [59]. While some of these compounds play a role in defense, others mediate the bacteria's interaction with the host plant. Some of these compounds alter gene expression and stress-tolerance pathways within the plant, but for many of these bioactive compounds their actual role or purpose still needs to be elucidated.

A diverse array of bacterial endophytic species have already been isolated and identified from a wide range of plant species and many of these isolates have been shown to improve growth and tolerance to biotic and abiotic stresses in plants [8] [10] [15] [16] [22] [23]. Furthermore, much emphasis has been placed on the isolation of bacterial endophytes from vegetable and cereal crop species and on how these endophytes improve growth and stress tolerance in these plants. In addition to the plant species, the environment or stress the plant is exposed to will influence the type of bacteria populating the plant. In desert terrains, endophytic bacteria isolated from cardon cactus were found to promote the establishment of seedlings and plant growth on igneous rocks without soil [60]. Halotolerant bacteria isolated from saline habitats were shown to increase salt tolerance of inoculated plants [61] [62]. Endophytic bacteria were isolated and identified in three plants from the low arctic tundra and were subsequently shown to be cold-adapted and host-plant specific [63]. Growth-promoting heavy metal-resistant endophytic bacteria were isolated from two copper-tolerant plant species growing on copper mine wasteland [44]. Similarly, heavy metal-resistant bacterial endophytes isolated from the Cadmium-hyper accumulator *Solanum nigrum* L. found growing on mine tailings, these bacteria were found to improve growth and resistance to different heavy metals in test plants [45]. Endophytic bacteria were isolated from plants growing in hydrocarbon contaminated soils, these endophytes were shown to enhance growth and were capable of degrading

a wide range of hydrocarbons [49] [64]. Additionally, endophytic nitrogen-fixing bacteria that were isolated from dune grasses growing along the Oregon coast may contribute to the growth and persistence of these grasses on nutrient-poor sand [29].

Outside of sugarcane and the cereal grasses, there has been very little effort to investigate the diversity of bacterial populations in forage and turf related species [8] [15] [16] [17] [29] [49] [65] [66]. Grasses are found growing in a diverse range of environments from Antarctica, to the prairies, in alpine regions, to hot springs, to coastal shores, and even in the desert. Therefore grasses provide a unique opportunity and resource to isolate novel beneficial bacterial endophytes from plants associated with a specific environment, or plants providing an ecological niche, or plants exposed to specific types of stress. Furthermore, most of the current research on identifying plant growth-promoting bacteria that can improve growth under saline conditions has focused on isolating bacteria from the rhizosphere and the surrounding soils where the plant is growing. Far less has been done on the isolation and identification of endophytic bacteria from plants where they may impart these same benefits.

Grasses growing along the Oregon coast are exposed to poor quality soil, salinity and a variety of other abiotic and biotic stresses. In order to survive in this high stress environment, these grasses may contain an unique population of bacterial endophytes, which enhance the plant's ability to grow and survive. The long-term goal of this study is to identify bacterial endophytes that have the potential to increase stress tolerance, growth, and persistence in diverse environments for grasses and other crop species. This paper describes the initial isolation and identification of bacterial endophytes from various grasses found growing in sandy soils along the Oregon coast exposed to saline environments.

2. Materials and Methods

Plant Collection and Endophyte Isolation

Various grass species found growing in areas exposed to ocean spray, mists and tides were collected at four different sites along the Oregon coast. Sites designated using the UTM(Universal Transverse Mercator) coordinate system were located near Harbor Vista (UTM Easting 409358.11; UTM Northing 4874316.08; UTM Zone 10T), Coos Bay (UTM Easting 394491.19; UTM Northing 4805343.08; UTM Zone 10T), Bob Creek Wayside (UTM Easting 411168.70; UTM Northing 4899584.52; UTM Zone 10T), and Yachats (UTM Easting 412067.47; UTM Northing 4906654.63; UTM Zone 10T) (**Table 1**). Identifications of collected plant species were confirmed with the assistance of Dr. Richard Halse at Oregon State University Herbarium.

All collected plants samples were stored in plastic bags in a cooler on ice after collection and stored at 4˚C until processing. Samples were processed within 48 h of collection as described in [67]. Briefly, plants were rinsed with water to remove soil and debris, dead or damaged plant tissue, and the majority of the roots, were removed prior to processing. The remaining plant was dissected by

tissue type corresponding to the root crown, stems, and leaves. Stems and root crowns were surface sterilized by placing in 90% ethanol for 1 min, 3% chlorine bleach with 2 drops of Tween-20/100ml for 3 min, sterile double distilled water (DDW) for 1 min, 70% ethanol for 1 min, and a quick rinse in sterile DDW. Leaf tissue was sterilized by placing leaves in 70% ethanol for 2 min, 2% bleach for 3 min, sterile DDW for 1 min, followed by a quick dip in 90% ethanol. After sterilization, the end (~2 - 3 mm) of stem, leaf or root crown was cut off and discarded. The remaining sample was cut into 2 - 3 mm sections. To ensure the effectiveness of the sterilization technique, randomly selected samples from all tissue types were dipped and swirled in 500 mL liquid LB and plated onto Luria Broth (LB) agar plates, no growth was observed in any of the tested samples.

For bacterial isolation, we ground the tissue by either of the following methods: 1) Sections were placed into a sterile 1.5 mL Eppendorf tube with 500 mL of liquid LB media. The tissue was macerated using a sterile pestle. 2) Tissue sections were placed into 2 mL tubes containing 4 beads per tube and 500 mL of LB media. The tissue was macerated using the 2000 Geno/Grinder (SPEX Certiprep, New Jersey USA) for 1 min at setting 1100 strokes per min for stems and root crowns, and 15 sec for leaf tissue. The ground tissue suspension was then was spread on LB agar plates and incubated at 28˚C for 1 to 2 days.

Colony Isolation and DNA Prep

Some tissues samples (mostly leaf) yielded either very few or no isolates. Most stem and root crown tissue produced a range from ten to hundreds of colonies per plate. On plates with large numbers of bacteria, selection of colonies was based on observable differences such as color and colony morphology in order to obtain a representative sampling of the bacteria. Where possible we attempted to select at least 2 - 3 colonies per colony type per plate.

These initial bacterial cultures were sub-cultured on LB agar plates until single, isolated colonies were obtained. Single colonies were selected and grown in LB at 28˚C until turbid (typically 24 hours). Bacterial DNA was extracted by concentrating 1.5 mL of cell suspension via centrifugation for 1 min at 14,000 rpm using a Eppendorf 5417C centrifuge followed by resuspension in 400 uL CLS-TC lysis buffer (MP Biomedicals, Santa Ana, CA) with 0.1 mg/mL Proteinase K and 1mg/mL Lysozyme. Samples were incubated for 10 min at 55˚C, followed by inactivation of Proteinases by incubating 10 min at 80˚C. The DNA was isolated by applying the lysates to QiagenDNeasy Plant Mini Kit purification columns followed by centrifugation for 1 min at 14,000 rpm using an Eppendorf 5417C centrifuge, followed by two washes with 500 mL Buffer AW (Qiagen, Germany), and resuspension in 50 uL EB elution buffer (Qiagen, Germany) from the QiagenDNeasy Plant Mini Kit.

