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Review

Ecochemical Studies of Interrelationships between Epiphytic Bacteria and Host Plants *via* Secondary Metabolites

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The plant surface, which is representative of the phylloplane and rhizoplane, is a characteristic habitat for microorganisms. In this review, the ecological roles of phytoepiphytic bacteria will be described. The phylloplane and rhizoplane, which are adjacent to the atmosphere and soil sphere respectively, accumulate topically and/or selectively release secondary metabolites that are specific to the plant genera and species which reside within these regions. Some epiphytes have abilities to decarboxylate xenobiotic phenolic acids that have accumulated in the plant tissues and surfaces as a majority of such secondary metabolites. In physicochemically stressed soil, rhizosphere microflora often remedy such microenvironments within the rhizosphere in order to assist in the survival of the host, and some of the microfloral compositions behave as if they were symbionts. Specifically, some *Sphingomonas* spp., which are frequently isolated from the rhizosphere of acidic soil-tolerant plants in tropical zones, make possible the development of a rhizo-biocomplex. In this review, the possibility of rhizosphere regulation utilizing such a rhizo-biocomplex is discussed.

Key words: decarboxylation of phenolic acids; gellan gum medium; glandular trichomes of *Rosa rugosa*; *Sphingomonas*; rhizo-biocomplex

I. Phylloplane and Rhizoplane, a Thin but Deep Black Box

The plant surface, which is representative of the phylloplane (for leaf) and the rhizoplane (for root), is a characteristic habitat for epiphytic microorganisms. Under strong sunlight conditions, the phylloplane exists in the atmospheric open space and may resemble a severe dry area,[1] as if it were a desert-like environment for microorganisms. On the other hand, the rhizoplane is shaded from sunlight by bulk soil and it is therefore maintained under relatively stable conditions with respect to humidity and temperature. The stability of these conditions and the active nutrient circulation in the rhizosphere are thought to be reasons why high populations of microorganisms can grow in the rhizosphere.[2] Hence, the rhizoplane is described to resemble an environment like a tropical forest where elemental circulation is also topical but highly active on the micro scale.

This particular study, which will be discussed in this review, was started based on natural product chemistry, particularly phytochemistry,[3] and the focus shifted to plant-microbe interactions on the basis of chemical ecology. With respect to ecologically significant epiphytisms, plant-epiphyte cross-talk *via* chemical substances is a topic that attracted my research interest. Discoveries of mycorrhizal fungi have shown that plants often incorporate phytoepiphytes in their strategy to survive under stressed conditions,[4] and it is thus more likely that the plant and phytospherous microorganisms play important roles in plant defense and/or environmental remediation of physical and chemical properties of rhizosphere soils. In fact, we could often isolate some functional microorganisms from plant surfaces that survived under certain stressed conditions. In such incorporation between plants and phytoepiphytes to form a plant-microbe biocomplex, communication webs among them may be established as a signal network regulated by chemical substances.

Although this interdisciplinary field of study is not fully understood at this time, this review attempted to shed light on the dynamic nature of the interaction among plants and epiphytic microbes. In addition, our results in relation to this field of study are included, along with summaries from important literature which pertains to this field. Some of our results contain unpublished data, but they are helpful in allowing us to advance our understanding for the importance of epiphytism and biocomplex formation between plants and microorganisms.

II. Microcosms on the Plant Surface Where Topical Accumulation of Certain Secondary Metabolites of Plant Origins Often Occurs

In the phyllosphere, many unicellular and/or multicellular trichomes on the phylloplane stock large volumes of moisture inside the swollen cells. On trichome-rich leaves, many leaf epiphytes often attach

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to the base of the trichomes.[5] When the leaf is mechanically stressed, the moisture-rich trichomes collapse and crush at their base. Subsequent to this mechanical perturbation, such injured cells exude moisture and nutrients, both of which are necessary for the growth of epiphytic bacteria. In fact, it is known that epiphytic bacteria drastically increase their population size on injured leaves, with a particular enrichment which occurs at the base of the trichomes.[6] Interestingly, many of the bacteria which inhabit the phylloplane are airborne. The main source of the phylloplane bacteria is not soil dusts; rather it is believed to be bacterial aerosols from the plant leaves themselves.[7] As a matter of a fact, on sunny days, the dispersal of bacterial aerosols into the atmosphere occurs actively in the plant canopies.

