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# Plant Growth-Promoting Potential of Endophytic Bacteria Isolated from Roots of Coastal Sand Dune Plants

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**Abstract** Endophytic bacteria associated with the roots of coastal sand dune plants were isolated, taxonomically characterized, and tested for their plant growth-promoting activities. Ninety-one endophytic bacterial isolates were collected and assigned to 17 different genera of 6 major bacterial phyla based on partial 16S rDNA sequence analyses. *Gammaproteobacteria* represented the majority of the isolates (65.9%), and members of *Pseudomonas* constituted 49.5% of the total isolates. When testing for antagonism towards plant pathogenic fungi, 25 strains were antagonistic towards *Rhizoctonia solani*, 57 strains were antagonistic towards *Pythium ultimum*, 53 strains were antagonistic towards *Fusarium oxysporum*, and 41 strains were antagonistic towards *Botrytis cinerea*. Seven strains were shown to produce indole acetic acid (IAA), 33 to produce siderophores, 23 to produce protease, 37 to produce pectinase, and 38 to produce chitinase. The broadest spectra of activities were observed among the *Pseudomonas* strains, indicating outstanding plant growth-promoting potential. The isolates from *C. kobomugi* and *M. sibirica* also exhibited good plant growth-promoting potential. The correlations among individual plant growth-promoting activities were examined using phi coefficients, and the resulting data indicated that the production of protease, pectinase, chitinase, and siderophores was highly related.

**Keywords:** Endophytic bacteria, sand dune, *Pseudomonas*, phi coefficient

Coastal sand dune plants harbor a variety of microbes associated with their rhizosphere and roots [16, 21], and these plant-microbe interactions may be vital for sand dune plants, as seen in studies on other types of vegetation, for example, the association between plants and arbuscular mycorrhizal fungi [15, 31], *Frankia* [10], and nitrogen-fixing bacteria [5].

Several mechanisms for plant growth promotion by microorganisms have already been suggested, including the facilitation of nutrient uptake, such as phosphorus, nitrogen fixation for plant use, the sequestration of iron for plants by siderophores, the production of plant hormones like auxins, cytokinins, and gibberellins, the lowering of plant ethylene levels, the excretion of plant growth hormones or antagonization of plant-pathogenic microbes by reducing the iron available to phytopathogens in the rhizosphere, the synthesis of fungal cell-wall-lysing enzymes, and the competition with detrimental microorganisms [8, 11, 12, 17, 18, 24, 25, 28, 29].

The current authors recently reported on the community diversity of heterotrophic bacteria associated with the rhizosphere and roots of two coastal sand dune plants using culture-based methods [21], where species of *Pseudomonas*, *Chryseobacterium*, *Microbacterium*, *Paenibacillus*, and *Acinetobacter* were found to form the majority of the root endophytic bacteria. In a separate report, the analysis of environmental clones revealed that members of *Lysobacter* were predominant in all root samples of sand dune plants [16].

Endophytic bacteria are defined as bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plant, and do not visibly harm the plant [9]. It has recently been demonstrated that bacterial endophytes may also have beneficial effects on host plants, such as growth promotion and biological control of pathogens [6, 24, 30]. Some studies have indicated that the plant growth-promoting potential of endophytes is higher

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than that of rhizosphere microbes [23, 32], but the roles of bacterial endophytes in plant growth are not yet fully understood. The study of root-associated bacteria and their corresponding antagonistic potential is important to understand their ecological roles in the rhizosphere and interaction with plants, and for biotechnological applications, such as the biological control of soilborne plant pathogens. The mechanisms of bacterial antagonism towards plant-pathogenic fungi include competition for nutrients and spaces, as well as the production of antibiotics and fungal cell-wall-degrading enzymes.

In this study, root-endophytic bacteria were isolated from the major sand dune plant species inhabiting the sand dune areas along the west coast of Korea, and screened for the plant growth-promoting potential, where the ultimate aim was to apply them to facilitating the growth of sand dune plants as a basic restoration strategy for vegetation in the sand dune ecosystem.