Amplification of 16S Ribosomal ITS Region

The 16S rRNA ITS region was amplified by PCR with primers derived from primer set 1 5'-AGAGTTTGATCCTGGCTCAG-3' and 5'-AAGGAGGTGATCCAGCCGGA-3' [68]. Note: some samples gave mixed products when sequenced, so a second primer pair, primer set 2 5'-AGAGTTTGATYMTGGC-3'

and 5'-TACCTTGTTACGACTT-3' was designed to amplify the ITS region from these bacterial isolates [69]. Most amplicons were ~1402 base pairs in length. The DNA was amplified using 1× HotStarTaq Master Mix (Qiagen, Germany) following the manufacturer's instructions, 20 µL reaction volumes containing with 10 p moles of each primer, 1 µL DNA, and water to volume. Amplification was performed on an MJ Research PTC 200 (BioRad; Hercules, CA) with the following program: initial denaturation at 94˚C for 15 minutes; 35 cycles of 94˚C for 1 min, annealing temperature for primer set 1 was at 60˚C and for primer set 2 at 56˚C for 1 minute, 72˚C for 1 min; and a final extension at 72˚C for 10 min; and then kept at 4˚C until removal.

Amplification was verified by gel electrophoresis and those with multiple bands were run on agarose gels, followed by excision of desired products and purification with Qiagen Gel Purification Kits. The PCR products were run on a 1% TAE agarose gels to analyze purity. Single-band products were purified using Qiagen PCR Purification Kit (Qiagen, Germany); those with multiple bands were run on 1% TAE agarose gels, followed by excision of desired products and purification with Qiagen Gel Purification Kits (Qiagen, Germany). All samples were measured on a Nanodrop for quantity and quality.

Sequencing and Identification

Sanger sequencing was performed at either Oregon State University's Center for Gene Research and Biocomputing, Corvallis, OR or by Eurofins, Louisville, KY. Sequencing primers used were 5'-GTATTACCGCGGCTGCTGG-3' for products amplified with primer set 1, and 5'-ATTACCGCGGCTGCTGG-3' for products amplified with primer set 2. This generated sequences of 433 - 460 base pairs in length. Resulting fasta files were subjected to a Nucleotide-Nucleotide BLAST 2.2.29+ search against the nr/nt database using command line syntax with default settings at National Center for Biotechnology Information (NCBI, GenBank; www.ncbi.nih.gov).

ACC Deaminase Activity Assay

The bacterial isolates were screened for ACC utilization. We followed the protocol described in [70] [71] with the following modifications: Bacteria were first grown on LB plates at 28˚C until colonies were visible, 24 - 48 hours depending on rate of growth of a particular isolate. Selected colonies from the bacterial plates were grown in liquid cultures in LB media to visible turbidity, 24 - 48 hours prior to use in the assay. Transformation bacterium *E. coli* DH5*α* was used as a negative control in addition to the blank sample since it was confirmed to show no ACC utilization via this assay. A *Pseudomonas* strain with known ACC deaminase activity (Joyce Loper USDA-ARS Corvallis Oregon and Ann Kennedy, USDA-ARS Pullman WA) was used as a positive control on all plates. Quantities of the 10-fold dilution of DF-ACC medium [70] [71] culture and ninhydrin reagent were increased on the reaction plates to 80 µl and 160 µl per well respectively for improved results (Q. An, personal communication). Each diluted supernatant was run in duplicate. After visual scoring, absorbance was measured at 595 nm with the Bi-Tek EL808 plate reader.

3. Results and Discussion

Thirty-four different plant samples comprising eight different genera of grasses were collected from various sites along the Oregon coast. Table 1 shows the plant genera and their distribution for each site; four plants collected from Coos Bay, fourteen from Harbor Vista, nine from Bob Creek Wayside, and seven from Yachats. The two most prevalent grasses collected from these locations were *Bromus* and *Festuca*, with ten plants each. *Bromus* was the only genus collected from all four sites. A total of 173 culturable single colony isolates were generated from the various tissue types from 34 grass plants collected along the Oregon coast. DNA was isolated from each of these cultures and a portion of the 16S rRNA ITS region was PCR-amplified and sequenced. The resulting sequences were BLASTed against the nucleotide database collection at National Center for Biotechnology Information. Based on their 16S rRNA ITS sequences, duplicate isolates from each individual plant (same strain originating from the same plant, found in any tissue type) were eliminated from further analysis. This resulted in 133 individual isolates. The 133 bacterial isolates were classified into 36 taxonomic groups based on their 16S rRNA ITS sequences. The classification and distribution (plant genera and locations) of these bacterial groups are listed in Table 2.

Based on the BLAST sequences, the isolates were classified and grouped by their lowest common taxonomic rank. Twenty-six were classified into taxonomic groups at the genus level, five were identified to the species level, four others could only be assigned to the class, order or family taxonomic rank, and three bacterial isolates could not be defined. (**Supplementary** Table S1, all 133 bacterial isolates are listed with their hit descriptions and sequence id information at the end of the manuscript). Over 50% of the total bacteria isolates are from just five taxonomic groups. *Pseudomonads* were the most predominant bacteria isolated from these grasses, making up 20.3% of the total isolates, followed by *Curtobacterium* and *Microbacterium* each at 8.2%, *Bacillus* at 7.5% and *Xanthomomas* at 6%, whereas 47% (17 of 36) of the taxonomic groups contained only a single

Table 1. Plant collection and distribution.

Plant Genus	Bob Creek	Coos Bay	Harbor Vista	Yachats	Total Plants	Total Isolates	ACC+
Agrostis			1	2	3	3	1
Ammophilia		1	1		2	9	1
Bromus	3	1	4	2	10	49	5
Descampsia				1	1	7	
Festuca	3	1	6		10	41	7
Hordeum		1			1	7	
Lolium	1				1	2	
Phalaris	2		2	2	6	15	
Totals:	9	4	14	7	34	133	14

Table 2. Classification and distribution of bacterial isolates.

Taxonomic Group*		Total # isolates	Unique Seq	ACC+	Location(s)**	Plant Genus***
Agreia	G	4	2		H	B F P
Achromobacter	G	1	1	1	C	Am
Actinomycetales bacterium	O	2	2		C Y	B D
Aeromicrobium	G	1	1		B	B
alpha proteobacterium	C	2	2		Y	Ag B
Alter erythrobacter	G	1	1	1	Y	Ag
Agrobacterium	G	1	1		C	B
Bacillus	G	10	6		C B H Y	Ag Am B F P
Betaproteobacteria bacterium	C	1	1		Y	P
Bordetella	G	1	1		C	B
Brachybacterium tyrofermentans	Sp	1	1		H	F
Brevundimonas	G	1	1		Y	B
Caryophanon	G	1	1		H	F
Chryseobacterium	G	1	1		Y	D
Clavibacter michiganensis	Sp	1	1		H	B
Curtobacterium	G	11	4		B C H Y	B D F P
Enterobacteriaceae bacterium	F	1	1		B	F
Exiguobacterium	G	4	3		H Y	B F
Flavobacterium	G	3	3		B H Y	B F P
Frigoribacterium faeni	Sp	3	3		B C Y	B F
Luteimonas aestuarii	Sp	1	1		H	F
Lysobacter	G	1	1		H	F
Kocuria	G	3	3		B H	F P
Microbacterium	G	11	9		B C H Y	Am B D F H P
Oerskovia turbata	Sp	1	1		Y	B
Pantoea	G	5	5		B C	B F
Plantibacter	G	7	2		C H Y	B D H P
Pseudomonas	G	27	13	10	B C H Y	Am B D F H P
Ralstonia	G	2	2		B C	Am P
Rhizobium	G	4	4	1	B H Y	Am F P
Rhodococcus	G	2	2	1	C Y	B P
Roseomonas	G	1	1		C	H
Sphingomonas	G	1	1		B	B
Stenotrophomonas	G	5	4		H Y	Am B F P
Uncultured bacterium	nd	3	3		B C H	Am F P
Xanthomonas	G	8	5		B C H	B F H L
Total Taxa: 36		133	94	14		