On the other hand, the rhizoplane is surrounded by root hairs which extend from the root surface to form a complex three-dimensional structure on the rhizosphere. In addition, some plants are capable of exuding a large amount of polysaccharides from the root surface, while others accumulate root polyphenolics to levels which can exceed 10% of root fresh weight.[8] Some plants that are tolerant to highly stressed soil often provide organic matter such as mucigel, decidual epidermis cells, border cells, and/or dead root hairs into the rhizosphere. These contributions of organic matter lead to the establishment of "active and healthy" rhizospherous microfloral communities.[9] This trend is similar to what occurs in lowlands where swampy forest trees re-use elements from decomposing litter to complement the low nutrients of tropical peat soils.[10]

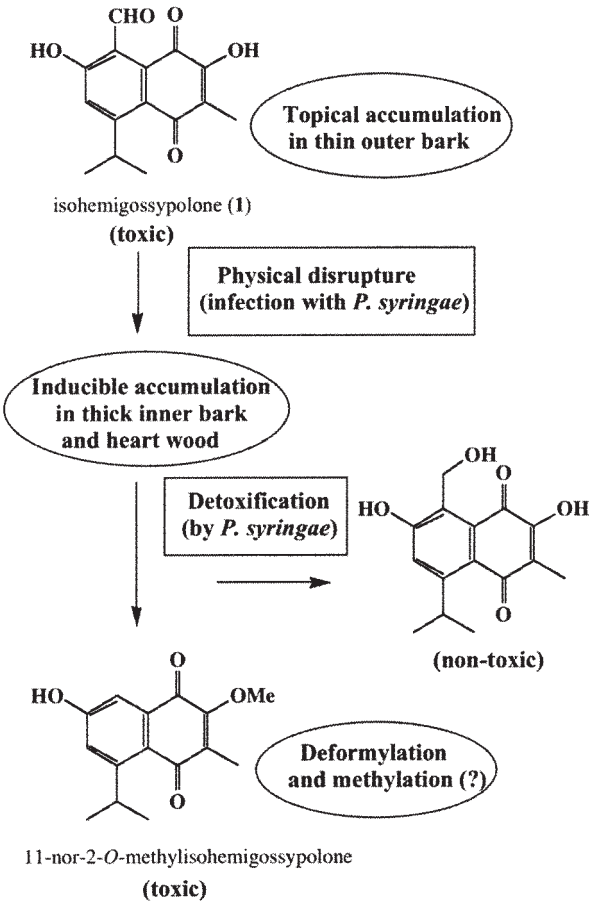
Both phylloplane and rhizoplane often accumulate remarkable amounts of secondary metabolites that are characteristic of the plant species which reside there. Many plants contain several types of leaf secondary metabolites including pigments, alkaloids, terpenoids, flavonoids, some other polyphenolics and additional related compounds. When the leaves are mechanically wounded, such secondary metabolites are often exuded from the injured parts.[11] On the leaf surface, some plants possess specific organs, known as glands or glandular trichomes, and topically exude oily drops or syrups of terpenoids, sugars, and sugar esters, fatty acid derivatives, or other secondary metabolites.[12] However, in root systems, organic matter is actively supplied into the rhizosphere along with active replacements of the epidermal cells, root hairs, and the fibrous root tissues themselves. Since root bark tissue often accumulates relatively high concentrations of secondary metabolites, an effective supply of such secondary metabolites diffused into the rhizosphere also occurs.