## MATERIALS AND METHODS

### Sampling and Isolation of Bacteria

Eight plant species were collected from four coastal sand dune areas in the Tae-An area (Baramarae, Sambong, Shindu, and Hagam), Chungnam Province, during August 2003 (Table 1). The root treatment and bacterial isolation were carried out as described previously [21]. R2A and nutrient agar plates were used as the isolation media for all the cultivation experiments, and incubated in the dark for 2 days at 25°C. Based on the colony characteristics, single colonies were selected and stored in 15% glycerol at −80°C for subsequent characterization. The colonies from each plate that could be distinguished based on their morphology were selected and subcultured.

### Identification Using 16S rDNA Sequences

The isolation of genomic DNA from the bacteria and PCR amplification of the 16S rDNA sequences were performed using previously described methods [3]. The sequencing of the partial 16S rDNA was carried out using the service of MicroID Co. (Daejeon, Korea), and the obtained sequences identified using nucleotide-nucleotide BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The individual sequences were identified to the genus level, and also assigned to classes or phyla according to the hierarchical classification system, as suggested by Garrity and Holt [7].

### Antagonistic Activities Against Plant Pathogenic Fungi

To test the antagonistic effects against fungal pathogens, the bacterial isolates were streaked onto one side of a Petri dish (1 cm from the edge) containing a potato dextrose agar (PDA) medium (Difco, Detroit, MI, U.S.A.). A 6-mm mycelial disc from a 7-day-old PDA culture of four fungal pathogens was then placed on the opposite side of the Petri dish perpendicular to the bacterial streak, and the plates incubated at 25°C for 4 days. Thereafter, the zones of inhibition (mm) were recorded by measuring the distance between the edges of the fungal mycelium and the bacterial streak. All the strains were tested in three independent replicates with 4 common plant pathogenic fungi, namely *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium oxysporum*, and *Botrytis cinerea*.

### Production of Secondary Metabolites and Hydrolytic Enzymes

The ability of the bacterial isolates to produce indole-3-acetic acid (IAA) was determined following the microplate method developed by Sawar and Kremer [26], whereas the siderophore production was determined following the method of Sehwyn and Neilands [27]. The production of

**Table 1.** Sand dune plants from which root samples were taken for isolation of endophytic bacteria.

| Plant species (common name) | Sample code[a] | No. of isolates | Dominant group (no. of strains) |
|---|---|---|---|
| 1. *Calystegia soldanella* (beach morning glory) | BA01 | 3 | NA[b] |
| | SB01 | 24 | *Chryseobacterium* spp. (8) |
| | SD01 | 5 | *Microbacterium* spp. (2) |
| 2. *Lathyrus japonica* (wild pea) | HA02 | 1 | NA |
| | SB02 | 3 | *Pseudomonas* spp. (3) |
| 3. *Elymus mollis* (wild rye) | HA03 | 19 | *Pseudomonas* spp. (17) |
| | SB03 | 12 | *Pseudomonas* spp. (12) |
| 4. *Vitex rotundifolia* (roundleaf chastetree) | HA04 | 1 | NA |
| | SB04 | 8 | NA |
| 5. *Carex kobomugi* (Asiatic sand sedge) | SB05 | 3 | *Pseudomonas* spp. (2) |
| 7. *Artemisia fukudo* (beach mugwort) | SB07 | 7 | *Pantoea* spp. (3) |
| 8. *Messerschmidia sibirica* (sea rosemary) | SD08 | 2 | *Pseudomonas* spp. (2) |
| 9. *Glehnia littoralis* (beach silvertop) | BA09 | 3 | NA |
| Total | | 91 | |

[a]BA, Baramarae; HA, Hagam; SB, Sambong; SD, Shindu.