*Taxonomic Group: C = Class; O = Order; F = Family; G = Genus; Sp = Species nd = not determined; **Location: B = Bob Creek Wayside; C = Coos Bay; H = Harbor Vista; Y = Yachats; ***Plant Genus: Ag = Agrostis; Am = Ammophilia; B = Bromus; D = Descampsia; F = Festuca; H = Hordeum; L = Lolium; P = Phalaris.

isolate. A survey of endophytic bacteria isolated from dune grasses collected from the Oregon Coast also found that that *Pseudomonads* were the most prevalent microorganism [29]. In addition, in a study of endophytic bacteria isolated from *Lolium perenne* plants growing in hydrocarbon contaminated soil, *Pseudomonas*, *Bacillus* and *Curtobacterium* were the most prevalent bacteria isolated [49]; and in poplar grown under field conditions, the most abundant genera among the isolated bacterial endophytes were *Pseudomonas* and *Curtobacterium* [72]. Furthermore we found that potentially pathogenic strains of bacteria were present in some of the grasses we collected along the Oregon Coast, such as *Clavibacter michiganensis* and members of the genus *Xanthomonas*. This is not surprising since these types of pathogens infect the surface of the plant and can live inside the plant, so they would have survived the sterilization treatments.

Sixty-seven percent of the bacteria were isolated from root crown tissue, 23% were derived from the stem, and only 10% came from leaf tissue (**Table 3**). Note, in **Table 3**, three plants had the same isolate in two different tissue types, which added three additional isolates to the overall total. This is not surprising, since most colonization of plants occur initially via the roots, and in most plants the roots have the highest number of endophytes when compared other parts of plant. [15] [16] [73] [74]. Not surprisingly, the genera containing the largest number of isolates, *Pseudomonads*, *Curtobacterium*, *Microbacterium* and *Bacillus*, were found in grasses at all the sites, while 19 taxonomic groups were isolated from plants found at only one of the locations. No taxonomic group had members isolated from all the eight plant genera. Two of the most predominant genera, *Pseudomonas* and *Microbacterium*, were found in six of the eight plant genera. Bacteria from the taxonomic groups, *Actinomycetales bacterium*, *Flavobacterium*, *Frigoribacterium faeni*, *Ralstonia*, *Rhodococcus* and *Uncultured bacterium* with two or three isolates each, had each isolate originating from different locations. All but *Frigoribacterium faeni* were isolated from different plant genera as well. *Agrostis* had the lowest number of isolates per plant (one each), but all the isolates were from different genera, *Alpha proteobacterium*, *Alter erythrobacter* and *Bacillus*, while the only *Lolium* plant yielded only two isolates belonging to *Xanthomonas* genus. A single *Bromus* plant collected from the Coos Bay site yielded fourteen distinct isolates belonging to ten different taxonomic groups; similarly a single *Bromus* plant from the Yachats site generated eleven different isolates from seven different taxonomic groups. The only *Descampsia* plant collected yielded seven different isolates from six different taxonomic groups.

Table 3. Tissue distribution of bacterial isolates.

Tissue	Total isolates
Root Crown	91
Stem	31
Leaf	14
Total	136

Table 4. Distribution of bacterial isolates by collection site.

Location	Total Isolates	Total Plants	Avg. # Isolates/Plant	Total Taxa
Bob Creek Wayside	28	9	3.1	15
Coos Bay	30	4	7.5	16
Harbor Vista	43	14	3.1	18
Yachats	32	7	4.6	18

Interestingly, while fewer plants were collected at Coos Bay, they displayed a much higher average number of isolates per plant (7.5, **Table 4**) than plants from other areas (3.1 - 4.6 isolates/plant). However this higher number of isolates per plant from grasses collected from Coos Bay was similar to the findings for fungal endophytes isolated from these same plants [67]. Despite differences in the total number of isolates per location as well as the total number of plants per location, all four locations yielded a similar number of taxonomic groups per locale; from 15 at Bob Creek Wayside, 16 at Coos Bay and 18 for both Harbor Vista and Yachats. These numbers suggest that plants collected from Coos Bay appear to have on average a higher number of isolates per plant, but also a greater diversity of bacteria than found in plants from the other three sites. One can speculate why this would be, the Coos Bay site was more remote and there were no cultivated grasses growing in close proximity to where the plants were collected. This was not the case at the other sites, where some of the samples collected could have been escapes from cultivated grasses, from nearby home sites, recreational areas, or erosion-control landscaping. This could potentially affect the types of endophytes and grasses isolated from each area. Furthermore, while the average number of isolates per plant provides some insight into the diversity present at a particular location, it still is only a rough approximation. Since the number of bacteria from some tissue types in some plants could yield hundreds of bacteria per plate. A particular bacterial plate could yield hundreds of colonies and only a representative sample from each plate was selected. Furthermore the bacteria isolated were only those that were culturable, therefore these numbers by no means represent the totality or diversity of bacteria that may be present in any of the plants collected.

In order to determine the number of unique isolates identified in our study, we aligned and compared the partial 16S rRNA ITS sequences in each taxonomic group for the 133 bacterial isolates. Based on this analysis, we identified potential identical isolates present in different plants in nine of the 36 taxonomic groups (**Table 5(a)** and **Table 5(b)**). Each unique sequence was assigned an arbitrary letter within a taxonomic group. For example, in *Bacillus*, there were ten total isolates but only six unique sequences. In sequence groups A-E there was only a single isolate, while in group F, five isolates had the same ITS sequence but they were isolated from different plants (see **Supplemental Table S1**). **Table 5(a)** and **Table 5(b)** summarize only the sequence groups from each taxonomic group that have more than one isolate. By eliminating these duplicate isolates, the total number of unique bacterial isolates was reduced to 94. For example in

Table 5. (a) Distribution of the same bacteria isolates found in different plants by location; (b) Distribution by plant genera containing the same bacteria isolates.

(a)

Taxonomic Group	Seq Group	Bob Creek*	Coos Bay	Harbor Vista*	Yachats*
Agreia pratensis	B			3	
Bacillus	F	1	1	2	1
Curtobacterium	D	4	2	1	1
Exiguobacterium	C				2
Microbacteriaceae	F		1		1
Plantibacter	B		1	2	3
Pseudomonas	B	1		3 (3)	1 (1)
Pseudomonas	C	2	1	1	1
Pseudomonas	D			1 (1)	2
Pseudomonas	H	1 (1)		1 (1)	1 (1)
Pseudomonas	M		1	1 (1)	1
Stenotrophomonas	D			1	1
Xanthomonas	E	1	1	1	

Letter designates isolate with same ITS Sequence but isolate found in a different plant. Numbers represent the # of isolates found with that particular sequence. *The number in parenthesis represents the # of isolates displaying ACC deaminase activity.