Under nutrient-rich conditions, some limited groups of oligopsonistic and saprophytic microbes often occupy a rhizospherous microfloral niche. In contrast, when rhizospherous microfloral communities exist under nutrient-poor conditions, they tend to exhibit high biodiversity and synergistic bioactivity which functions to increase the bioavailability of limited resources and elements in the rhizosphere soil. One of the best known models for ecosystems under nutrient-poor conditions is the rhizosphere of tropical plants that are tolerant to highly stressed soil, in which limited elements are effectively mineralized, re-used, and circulated. As seen in our investigations, there is a clear tendency for oligotrophic microorganisms to occupy predominantly the microfloral niche in the organic matter-poor and/or physicochemically stressed rhizosphere soil.

There are species-specific relationships between particular plants and certain microorganisms in the rhizosphere. At present, it is still unclear whether host specificity for rhizospherous microflora is due mainly to selection by the host plants or to soil conditions. However, our investigation of rhizoplane microflora from *Aegopodium podagraria* (a chlorogenic acid-rich plant, Umbelliferae) and *Geranium robertianum* (a hydrolysable tannin-rich plant, Geranaceae) shed some light on this enigma. Although both plant types established pure communities when grown under similar conditions on the Hokkaido University Campus in Sapporo, the bacterial compositions in the rhizoplane of *A. podagraria* was completely different from those obtained from *G. robertianum*, even though they shared the same bulk soil within their root systems. To the point, it was found that the major rhizobacteria isolated from these host plants show variation in their ability to decarboxylate polyphenolic acids, variation dependent upon the host plant they were associated with.[13] From this preliminary case study, we were able to conclude that the metabolic properties of rhizobacteria are highly dependent on the polyphenolic profile of their host plants.

Leaf-attaching and/or root-associating microorganisms are presented with frequent opportunities to encounter such plant-originated secondary metabolites. Such secondary metabolites often cause an arms race between a phytopathogenic bacterium and the plant, similar to those between plants and herbivorous animals.[14] In the case of *Pachira aquatica*, this Bombacaceae plant topically accumulates a sesquiterpene quinone aldehyde, isohemigossypolone (**1**), in the thin outer bark which surrounds a swollen trunk near the underground part of the plant.[15] When *Pseudomonas syringae* invades swollen tissues that have suffered mechanical injury, *P. aquatica* starts to induce and accumulate this particular antimicrobial compound within the inner bark of the injured tissue region. The bacterium that has been exposed to compound **1** acquires an ability to detoxify **1** completely by reduction at the formyl group.[16] The plant, however, is able to accumulate a deformylated derivative of **1** that still exhibits significant antimicrobial activity (Scheme 1).

Although the previous example is descriptive of a hostile relationship between plants and microbes, it is likely that some microbes and plants establish a synergism and symbiosis by using chemicals that



Scheme 1. Arms Race between *Pachia aquatica* and *Pseudomonas syringae*.

Events from plant and phytopathogenic bacterium are indicated by arrows. This arms race from the plant side (in oval circles) and the bacterial side (in rectangles) show a competitive relationship between these two organisms, due to the phytopathogenicity of *P. syringae*.

topically accumulate in plant roots as one-way or two-way signals between them. In particular, it is of great interest to discover two-way signaling systems that can amplify such synergistic efficacy in terms of positive feedback regulation.

III. Rugosa Rose (*Rosa rugosa* Thunb.) as a Plant That Topically Accumulating Secondary Metabolites

This study originated from a chemical study on natural products that were identified from the leaves of *Rosa rugosa*. *R. rugosa* belongs to the genus *Rosa* in the family Rosaceae, a tannin-rich family known to contain a large amount of condensed and/or hydrolyzable tannins. Despite this accumulation, it is believed that Rosaceae is unable to produce sesquiterpenoids.[17] However, during our investigation of antifungal substances in the leaves of *R. rugosa*, we found that the major antifungal substance of this plant is an unknown C15-peroxide compound. By means of spectroscopic analysis and chemical derivatization and synthesis, the chemical structure of this antifungal sesquiterpene was elucidated, including its absolute configuration. It was determined that this novel carotane (also called daucane)-type sesquiterpene aldehyde possesses epidioxy linkage in the molecule. It was subsequently named rugosal A (**3**).[18,19]