[b]NA, not applicable owing to lack of dominance by single group.

cell-wall-degrading enzymes and secondary metabolites is a common mechanism used by bacteria to inhibit the growth of other microorganisms. Thus, to better characterize the degree of antagonism of the bacterial isolates, the production of hydrolytic enzymes and secondary metabolites was tested. Chitinase activity (degradation of β-1,4-*N*-acetylglucosamine polymer) and pectinase activity were tested in a minimal medium according to Chernin *et al.* [2], where clearing zones were detected after a 5-day incubation period at 30°C. Protease activity, indicated by casein degradation, was determined by observing clearing zones in skim milk agar plates (50 ml sterilized skim milk mixed at 55°C with 50 ml of a 1/5 tryptic soy agar and 4% agar) after incubation for 3 days at 30°C.

## RESULTS

### Diversity of Sand Dune Plant-associated Bacteria

Ninety-one isolates were recovered from the roots of eight coastal sand dune plant species (Table 1). The 17 different

**Table 2.** Plant growth-promoting potential of individual isolates: + weak positive, ++ positive, and +++ strong positive.

| Strain[a] | Identification | Antifungal activity against | | | | Production of | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *R. solani* | *P. ultimum* | *F. oxysporum* | *B. cinerea* | IAA | Siderophore | Protease | Pectinase | Chitinase |
| PSB01-06 | *Acinetobactor* sp. | | | | ++ | | ++ | | + | + |
| PSB01-07 | *Acinetobactor* sp. | | | | ++ | | + | | ++ | + |
| PSB01-26 | *Acinetobactor* sp. | + | | ++ | | | + | | + | + |
| PBA01-06 | *Agrobacterium* sp. | | | | ++ | | | | | |
| PSB01-08 | *Agrobacterium* sp. | | | | ++ | ++ | | | | |
| PSB04-01 | *Agrobacterium* sp. | | | +++ | | | | | | |
| 2PHA03-02 | *Agrobacterium* sp. | | +++ | | | | | | | |
| PBA09-13 | *Bacillus* sp. | | | ++ | | | | | | |
| PSB01-30 | *Bacillus* sp. | | ++ | +++ | | | + | + | + | + |
| 2PBA01-02 | *Bacillus* sp. | | + | | + | | | | | |
| PSB01-15 | *Brevibacillus* sp. | | | | ++ | | | | | |
| PSB01-25 | *Brevibacillus* sp. | + | | ++ | | | | | | |
| PSB01-29 | *Brevibacillus* sp. | | | + | ++ | | | | | |
| PSB01-01 | *Chryseobacterium* sp. | | | ++ | ++ | | | + | + | + |
| PSB01-03 | *Chryseobacterium* sp. | | | | ++ | | | + | + | + |
| PSB01-19 | *Chryseobacterium* sp. | ++ | | ++ | | | | | | |
| PSB01-20 | *Chryseobacterium* sp. | | | +++ | | | | +++ | + | + |
| PSB01-21 | *Chryseobacterium* sp. | ++ | + | ++ | | | | | | |
| PSB01-22 | *Chryseobacterium* sp. | ++ | + | ++ | ++ | | | | | |
| PSB01-23 | *Chryseobacterium* sp. | | | ++ | ++ | | | | | |
| PSD01-04 | *Chryseobacterium* sp. | | | ++ | | | | | | |
| 2PSB01-02 | *Curtobacterium* sp. | | ++ | +++ | | | | | | |
| PSB04-08 | *Erwinia* sp. | + | + | +++ | | | | | + | ++ |
| PSB04-14 | *Erwinia* sp. | ++ | +++ | | ++ | | + | | + | ++ |
| PSB07-13 | *Erwinia* sp. | | ++ | | | | | | | |
| PSB07-14 | *Erwinia* sp. | + | ++ | | | | | | | |
| PSD01-05 | *Microbacterium* sp. | | | ++ | | | | | | |
| PSD01-17 | *Microbacterium* sp. | ++ | + | + | ++ | | | | | |
| PSB04-10 | *Novosphingobium* sp. | | | ++ | | | | | | |
| PSB04-12 | *Novosphingobium* sp. | | + | ++ | | | | | | |
| PHA02-04 | *Paenibacillus* sp. | | ++ | | | | | | | |
| PSB01-24 | *Paenibacillus* sp. | | | ++ | | | | | | |
| PSB01-27 | *Paenibacillus* sp. | | + | | | | | | | |
| 2PSB07-17 | *Paenibacillus* sp. | +++ | | | | | | | | |
| PBA09-10 | *Pantoea* sp. | | ++ | ++ | | | + | | + | +++ |
| 2PSB05-11 | *Pantoea* sp. | +++ | + | | | | + | | + | +++ |
| 2PBSB7-1 | *Pantoea* sp. | +++ | +++ | ++ | | | | | | |
| 2PSB07-06 | *Pantoea* sp. | | +++ | ++ | | | + | | + | + |
| 2PSB07-17 | *Pantoea* sp. | | +++ | ++ | | | | | | |
| PSB01-04 | *Pseudomonas* sp. | | ++ | ++ | +++ | | + | + | + | + |