(b)

Taxonomic Group	Seq Group	*Agrostis*	*Ammophilia*	*Bromus**	*Descampsia*	*Festuca**	*Hordeum*	*Lolium*	*Phalaris*
Agreia pratensis	B			1		1			1
Bacillus	F	1		2		1			1
Curtobacterium	D			3		4			1
Exiguobacterium	C			2					
Microbacteriaceae	F			1	1				
Plantibacter	B			3	1		1		1
Pseudomonas	B			2 (2)		3 (2)			
Pseudomonas	C		1	1	1	2			
Pseudomonas	D			2		1 (1)			
Pseudomonas	H			2 (2)		1 (1)			
Pseudomonas	M			1		1 (1)			1
Stenotrophomonas	D			1		1			
Xanthomonas	E					1	1	1	

Letter designates isolate with same ITS Sequence but isolate found in a different plant. Numbers represent the # of isolates found with that particular sequence. *The number in parenthesis represents the # of isolates displaying ACC deaminase activity.

the genus *Pseudomonas* there were 27 individual isolates, but after aligning their ITS sequences, it was determined that only 13 of the isolates were unique. Of the 13 unique bacterial isolates, five of them included a number of isolates with identical sequences, even though they were found in different plants, either from

the same site or in different locations (Table 5(a)). Specific bacterial isolates from the genera *Bacillus*, *Curtobacterium* and one from *Pseudomonas* were found in plants at all four sites (Table 5(a)). While *Bacillus* F, *Plantibacter* B and *Pseudomonas* C isolates were found in four different plant genera. It should be noted that the four collection sites were distributed along a 75 mile stretch of the Oregon coast, ranging from Coos Bay to Yachats. These data give some insight into how widespread or common some of these bacterial endophytes are in plants along the Oregon coast, and how amenable a particular isolate might be at colonizing different plant species. Surprisingly, one of the isolates from *Pseudomonas* seq group B, isolated from Bob Creek Wayside did not display ACC-deaminase activity (Table 5(a)). It is possible that it was only a closely related strain to the other members of the group, but could not be distinguished from them since only a partial sequence (433 - 460 bp) for the 16S rRNA region was used for its identification; or it is possible that this particular isolate had a potential mutation that affected its ability to produce an active enzyme. It should be noted that using partial sequence information can make it difficult to definitively identify a species [75].

Identification of Isolates displaying ACC Deaminase Activity

The main goal of this study was to isolate and identify endophytic bacteria that can increase the growth and persistence of plants when subjected to salt stress. Growth-promoting bacteria use a variety of mechanisms to improve the growth potential of the plant in different environments and stresses [8] [9] [10] [16] [24] [25]. One of the key mechanisms utilized by plant growth-promoting bacteria is the lowering of plant stress ethylene levels by the enzyme 1-amino-cyclopropane-1-carboxylate (ACC) deaminase [25] [40]. Under stress, plants produce higher levels of ethylene, which can inhibit their growth and reduce overall health [38] [39]. The ACC-deaminase enzyme cleaves the ethylene precursor, ACC, thereby decreasing ethylene levels in the plant. This in turn promotes growth and improves the plant's performance under stress [25] [40]. Furthermore, in addition to the modulation of ethylene levels, the synergistic interactions between ethylene and auxin during stress also regulate plant and root growth [25]. These ACC-deaminase containing bacteria not only promote plant growth, they also have been shown to improve the plant's tolerance to salt stress and other biotic and abiotic stresses including: drought, flower wilting, flooding, metals, organic contaminants, and pathogens [8] [9] [25]. Therefore endophytic bacteria possessing ACC deaminase activity may provide tolerance to more than one type of stress, which could improve the plant's adaptability to an ever-changing environment.

Realizing the value of this particular enzyme and its link to stress tolerance, we tested our endophytic bacterial isolates for ACC-deaminase activity [70] [71]. Our analysis found that 14 of 133 isolates tested positive for ACC deaminase activity (Table 6). Five different taxonomic groups had a positive isolate, with *Pseudomonas* containing 10 of the 14 positive isolates; *Achromobacter*, *Altererythrobacter*, *Rhodococcus* and *Rhizobia* each had one positive isolate.

Table 6. Bacterial isolates displaying ACC deaminase activity.

Taxonomic Group	Seq Group*	Seq ID**	Location	Plant Genus	Tissue***
Achromobacter	A	CB3BRC-3B	Coos Bay	*Ammophilia*	RC
Altererythrobacter	A	YH7SS-1A	Yachats	*Agrostis*	S
Rhizobium	C	BS7RC-2A	Bob Creek Wayside	*Festuca*	RC
Rhodococcus	B	CB2ALF-2A	Coos Bay	*Bromus*	L
Pseudomonas	B	HV11RC-1A	Harbor Vista	*Bromus*	RC
Pseudomonas	B	YH3RC-7A	Yachats	*Bromus*	RC
Pseudomonas	B	HV9RC-3A	Harbor Vista	*Festuca*	RC
Pseudomonas	B	HV6RC-2A	Harbor Vista	*Festuca*	RC
Pseudomonas	D	HV13RC-1A	Harbor Vista	*Festuca*	RC
Pseudomonas	E	BS5RC-9A	Bob Creek Wayside	*Festuca*	RC
Pseudomonas	H	BS5RC-8A	Bob Creek Wayside	*Festuca*	RC
Pseudomonas	H	HV14SS-3A	Harbor Vista	*Festuca*	S
Pseudomonas	H	YH3RC-12C-A	Yachats	*Bromus*	RC
Pseudomonas	M	HV11RC-3A	Harbor Vista	*Bromus*	RC

*Letter designates isolate with same ITS Sequence but isolate found in a different plant. **Seq ID: Location, Plant #, TissueType - Isolate #. ***RC = Root Crown; S = Stem; L = Leaf.