A precursory compound that lacked peroxidation was also searched for in the mono-carbonyl sesquiterpene fraction that was obtained from young leaves of *R. rugosa*, leading to the discovery of carota-1,4-dienaldehyde (**2**).[20] Compound **2** possessed a 1,4-diene structure, of which the bis-allyl methylene moiety triggered a radical-mediated peroxidation reaction. This in turn allowed it to be easily converted into a 2-hydroperoxy intermediate of compound **3**.[21] Peroxidation of **2** and carota-1,4-dien-14-ol was found to occur from the primary formation of a hydroperoxy group at the C-5 positions and successive endoperoxy linkage between the C-5 and C-1 positions to induce another exoperoxy radical at the C-2 position. This process was demonstrated in the autoxidation of carota-1,4-dien-14-ol benzoate to yield more stable intermediary products.[22] In contrast, carota-1,4-diene, which did not become oxidized at C-14, accumulated C-4-hydroperoxy derivatives to give evidence for a carbon radical shift from C-3 to C-4 in forming C-14-norsesquiterpene.[22]

We also isolated several new carotane-type and (+)-*epi*-α-bisabolol-type sesquiterpenoids as representative constituents of *R. rugosa* leaves, including rugosic acid A (**4**) and bisaborosaol A (**5**), respectively (Fig. 1).[23–25] In particular, compound **4** was the predominant compound that accumulated in the leaves (often over 3 g/kg fresh w.t.). Compound **4** exhibited a clear response to a peroxide detecting reagent (*N,N*-dimethyl-*p*-phenylenediamine sulfate), which was used for detection of the epidioxy linkage in this experiment.

In parallel with structural elucidation of the sesquiterpenes, their topical location in the leaves was also discovered. Glandular trichomes possessing oil droplets on the trichome tips were identified as the organ where the majority of the sesquiterpenes were produced and accumulated.[26,27] Thus we reidentified the glandular trichomes of *R. rugosa*, which are distributed along the leaf vein of the lower leaf surface, as a sesquiterpene-producing organ of this particular Rosaceae plant.

We further examined over 60 horticultural varieties of hybrid rugosa roses, in addition to some wild roses (*R. acicularis*, *R. multiflora*, *R. woodsii*, and *R. damasceana*). In all investigations, plant samples were studied to determine whether similar glandular trichomes (similar to those of *R. rugosa*) are present on the leaf surface. When similar trichomes were identified, the correlation between the glandular trichome density and the amount of exuded sesquiterpenes was individually determined, together with their respective gas-chromatographic profiles.[28] Some of the hybrid rugosa that possessed dense glandular trichomes mainly produced character-