**Table 2.** Continued.

| Strain[a] | Identification | Antifungal activity against | | | | Production of | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *R. solani* | *P. ultimum* | *F. oxysporum* | *B. cinerea* | IAA | Siderophore | Protease | Pectinase | Chitinase |
| PSB02-16 | *Pseudomonas* sp. | | ++ | | | | + | | + | + |
| PHA03-03 | *Pseudomonas* sp. | | ++ | +++ | | | + | ++ | + | + |
| PHA03-17 | *Pseudomonas* sp. | ++ | ++ | +++ | | + | ++ | | + | + |
| PHA03-19 | *Pseudomonas* sp. | | | ++ | | +++ | + | | +++ | + |
| 2PSB03-01 | *Pseudomonas* sp. | | | | ++ | | + | + | + | + |
| 2PSB03-07 | *Pseudomonas* sp. | | ++ | | +++ | | + | + | | + |
| 2PSB03-08 | *Pseudomonas* sp. | | + | +++ | +++ | + | + | ++ | + | + |
| 2PSB03-14 | *Pseudomonas* sp. | | + | | ++ | | + | + | + | + |
| 2PHA03-10 | *Pseudomonas* sp. | | + | | ++ | | + | + | + | + |
| 2PHA03-11 | *Pseudomonas* sp. | | | +++ | +++ | | + | ++ | + | + |
| 2PHA03-14 | *Pseudomonas* sp. | | + | ++ | +++ | | + | | + | ++ |
| 2PHA03-15 | *Pseudomonas* sp. | | + | +++ | +++ | | + | + | + | + |
| 2PHA03-23 | *Pseudomonas* sp. | | + | +++ | ++ | | + | ++ | + | + |
| PSB04-03 | *Pseudomonas* sp. | | + | +++ | | | + | + | + | + |
| 2PHA04-09 | *Pseudomonas* sp. | ++ | +++ | | ++ | | + | + | + | + |
| 2PSB05-06 | *Pseudomonas* sp. | + | ++ | | +++ | | + | + | + | + |
| 2PSB07-18 | *Pseudomonas* sp. | +++ | | | | | + | + | + | + |
| 2PSD08-03 | *Pseudomonas* sp. | +++ | +++ | +++ | +++ | | + | + | + | + |
| PBA09-15 | *Pseudomonas* sp. | ++ | | ++ | | ++ | + | | + | + |
| PHA03-15 | *Pseudomonas* sp. | | ++ | +++ | ++ | | | | | |
| PHA03-16 | *Pseudomonas* sp. | | | ++ | ++ | | | | | |
| 2PSB03-05 | *Pseudomonas* sp. | | | ++ | ++ | | | | | |