While *Pseudomonas* was by far the most prevalent genus found in our study, this is not surprising since there have been many reports of different growth-promoting *Pseudomonas* species that display ACC-deaminase activity and that have been shown to improve growth and stress tolerance [8] [9] [23]. An ACC-deaminase-active *Pseudomonas fluorescens* strain mediates saline resistance in groundnut plants [76]. Similarly, *Pseudomonas putida* containing ACC-deaminase was shown to improve growth and yield of wheat under salt-stressed conditions [77]. In addition strains of ACC deaminase-producing *Pseudomonas fluorescens* and *Pseudomonas putida* were shown to improve growth and alleviate the effects of salt stress in canola [78]. Furthermore ACC-deaminase-containing *Pseudomonas syringae* and *Pseudomonas fluorescens* improved the growth of maize plants under high-salt conditions [79]. Additionally, studies have demonstrated the role of various ACC deaminase-containing *Pseudomonas* bacteria in improving growth and tolerance to plants subjected to salt stress. These include *Pseudomonas syringae* and *Pseudomonas fluorescens* in mung bean [80], *Pseudomonas putida* and *Pseudomonas fluorescens* in wheat [81], and *Pseudomonas mendocina* containing a plasmid carrying the gene encoding ACC deaminase in tomato [82]. In one recent study, ACC-deaminase-containing bacterial endophytes, *Pseudomonas fluorescens* and *Pseudomonas migulae*, and their ACC-deaminase-deficient mutants were compared for their ability to improve growth of tomato plants under salt stress. The ACC-deaminase-active strains improved growth, while their mutant counterparts did not. Since the only difference between the wild-type and mutant bacterial endophytes was ACC deaminase activity, this indicated that ACC-deaminase was directly

responsible for the improved growth to salt-stressed tomato plants [83]. *Pseudomonas* strains have also been shown to be effective biocontrol agents against pathogenic fungi and bacteria [51] [55] [83] [84] [85], as well as other types of stresses. For example, in *Lolium perenne* plants growing in hydrocarbon- contaminated soils, a number of ACC-deaminase containing *Pseudomonas* strains were isolated and shown to possess a variety of growth-promoting characteristics [49]. In addition an ACC-containing strain of *Pseudomonas fluorescens* stimulated plant growth and promoted heavy metal uptake in rape [41].

Since ACC deaminase positive *Pseudomonas* strains have been shown to alleviate salinity stress, and our *Pseudomonas* isolates (**Table 6**) are from plants growing in high saline environments on the Oregon coast, these isolates are good candidates for improving growth and salt stress tolerance in other forage and turf grasses, as well as other crop species. The ACC-deaminase positive *Pseudomonas* isolates B and H are of particular interest, since they were found in multiple plant genera, indicating their ability to infect different plant species. They were also found in more than one location, which may mean that they are more common in Oregon (**Table 5**).

There are a great number of different bacteria species from a large number of genera that possess ACC deaminase activity [8] [9] [23] [49] [62] [86]. In our analysis, we found four other genera in addition to *Pseudomonas* that possessed ACC deaminase activity. However, they are not as well characterized for their growth and stress tolerance enhancing capabilities. In the genus *Achromobacter*, the endophytic bacterium *Achromobacter xylosoxidans* was shown to promote growth in wheat [87]. The endophytic bacterium *Achromobacter xylosoxidans*, was also shown to improve phytoremediation of phenolic pollutants in *Arabidopsis* and in vetiver grass [46] [47]. A different strain of *Achromobacter xylosoxidans* increased growth and resistance to blast fungus in rice [88]. Most relevant to our study, the plant-growth-promoting bacteria ACC deaminase containing *Achromobacter piechaudii* was shown to improve growth of tomato and pepper when subjected to drought [89], and also improved the growth of tomato when subjected to salt stress [90]. This demonstrated that the same ACC-deaminase-containing bacteria has the potential to improve tolerance to more than one type of stress. There also have been a number of endophytic *Rhodococcus* strains that have been identified to possess ACC deaminase activity and other common attributes associated with plant growth promoting bacteria [23] [49], however they have not been extensively study for their potential to improve plant growth under stress. Interestingly, the first report of *Rhizobium* strains containing ACC deaminase activity resulted from an examination of 13 *Rhizobium* strains that were capable of nodulating different legumes [91]. There were also 27 *Rhizobium* strains having ACC deaminase activity identified from a survey of 233 *Rhizobia* strains collected from sites across Canada [86]. To our knowledge, our study is the first report of ACC deaminase activity reported in the genus *Altererythrobacter.*

In our study, we found over 36 different taxonomic groups of endophytic

bacteria, of which only five taxa contained strains that possessed ACC deaminase activity. Of the remaining bacterial isolates, some may not normally exist as endophytes, but may have been opportunistic in gaining access to the interior of the plant as a result of damage to the plants exterior surfaces. After gaining access these bacteria may have migrated to other locations within the plant. Some of the bacteria may be pathogens that infect the surface as well as the interior of the plant. Some may be benign symbionts, which provide no real benefit to their host. In addition to the ACC deaminase containing bacterial strains identified in this study, there most likely are other bacteria in our collection that are providing benefits to the host plant in this austere and harsh environment. *Bacillus* is one of the genera that may yield potential beneficial endophytes. While there have been *Bacillus* strains displaying ACC deaminase activity [23] [49] [92], in our survey none of the ten *Bacillus* isolates tested positive for ACC deaminase activity. However *Bacillus* species have been shown to improve growth in plants in other ways, such as through the release of volatile compounds [93] [94], the activation of signaling pathways [52], or the production and manipulation of phytohormones [34] [95]. Furthermore, a number of different strains of *Bacillus* have been shown to be good biological control agents against pathogens [50] [52] [96] [97]. Recently it was shown that, when introduced into *Brachypodium distachyon*, an endophytic strain of *Bacillus subtilis* improved growth and alleviated the effects of drought stress [98]. Another interesting aspect to this study was how efficiently it colonized the plant. The bacterium was able to spread systemically throughout the plant, establish itself in the roots, aerial plant tissues and organs, and was vertically transmitted to seeds.

The long-term utility of a beneficial organism in an agricultural setting depends on the ease of establishing the microorganism's association with the target plant, its persistence under different environmental conditions, and the length of time the organism will be able to provide a benefit to the plant. The benefit of utilizing an endophyte over a soil-based bacterium is that an endophyte is already established in the plant, and if seed-borne it can be maintained, and provide continued benefits to the next generation with no additional inputs. Endophyte infected plants require less inputs since they are already in the plant or seed. However, when using soil-based bacterium, pretreatment of soil, seed or seedlings would be necessary prior to planting. In an agricultural setting endophytes provide benefits directly to the host plant, whereas soil associated bacteria could also have the potential to improve the growth of weeds in the field. Furthermore, endophytic bacteria would be less prone to effects of soil composition, competition from other microorganisms present in the soil, and environmental effects that cause changes to the composition and dynamics of the microbiome where the crop is being grown, than their rhizospheric counterparts.

4. Conclusion

Grasses are a critical component for livestock production and maintaining the food supply. Grasses also provide feedstock for bioenergy, erosion control, habi-

tat for biologically diverse animals, buffers for watersheds, sinks for carbon sequestration and valuable landscape/recreational surfaces. As water resources diminish throughout the US and worldwide due to overuse and climate change, innovative approaches such as the discovery, development, and utilization of novel endophytes will be necessary to improve water stress tolerance in grasslands and other crop species. The purpose of this study was to identify new bacterial endophytes that exist in Oregon that could potentially improve grass stress tolerance without using direct genetic modification, and without introducing foreign or exotic species into this diverse agricultural production area. Our future research will be directed at investigating the ability of the ACC deaminase positive isolates identified in this study to improve growth in grass species and crop plants when subjected to salt and drought stress. In addition, since endophytic bacteria can utilize other mechanisms to improve growth, research will also focus on investigating the ACC deaminase positive isolates and other selected isolates for phytohormone production, bioactive compounds, phosphate solubilization, nitrogen fixation, as well as their utility as biocontrol agents against pathogens. The discovery and utilization of bacterial endophytes as a biological resource have the potential to improve yield and persistence, as well as increase the adaptability of grasses and other crops grown in diverse environments and to meet the challenges associated with an unpredictable climate.