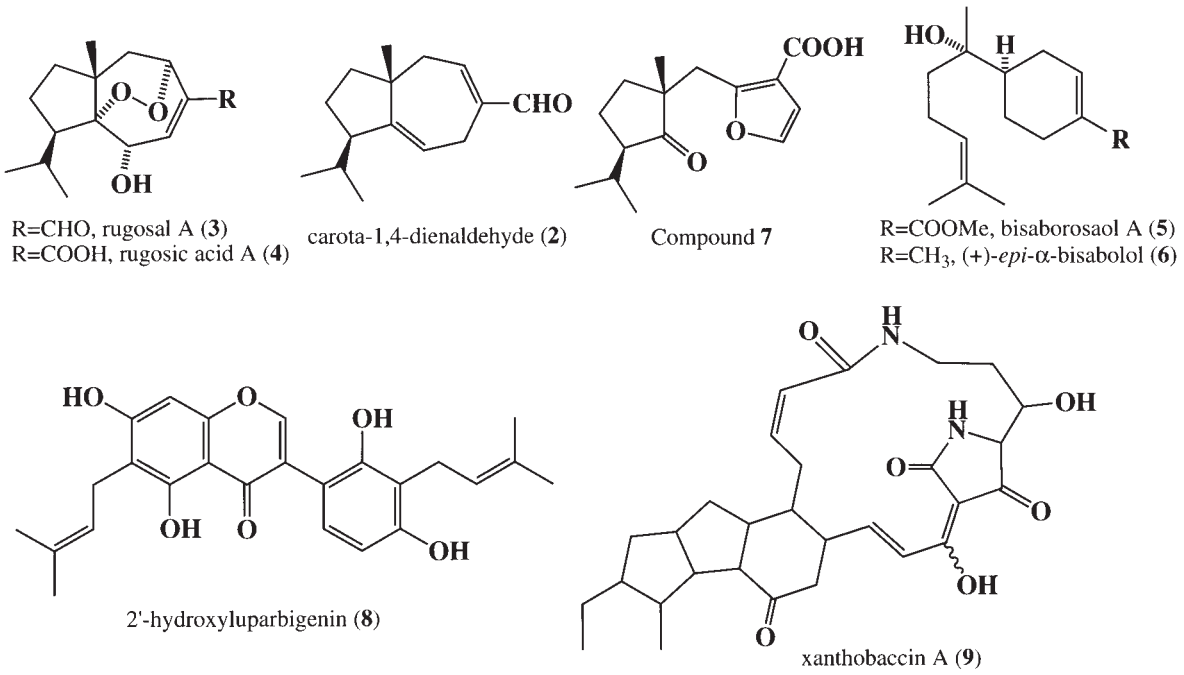


Fig. 1. Chemical Structures of Important Secondary Metabolites and Related Compounds Associated with Epiphytisms in the Phytosphere.

istic sesquiterpenoids which were not the same as those identified as major sesquiterpenoids from *R. rugosa* (*e.g.* compounds **2–5**). A particular hybrid rugosa, Martin Frobisher, produced 400-fold amounts of (+)-4-*epi*-α-bisabolol (**6**) as a large proportion of sesquiterpene from the glandular trichomes.[29] Recently, compound **6** has been isolated and identified from *Peperomia galiodes* (Piperaceae) and described as the wound-healing principle.[30]

In conclusion, many plants produce and accumulate species- and/or family-characteristic secondary metabolites topically from certain organs of plant surfaces, and sesquiterpenoids exuding from the glandular trichomes of *R. rugosa* are an example of the species-specific chemical compounds topically accumulated on the leaves.

IV. Phylloplane Microorganisms Having an Ability to Metabolize Plant Secondary Metabolites

When mechanically damaged *R. rugosa* leaves were soaked in water for 48 h and the aqueous layer was recovered and extracted with EtOAc, we obtained a remarkable amount of cleaved product (compound **7**) from compound **4**, of which the chemical conversion was similar to the production of furopelargone B from hanalpinol, a guaiane-type sesquiterpene peroxide.[31] In contrast, only a trace amount of compound **7** was detected in fresh leaf rinsate of *R. rugosa* with 95% ethanol. Hence we speculated that a certain leaf microorganism(s) covert **4** into **7** with this characteristic cleavage of the epidioxy-linkage on **4**. Due to our interest in the identification of this particular hypothetical organism(s), we started to screen phylloplane microorganisms that possess unique metabolic properties. For the preliminary screen, younger leaves and senescent leaves were collected in June and October, respectively. For culturing media, *R. rugosa* leaves were chopped into small pieces and soaked in water, and the resulting aqueous extracts were retrieved after passage through a sterilized membrane filter (0.45 μm pore size).

We were unable to obtain any **4**-cleaving microorganisms from the leaves, whereas two strains of *Rahnella aquatilis* of γ-proteobacteria were isolated from emerging and mature leaves respectively. These particular isolates showed high abilities to decarboxylate polyhydroxybenzoic acids, including gallic acid and protocatechuic acid, due to expression of gallate decarboxylase (GD).[32] This GD activity was substrate-inducible, and the decarboxylative product exhibited strong antifungal activity[33] (Fig. 2). When excess (10 mM) gallic acid was added as a GD-inducer, bacterial cell growth was rarely inhibited and the GD activity per bacterial cell as compensated $O.D._{660\,nm}$ was 3-fold increased in comparison to those induced with applications of 1 mM gallic acid.