| PSB04-09 | *Pseudomonas* sp. | + | + | ++ | | | | | | |
| PSB02-10 | *Pseudomonas* sp. | | ++ | + | | | | | | |
| PHA03-01 | *Pseudomonas* sp. | | ++ | | | | | | | |
| PHA03-08 | *Pseudomonas* sp. | | ++ | | | | | | | |
| 2PSB01-04 | *Pseudomonas* sp. | | ++ | | | | | | | |
| PSB02-19 | *Pseudomonas* sp. | + | +++ | ++ | | | | | | |
| PHA03-05 | *Pseudomonas* sp. | ++ | +++ | +++ | | | | | | |
| 2PBA01-09 | *Pseudomonas* sp. | | + | + | + | | | | | |
| 2PSB01-12 | *Pseudomonas* sp. | + | + | | | | | | | |
| 2PSB03-02 | *Pseudomonas* sp. | | | | ++ | | | | | |
| 2PSB03-10 | *Pseudomonas* sp. | | | | ++ | | | | | |
| 2PSB03-11 | *Pseudomonas* sp. | | + | +++ | | | | | | |
| 2PSB05-09 | *Pseudomonas* sp. | | ++ | | +++ | | | | | |
| 2PSB03-18 | *Pseudomonas* sp. | | + | | ++ | | | | | |
| 2PSB03-15 | *Pseudomonas* sp. | | + | + | | | | | | |
| 2PSB03-16 | *Pseudomonas* sp. | | + | + | ++ | | | | | |
| 2PHA03-06 | *Pseudomonas* sp. | | | | +++ | | | | | |
| 2PHA03-07 | *Pseudomonas* sp. | + | + | | ++ | | | | | |
| 2PHA03-08 | *Pseudomonas* sp. | | + | | ++ | | + | + | + | + |
| 2PHA03-12 | *Pseudomonas* sp. | | +++ | | | | | | | |
| 2PSB03-19 | *Pseudomonas* sp. | | + | | ++ | | + | + | + | + |
| 2PSD08-02 | *Pseudomonas* sp. | | | | ++ | | + | + | + | + |
| 2PHA03-03 | *Rahnella* sp. | | +++ | | | | + | | + | ++ |
| PSB01-13 | *Rhizobium* sp. | | | +++ | | | | | | |
| PSB01-32 | *Rhizobium* sp. | | + | + | | + | | | + | ++ |
| PSB04-02 | *Rhizobium* sp. | | | +++ | | | | | | |
| PSD01-03 | *Rhodanobacter* sp. | ++ | ++ | + | ++ | + | | | | |
| 2PSD01-04 | *Xanthomonas* sp. | + | | ++ | | | + | + | ++ | + |
| PSB01-33 | *Variovorax* sp. | | + | + | | | | | | |