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Supplemental Tables

Table S1. Hit descriptions of isolates.

Hit Description	SEQ Designation*	Plant Species	Location	Tissue**	Seq ID***	Seq Length	Alignment Length	% Identical Matches	Hit ID
Achromobacter sp.	A	Ammophilia	Coos Bay	RC	CB3BRC-3B	460	437	99.54	gi|565666205|emb|HG324052.1|
Actinomycetales bacterium	A	Descampsia	Yachats	RC	YH4RC-3A	460	436	98.85	gi|222427416|dbj|AB461704.1|
Aeromicrobium sp	A	Bromus	Bob Creek Wayside	RC	BS6RC-1A	460	441	100	gi|322162258|gb|JF176853.1|
Agreia pratensis	A	Bromus	Harbor Vista	RC	HV12RC-4A	460	437	100	gi|342067998|gb|JF632813.1|
Agreia pratensis strain	B	Phalaris	Harbor Vista	RC	HV2RC-2A	460	444	99.77	gi|118193424|gb|EF010578.1|
Agreia pratensis strain	B	Bromus	Harbor Vista	S	HV12SS-1A	460	436	100	gi|219878321|ref|NR_025460.1|
Agreia bicolorata strain	B	Festuca	Harbor Vista	RC	HV13RC-4A	460	445	99.55	gi|322182817|gb|JF197412.1|
Agrobacterium tumefaciens strain	A	Bromus	Coos Bay	RC	CB2BRC-3A	433	413	99.76	gi|151303412|gb|EF620461.1|
Alphaproteobacterium	A	Bromus	Yachats	RC	YH3RC-6A	443	430	99.53	gi|411113074|gb|JQ387405.2|
Alphaproteobacterium	B	Agrostis	Yachats	RC	YH5RC-3A	460	432	98.61	gi|333122829|gb|JF745377.1|
Altererythrobacter marenisis	A	Agrostis	Yachats	S	YH7SS-1A	449	422	98.34	gi|636632492|gb|KJ549198.1|
Arthrobacter sp.	A	Bromus	Coos Bay	RC	CB2BRC-4B	460	440	99.77	gi|332002558|gb|JF683267.1|
Bacillus sp.	A	Phalaris	Harbor Vista	RC	HV1RC-2A	460	453	99.34	gi|347812460|gb|HM584282.1|
Bacillus sp.	B	Bromus	Bob Creek Wayside	RC	BS3RC-2A	460	453	98.9	gi|347812460|gb|HM584282.1|
Bacillus simplex strain	C	Ammophilia	Coos Bay	RC	CB3ARC-1A	460	441	100	gi|310780859|gb|HQ432812.1|
Bacillus safensis	D	Festuca	Harbor Vista	L	HV9LF-3A	460	446	100	gi|340003212|emb|FR877571.1|
Bacillus megaterium strain	E	Bromus	Coos Bay	L	CB2BLF-3A	460	447	99.78	gi|449040652|gb|KC414707.1|
Bacillus sp.	F	Bromus	Coos Bay	L	CB2BLF-1A	460	452	99.78	gi|347812460|gb|HM584282.1|
Bacillus sp.	F	Festuca	Bob Creek Wayside	RC	BS5RC-13A	460	451	99.33	gi|347812460|gb|HM584282.1|
Bacillus sp.	F	Agrostis	Yachats	RC, L	YH7LF-1A	460	452	99.56	gi|347812460|gb|HM584282.1|
Bacillus sp.	F	Phalaris	Harbor Vista	RC	HV1RC-1A	460	450	99.56	gi|485650999|gb|KC545293.1|
Bacillus sp.	F	Bromus	Harbor Vista	RC	HV5RC-1A	460	449	99.11	gi|485650999|gb|KC545293.1|
Uncultured Betaproteobacteria bacterium	A	Phalaris	Yachats	S	YH6SS-1A	460	445	99.1	gi|238000899|emb|CU922693.1|
Uncultured bordetella sp.	A	Bromus	Coos Bay	RC	CB2BRC-5A	460	138	85.51	gi|346988245|gb|JN590663.1|
Brachybacterium tyrofermentans strain	A	Festuca	Harbor Vista	RC	HV6RC-2A	460	449	98	gi|219846680|ref|NR_026272.1|
Brevundimonas sp.	A	Bromus	Yachats	RC	YH3RC-9A	445	430	99.3	gi|224027500|emb|AM988991.1|
Caryophanon sp.	A	Festuca	Harbor Vista	L	HV9LF-1A	460	443	99.77	gi|14537944|gb|AF385535.1|
Chryseobacterium		Descampsia	Yachats	RC	YH4RC-1A	460	436	100	gi|322163911|gb|JF178506.1|

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Clavibacter michiganensis	A	Bromus	Harbor Vista	S	HV8SS-1A	460	437	100	gi|444304176|ref|NR_074600.1|
Uncultured *Curtobacterium* sp.	A	Descampsia	Yachats	S	YH4SS-2A	460	449	99.55	gi|545341513|gb|KF504745.1|
Curtobacterium flaccumfaciens	B	Festuca	Coos Bay	S	CB4RCSS-1A	460	442	97.74	gi|602152779|emb|HG934367.1|
Curtobacterium oceanosedimentum strain	C	Bromus	Yachats	RC	YH3RC-12A	460	442	99.55	gi|559795249|ref|NR_104839.1|
Curtobacterium flaccumfaciens	D	Bromus	Coos Bay	RC	CB2BRC-2B	460	440	100	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Festuca	Coos Bay	S	CB4RCSS-3A	460	442	99.77	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Bromus	Bob Creek Wayside	RC	BS3RC-1A	460	448	99.78	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Festuca	Bob Creek Wayside	RC	BS5RC-1A	460	440	100	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Festuca	Bob Creek Wayside	RC, S	BS7RC-1A	460	435	100	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Phalaris	Bob Creek Wayside	S	BS9SS-1B	460	438	100	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Festuca	Harbor Vista	S	HV14SS-2A	460	450	99.78	gi|602152779|emb|HG934367.1|
Curtobacterium flaccumfaciens	D	Bromus	Yachats	RC	YH3RC-5A	460	444	100	gi|602152779|emb|HG934367.1|
Enterobacteriaceae bacterium	A	Festuca	Bob Creek Wayside	S	BS7SS-4A	460	432	99.77	gi|399764347|gb|JX067714.1|
Exiguobacterium sibiricum strain	A	Festuca	Harbor Vista	S	HV14SS-1A	460	422	86.26	gi|559101922|gb|KF815556.1|
Exiguobacterium sp.	B	Bromus	Harbor Vista	RC	HV11RC-2A	460	440	100	gi|619856028|gb|KJ456597.1|
Exiguobacterium sibiricum strain	C	Bromus	Yachats	RC	YH3RC-12B-A	460	442	99.77	gi|559101922|gb|KF815556.1|
Exiguobacterium undae strain	C	Bromus	Yachats	RC	YH3RC-3A	460	435	100	gi|545599219|gb|KF555609.1|
Flavobacterium sp.	A	Festuca	Bob Creek Wayside	RC	BS8RC-2A	460	441	99.55	gi|189231650|emb|FM161717.1|
Flavobacterium sp.	B	Bromus	Harbor Vista	S	HV11SS-1A	460	439	99.54	gi|224027433|emb|AM988924.1|
Flavobacterium sp.	C	Phalaris	Yachats	S	YH6SS-3A	460	442	99.32	gi|125988135|emb|AM492721.1|
Frigoribacterium faeni	A	Festuca	Coos Bay	S	CB4RCSS-2A	460	447	98.66	gi|590121420|emb|HE716910.1|
Frigoribacterium faeni	B	Bromus	Bob Creek Wayside	S	BS3SS-3A	460	447	98.66	gi|590121420|emb|HE716910.1|
Frigoribacterium faeni	C	Bromus	Yachats	RC	YH3RC-11A	460	438	100	gi|590121420|emb|HE716910.1|
Kocuria marina strain	A	Phalaris	Harbor Vista	RC	HV2RC-1A	460	447	100	gi|572540661|gb|KF777377.1|
Kocuria palustris	B	Festuca	Bob Creek Wayside	RC	BS5RC-12A	460	447	99.78	gi|590121451|emb|HE716941.1|
Kocuria palustris	C	Festuca	Harbor Vista	L	HV6LF-1A	460	442	98.42	gi|296963424|gb|HM269829.1|
Luteimonas aestuarii strain	A	Festuca	Harbor Vista	RC	HV4RC-3A	460	440	99.77	gi|583842931|gb|KF876901.1|
Uncultured *Lysobacter* sp.	A	Festuca	Harbor Vista	RC	HV14RC-3A	460	450	99.78	gi|307713682|gb|HM438532.1|
Microbacteriaceae bacterium	A	Bromus	Coos Bay	RC	CB2ARC-4A	460	447	84.79	gi|399764363|gb|JX067730.1|
Microbacterium sp.	B	Hordeum	Coos Bay	S	CB5SS-3A	460	448	97.99	gi|341867097|gb|JN196543.1|
Microbacteriaceae bacterium	C	Bromus	Coos Bay	L	CB2ALF-1A	460	442	95.48	gi|383850150|gb|JQ229710.1|