[a]Codes BA, HA, SB, and SD indicate sites from which strains were isolated. See Table 1 and text for code designation.

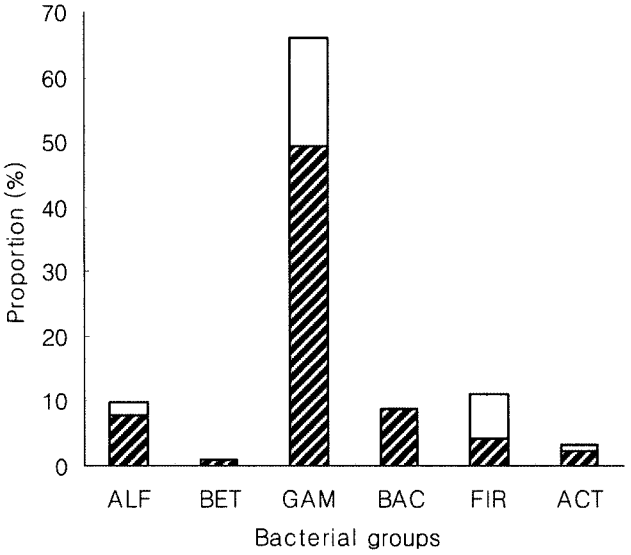


**Fig. 1.** Composition of endophytic bacterial isolates. ALF, *Alphaproteobacteria*; BET, *Betaproteobacteria*; GAM, *Gammaproteobacteria*; BAC, *Bacteroidetes*; FIR, *Firmicutes*; ACT, *Actinobacteria*.
Proportions of major genera within each phylum are indicated as a shadowed area: *Agrobacterium-Rhizobium* group (ALF), *Variovorax* (BET), *Pseudomonas* (GAM), *Chryseobacterium* (BAC), *Paenibacillus* (FIR), and *Microbacterium* (ACT), respectively.

genera identified (Table 2) were in turn assigned to 6 major bacterial groups, namely *Alphaproteobacteria* (9.9%), *Betaproteobacteria* (1.1%), *Gammaproteobacteria* (65.9%), *Bacteroidetes* (8.8%), *Firmicutes* (11.0%), and *Actinobacteria* (3.3%), based on the partial 16S rDNA sequence analysis (Fig. 1). *Pseudomonas* was the most common group, consisting of 49.5% of the total endophytic isolates from most of the plant species (Table 2). *Chryseobacterium* or *Microbacterium* was the major group primarily associated with beach morning glory (*C. soldanella*), and *Pantoea* primarily associated with beach mugwort (*A. fukudo*) (Table 1). *Chryseobacterium* (8.8%) and *Agrobacterium-Rhizobium* (7.7%) were also common constituents of the total isolates.

**Test for Antagonistic Activity Against Plant Pathogens**

The endophytic isolates were tested *in vitro* for antagonistic activity against four common fungal plant pathogens, namely *Rhizoctonia solani* (a basidiomycete with a chitin-glucan-containing cell wall, causing a variety of diseases, such as blight and rot, in a wide range of plants), *Pythium ultimum* (an oomycete with a cellulose-containing cell wall, causing root rot), *Fusarium oxysporum* (an ascomycete causing *Fusarium* wilt), and *Botrytis cinerea* (an ascomycete causing grey rot). Twenty-five strains (27.5% of the total isolates) were found to be antagonistic against *Rhizoctonia solani*, 57 strains (62.6%) were antagonistic against *Pythium ultimum*, 53 strains (58.2%) were antagonistic against *Fusarium oxysporum*, and 41 strains (45.1%) were antagonistic against *Botrytis cinerea* (Table 2). A number of groups exhibited characteristic antagonistic spectra. For example, the *Erwinia* and *Pantoea* groups, both members of *Enterobacteriaceae*, displayed strong antagonistic activity towards *P. ultimum*. All the strains in the *Rhizobium* group and almost all the *Chryseobacterium* strains displayed antagonistic activity towards *F. oxysporum*. However, no significant correlation was observed among the four antagonistic activities. Each bacterial isolate showed antagonism towards at least one fungal species. In contrast, only four isolates exhibited antagonism towards all four fungi. The average number of antifungal activities per individual strain was 1.93.

**Production of Hydrolytic Enzymes and Secondary Metabolites**

When testing for the production of metabolites that may facilitate plant growth, only 7 strains (7.7% of total isolates) were found to produce indole acetic acid (IAA), whereas 33 (36.3%) produced siderophores, 23 (25.3%) produced protease, 37 (40.7%) produced pectinase, and 38 (41.8%) produced chitinase (Table 2). Notably, all three strains from the *Acinetobacter* group produced siderophores, pectinase, and chitinase, yet not protease. In contrast, none of the *Brevibacillus* (3 isolates) and *Paenibacillus* (4 isolates) strains produced any of the 5 metabolites. The *Agrobacterium* strains (4 isolates) were also poor producers. Only one individual strain, designated *Pseudomonas* sp. 2PSB03-08, was found to produce all five metabolites, but 52 strains did not produce any of the metabolites. The average number of metabolites produced per individual strain was 1.52.