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Microbacterium sp.	D	Festuca	Harbor Vista	RC	HV4RC-1A	460	444	99.32	gi|341867097|gb|JN196543.1|
Uncultured bacterium	E	Descampsia	Yachats	RC	YH4RC-4A	460	438	100	gi|322199775|gb|JF214370.1|
Microbacteriaceae bacterium	F	Bromus	Coos Bay	RC	CB2BRC-6A	460	446	99.55	gi|399764363|gb|JX067730.1|
Microbacteriaceae bacterium	F	Descampsia	Yachats	S	YH4SS-3A	460	448	99.55	gi|399764363|gb|JX067730.1|
Microbacterium sp.	G	Ammophila	Harbor Vista	L	HV7LF-1A	460	451	99.33	gi|341867097|gb|JN196543.1|
Microbacterium sp.	H	Phalaris	Bob Creek Wayside	S	BS4SS-1A	460	443	98.87	gi|480360049|gb|KC768764.1|
Microbacterium phyllosphaerae	I	Bromus	Yachats	RC	YH2RC-4A	460	445	99.33	gi|110835896|emb|AM268326.1|
Microbacterium phyllosphaerae strain	I	Bromus	Harbor Vista	S	HV11SS-3A	460	436	100	gi|387568139|gb|JQ684246.1|
Oerskovia turbata strain	A	Bromus	Yachats	RC	YH3RC-10A	460	440	100	gi|118193595|gb|EF010749.1|
Pantoea agglomerans strain	A	Festuca	Bob Creek Wayside	S	BS8SS-3A	460	449	98.22	gi|440658039|gb|KC178591.1|
Pantoea agglomerans strain	B	Festuca	Bob Creek Wayside	S	BS8SS-4A	460	446	99.78	gi|440658039|gb|KC178591.1|
Pantoea ananatis	C	Festuca	Bob Creek Wayside	S	BS7SS-1A	460	446	97.76	gi|297373350|emb|FN691983.1|
Uncultured *Pantoea* sp.	D	Bromus	Coos Bay	RC	CB2ARC-3A	460	427	96.02	gi|545342379|gb|KF505611.1|
Pantoea sp.	E	Bromus	Coos Bay	RC	CB2ARC-2A	460	360	83.06	gi|353528968|gb|JN697999.1|
Plantibacter sp.	A	Bromus	Harbor Vista	RC	HV12RC-1A	460	443	85.1	gi|289185531|gb|GU726494.1|
Plantibacter flavus	B	Phalaris	Yachats	RC	YH1RC-2A	460	438	100	gi|590121428|emb|HE716918.1|
Plantibacter flavus	B	Bromus	Yachats	RC	YH2RC-2A	460	451	100	gi|590121428|emb|HE716918.1|
Plantibacter flavus	B	Descampsia	Yachats	RC	YH4RC-2A	362	332	98.19	gi|590121428|emb|HE716918.1|
Plantibacter sp.	B	Hordeum	Coos Bay	RC	CB5RC-3A	460	448	100	gi|384070530|emb|HE662660.2|
Plantibacter sp.	B	Bromus	Harbor Vista	RC	HV12RC-2A	460	440	100	gi|384070530|emb|HE662660.2|
Plantibacter sp.	B	Bromus	Harbor Vista	S	HV11SS-2A	460	445	99.33	gi|469665559|gb|KC355358.1|
Pseudomonas sp.	A	Festuca	Bob Creek Wayside	RC	BS5RC-7A	460	420	91.43	gi|576735116|gb|KJ140081.1|
Pseudomonas sp.	B	Festuca	Bob Creek Wayside	S	BS8SS-1A	460	444	99.77	gi|189231399|emb|FM161478.1|
Pseudomonas sp.	B	Bromus	Harbor Vista	RC	HV11RC-1A	460	443	99.55	gi|189231399|emb|FM161478.1|
Pseudomonas sp.	B	Bromus	Yachats	RC	YH3RC-7A	460	442	100	gi|189231399|emb|FM161478.1|
Pseudomonas sp.	B	Festuca	Harbor Vista	S	HV14SS-3A	460	446	99.55	gi|333774213|emb|FR775123.1|
Pseudomonas sp.	B	Festuca	Harbor Vista	RC	HV6RC-2A	460	451	99.56	gi|636774081|gb|KJ569377.1|
Pseudomonas sp.	C	Festuca	Harbor Vista	RC	HV14RC-2A	460	454	99.78	gi|189231281|emb|FM161360.1|
Pseudomonas sp.	C	Ammophilia	Coos Bay	RC	CB3ARC-3A	460	445	100	gi|189231466|emb|FM161545.1|
Pseudomonas sp.	C	Bromus	Bob Creek Wayside	S	BS2SS-1A	460	448	100	gi|189231466|emb|FM161545.1|
Pseudomonas sp.	C	Descampsia	Yachats	S	YH4SS-1A	460	447	100	gi|189231466|emb|FM161545.1|
Pseudomonas sp.	C	Festuca	Bob Creek Wayside	RC, L	BS8RC-1A	460	444	99.77	gi|346218346|emb|FR727809.1|
Pseudomonas gessardii strain	D	Festuca	Harbor Vista	RC	HV13RC-1A	460	443	100	gi|407280528|gb|JX514410.1|