## DISCUSSION

The dominance of proteobacteria, represented by *Pseudomonas*, and Gram-positive bacterial groups in the plant roots, has also been observed in other studies [19, 29]. Considering the condition of the plant samples used in this study, the bacterial isolates were likely typical inhabitants of healthy plants. Moreover, many of the representative taxa identified in this study, for example, *Pseudomonas*, *Paenibacillus*, *Pantoea*, and *Rhizobium*, are well known to exhibit plant growth-promoting potential through the production of plant growth-promoting hormones, antagonistic against plant pathogens, and nitrogen fixation [1, 4, 11–14, 18, 24, 29], although there is little information on the plant growth promotion for other taxa, particularly *Chryseobacterium*. However, frequent isolation of *Chryseobacterium* strains from the rhizosphere and root samples are indicative of their common presence in association with plants [16, 22, 33, 34].

When examining the relationships among the individual plant growth-promoting properties, plant species, sampling

**Table 3.** Distribution of plant growth-promoting activities according to host plant.

| Plant | Number of isolates | Antifungal activity against (%) | | | | Production of (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *R. solani* | *P. ultimum* | *F. oxysporum* | *B. cinerea* | IAA | Siderophore | Protease | Pectinase | Chitinase |
| 1. *C. soldanella* | 32 | 28.1 | 43.8 | 68.8 | 46.9 | 9.4 | 18.8 | 18.8 | 31.3 | 31.3 |
| 2. *L. japonica* | 4 | 25.0 | 100.0 | 50.0 | | | 25.0 | | 25.0 | 25.0 |
| 3. *E. mollis* | 31 | 9.7 | 74.2 | 48.4 | 64.5 | 9.7 | 48.4 | 35.5 | 45.2 | 48.4 |
| 4. *V. rotundifolia* | 9 | 44.4 | 66.7 | 77.8 | 22.2 | | 33.3 | 22.2 | 44.4 | 44.4 |
| 5. *C. kobomugi* | 3 | 66.7 | 100 | | 66.7 | | 66.7 | 33.3 | 66.7 | 66.7 |
| 7. *A. fukudo* | 7 | 57.1 | 71.4 | 42.9 | | | 28.6 | 14.3 | 28.6 | 28.6 |
| 8. *M. sibirica* | 2 | 50.0 | 50.0 | 50.0 | 100 | | 100 | 100 | 100 | 100 |
| 9. *G. littoralis* | 3 | 33.3 | 33.3 | 100 | | 33.3 | 66.7 | | 66.7 | 66.7 |
| Average | | 27.5 | 62.6 | 58.2 | 45.1 | 7.7 | 36.2 | 25.3 | 40.7 | 41.8 |

regions, and bacterial taxa, the broadest spectra of activities were observed for two *Pseudomonas* strains, 2PSB03-08 and 2PSD08-03, both of which exhibited eight positive properties out of nine. Five strains all belonging to *Pseudomonas*, namely PHA03-17, 2PHA03-15, 2PHA03-23, 2PHA04-09, and 2PSB05-06, exhibited 7 positive properties. The isolates from *C. kobomugi* (3 strains) and *M. sibirica* (2 strains) also showed a high plant growth-promoting potential, exhibiting average positive activities of 51.9% and 72.2%, respectively (Table 3). In contrast, the isolates from *L. japonica* (4 strains) and *A. fukudo* (7 strains) generally showed a low plant growth-promoting potential, exhibiting average positive activities of 27.8% and 30.2%, respectively.