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Pseudomonas sp.	D	Bromus	Yachats	RC	YH3RC-2A	460	446	99.33	gi|189231353|emb|FM161432.1|
Pseudomonas sp.	D	Bromus	Yachats	RC	YH2RC-1A	460	446	100	gi|189231437|emb|FM161516.1|
Pseudomonas sp.	E	Festuca	Bob Creek Wayside	RC	BS5RC-9A	460	443	97.52	gi|189231373|emb|FM161452.1|
Pseudomonas abietaniphila	F	Ammophilia	Coos Bay	RC	CB3BRC-1B	460	447	99.55	gi|483932156|emb|HF952541.1|
Pseudomonas anguilliseptica	G	Hordeum	Coos Bay	RC	CB5RC-2-1A	460	446	95.29	gi|99644529|emb|AM263523.1|
Pseudomonas sp.	H	Festuca	Bob Creek Wayside	RC	BS5RC-8A	460	448	99.78	gi|189231373|emb|FM161452.1|
Pseudomonas sp.	H	Bromus	Yachats	RC	YH3RC-12C-A	460	447	100	gi|189231373|emb|FM161452.1|
Pseudomonas viridiflava strain	H	Bromus	Harbor Vista	RC	HV11RC-3A	460	444	99.77	gi|584594659|gb|KF898146.1|
Pseudomonas sp. e	I	Hordeum	Coos Bay	RC	CB5RC-2-2B	460	443	98.42	gi|33150179|gb|AY336537.1|
Pseudomonas fulva strain	J	Bromus	Coos Bay	RC	CB2BRC-2A	460	451	99.78	gi|71493091|gb|DQ122353.1|
Pseudomonas anguilliseptica strain	K	Hordeum	Coos Bay	RC	CB5RC-4A	460	451	98.67	gi|406821997|gb|JX177687.1|
Pseudomonas graminis strain	L	Festuca	Bob Creek Wayside	RC	BS5RC-5A	460	444	98.65	gi|343469120|gb|JN390962.1|
Pseudomonas koreensis	M	Bromus	Coos Bay	RC	CB2BRC-1A	460	441	99.77	gi|397174178|emb|HE819905.1|
Pseudomonas moraviensis strain	M	Festuca	Harbor Vista	RC	HV9RC-3A	460	432	98.38	gi|605052202|gb|KJ186949.1|
Uncultured *Pseudomonas* sp.	M	Phalaris	Yachats	RC	YH6RC-1A	460	448	99.55	gi|34333932|gb|AY364050.1|
Uncultured Ralstonia sp.	A	Phalaris	Bob Creek Wayside	RC	BS9RC-1B	460	389	80.98	gi|189305371|gb|EU704960.1|
Uncultured Ralstonia sp.	B	Ammophilia	Coos Bay	RC	CB3BRC-4A	460	295	88.81	gi|209421022|gb|FJ191402.1|
Rhizobium sp.	A	Phalaris	Yachats	RC	YH6RC-3A	460	434	92.4	gi|456371517|gb|KC494332.1|
Rhizobium sp.	B	Festuca	Harbor Vista	RC	HV13RC-3A	460	430	97.21	gi|339521428|gb|JN030539.1|
Rhizobiaceae bacterium	C	Festuca	Bob Creek Wayside	RC	BS7RC-2A	460	443	99.77	gi|114440446|gb|DQ860031.1|
Rhizobiales bacterium	D	Ammophila	Harbor Vista	RC	HV7RC-1A	446	405	99.51	gi|296964105|gb|HM270510.1|
Rhodococcus erythropolis	A	Phalaris	Yachats	RC	YH1RC-1A	460	436	92.66	gi|229002248|dbj|AB499800.1|
Rhodococcus sp	B	Bromus	Coos Bay	L	CB2ALF-2A	460	449	98.89	gi|532529616|gb|KF494637.1|
Uncultured Roseomonas sp.	A	Hordeum	Coos Bay	S	CB5SS-2B	446	424	100	gi|545345985|gb|KF509217.1|
Uncultured Sphingomonas sp.	A	Bromus	Bob Creek Wayside	S	BS3SS-2A	460	439	98.41	gi|557520178|gb|KC907344.1|
Stenotrophomonas rhizophila	A	Ammophila	Harbor Vista	RC	HV7RC-2A	460	453	100	gi|111073240|emb|AM282567.1|
Stenotrophomonas sp.	B	Phalaris	Yachats	RC	YH1RC-4A	460	452	100	gi|333494190|gb|JF345182.1|
Uncultured Stenotrophomonas sp.	C	Bromus	Harbor Vista	RC	HV11RC-4A	460	447	99.55	gi|545339907|gb|KF503139.1|
Stenotrophomonas rhizophila strain	D	Festuca	Harbor Vista	RC	HV9RC-2A	460	446	100	gi|627787876|gb|CP007597.1|
Stenotrophomonas rhizophila strain	D	Bromus	Yachats	RC	YH3RC-4A	460	451	100	gi|627787876|gb|CP007597.1|
Uncultured bacterium clone	A	Phalaris	Bob Creek Wayside	RC	BS4RC-1A	460	417	83.93	gi|381149208|gb|JN835227.1|
Uncultured bacterium clone	B	Festuca	Harbor Vista	RC	HV4RC-2A	460	445	97.53	gi|296963526|gb|HM269931.1|

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Uncultured bacterium clone	C	Ammophilia	Coos Bay	RC	CB3BRC-2A	460	442	99.77	gi|323370055|gb|HQ906067.1|
Xanthomonadaceae bacterium	A	Lolium	Bob Creek Wayside	L	BS1LF-2A	460	354	75.42	gi|311919631|gb|HQ472388.1|
Xanthomonadaceae bacterium	B	Festuca	Harbor Vista	L	HV13LF-1A	460	444	100	gi|322161858|gb|JF176453.1|
Xanthomonadaceae bacterium	B	Festuca	Harbor Vista	RC	HV14RC-3A	460	440	99.77	gi|322161858|gb|JF176453.1|
Pseudoxanthomonas sp.	C	Festuca	Harbor Vista	L	HV14LF-1A	460	444	100	gi|326369573|gb|HQ256838.1|
Xanthomonas arboricola	D	Bromus	Harbor Vista	RC	HV12RC-3A	460	448	100	gi|629510152|dbj|AB911210.1|
Xanthomonas translucens strain	E	Hordeum	Coos Bay	S	CB5SS-1A	460	444	100	gi|443302145|gb|JX976312.1|
Xanthomonas translucens strain	E	Lolium	Bob Creek Wayside	L	BS1LF-1A	460	446	100	gi|443302145|gb|JX976312.1|
Xanthomonas translucens strain	E	Festuca	Harbor Vista	RC	HV13RC-2B	460	446	100	gi|443302145|gb|JX976312.1|

*Seq Designation letter signifies same bacterial isolate (by sequence) but also found in a different plant; **RC = Root Crown, S = Stem, L = Leaf; ***Seq ID: Location, Plant #, TissueType - Isolate #; Green Highlighted positive for ACC deaminase activity.