To examine the existence of a correlation between any two plant growth-promoting activities, the phi correlation coefficients were calculated using the following equation. The degree of response was not considered, and the data were treated as either positive or negative.

$$\varphi_{AB}=(ad-bc)/\sqrt{(efgh)}$$

$a$ ($d$): number of strains positive (negative) for both property A and B

$b$ ($c$): number of strains positive for property A (B), yet negative for B (A)

$e$ ($g$): number of strains positive for property A (B)

$f$ ($h$): number of strains negative for property A (B)

For example, antagonistic activity towards *R. solani* was found in 25 strains, whereas antagonistic activity towards *P. ultimum* was found in 57 strains. The number of strains antagonistic towards both *R. solani* and *P. ultimum* were 18, whereas the number of strains that showed no antagonism towards *R. solani* and *P. ultimum* was 27. The number of strains antagonistic towards *R. solani* yet not towards *P. ultimum* was 7, whereas the number of strains exhibiting the opposite was 39. The phi coefficient between the two properties is then

$$[18\times27-(25-18)\times(57-18)]/[25\times(91-25)\times57\times(91-57)]=0.12$$

The index of 0.12 implies no significant correlation between the two properties, as a zero value indicates that the character distribution is never affected by either property, a coefficient of 1 means both properties are absolutely related with each other, and a value below zero to −1 indicates a negative relationship between the two properties.

The phi coefficients for each pair of properties are presented in Table 4, where the pectinase-chitinase pair showed the highest correlation (0.98), followed by the

**Table 4.** Phi correlation coefficients between each pair of plant growth-promoting activities.

| Activity | Antifungal activity against | | | | Production of | | | |
|---|---|---|---|---|---|---|---|---|
| | *R. solani* | *P. ultimum* | *F. oxysporum* | *B. cinerea* | IAA | Siderophore | Protease | Pectinase |
| Antigungal activity against | | | | | | | | |
| *P. ultimum* | 0.12 | | | | | | | |
| *F. oxysporum* | 0.07 | −0.06 | | | | | | |
| *B. cinerea* | −0.16 | −0.08 | −0.26 | | | | | |
| Production of | | | | | | | | |
| IAA | 0.10 | −0.03 | 0.16 | −0.01 | | | | |
| Siderophore | 0.21 | 0.27 | −0.10 | 0.19 | 0.13 | | | |
| Protease | −0.08 | 0.03 | −0.07 | 0.34 | −0.06 | 0.61 | | |
| Pectinase | 0.04 | 0.04 | −0.03 | 0.15 | 0.18 | 0.87 | 0.65 | |
| Chitinase | 0.03 | 0.06 | −0.05 | 0.17 | 0.17 | 0.89 | 0.69 | 0.98 |

siderophore-chitinase (0.89), siderophore-pectinase (0.87), protease-chitinase (0.69), protease-pectinase (0.65), and siderophore-protease (0.61) pairs. Thus, the production of the three extracellular enzymes was clearly related, along with the production of siderophores. In contrast, no significant correlation was found for the antagonism towards the four fungal pathogens, except for one case, the *F. oxysporum* and *B. cinerea* pair, where the relationship was negative (-0.26), as antagonism towards one was slightly related with no antagonism towards the other. A weak correlation was also found between antagonism towards *B. cinerea* and the production of siderophores (0.34) and between the production of siderophores and antagonism towards *R. solami* (0.21) and *P. ultimum* (0.27).

Correlations among extracellular enzyme activities were also identified in a previous study [29], where significant correlations were observed for chitinase-pectinase (0.60), siderophore-protease (0.38), and siderophore-pectinase (0.35). Similarly, pectinase-chitinase exhibited a high correlation (0.58) in a separate study [20].

Consequently, the above results clearly indicate that many plant growth-promoting activities can be related with bacterial taxa, host plants, and sampling regions, and also that certain plant growth-promoting activities are clearly related with one another. In particular, the productions of degradative enzymes and siderophores were strongly related with one another.

A high level of diversity in the endophytic bacterial community associated with sand dune plants was confirmed in this study. Strains of *Pseudomonas* represented the primary endophytic bacterial group in the roots of most sand dune plant species, and were also outstanding in their plant growth-promoting potential. Clear correlations were found among certain plant growth-promoting activities. Thus, further study of such correlations may provide useful information for understanding the roles of bacteria in plant growth promotion.

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