Three additional strains were isolated from the senescing leaves, and identified by means of 16S rRNA gene sequences as *Pantoea agglomerans*, *Klebsiella terrigena*, and *Erwinia rhapotici*, all of which belong to γ-proteobacteria. These isolates showed high similarity to *R. aquatilis* in their morphological features and biochemical and physiological properties.[33] In particular, some enzymatic activities including oxidase and catalase, metabolic capabilities for sorbitol and H_2S production, were in better accordance than those recorded for standard strains in Bergey's Manual, 8th

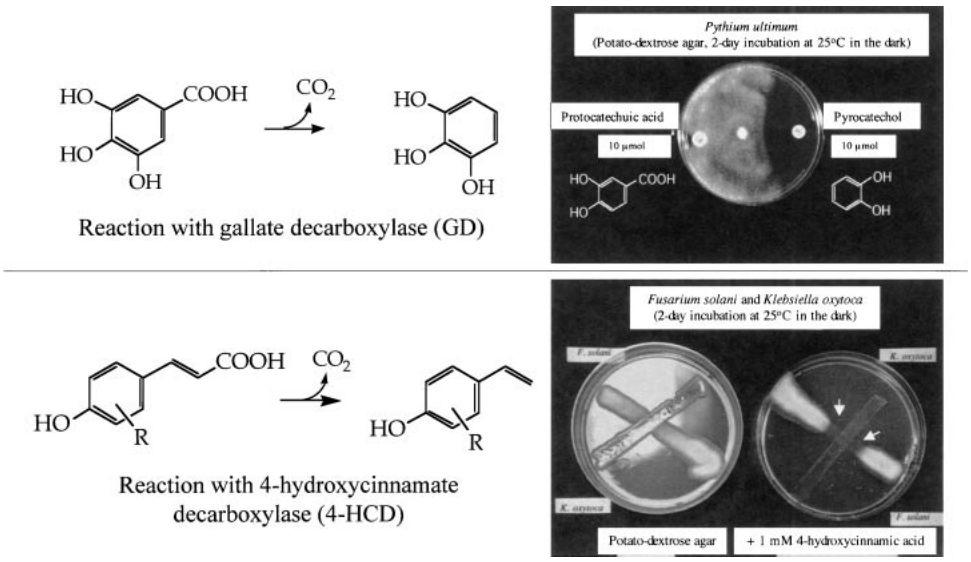


Fig. 2. Growth Inhibition of Phytopathogenic Fungal Mycelia with Decarboxylation Products Resulting from the Decarboxylation of Plant Phenolic Acids by Phytoepiphytic Bacteria.

and 9th editions.[34,35] After interpreting this phenomenon, these data were highly supportive of the hypothesis that these particular bacteria show convergence of their physiological properties due to the common habitat which they inhabit in the phylloplane of *R. rugosa*. It is the phylloplane environment that determines the morphological and primary metabolic properties of phylloplane and/or phyllosphere bacteria which occupy this region.[33] On the other hand, none of these isolates from the senescing leaves showed any GD activity, unlike *R. aquatilis*. This observation is not surprising, since the senescing leaves were found to contain only a trace amount of hydrolyzable tannins. The metabolic abilities of the microorganisms that have evolved towards the metabolism of xenobiotics are more specialized, but they are rarely coupled with primary metabolisms.

The phylloplane is thought to be under nitrogen-starved conditions. Within the phyllosphere of tannin-rich plants, even leaf proteins exuding from wounded tissues are not utilizable for many microorganisms because proteins are immediately tannized there. Therefore, many phylloplane bacteria are likely to have capabilities of nitrogen fixation.[1] *Phyllobacterium myrsinacearum* was first isolated from leaf nodules of *Ardisia* sp. (family Myrsinaceae),[36,37] and was described as a nitrogen-fixing phyllosphere bacterium. To date, *P. myrsinacearum* is known as a synergistic nitrogen-fixer with autotrophic *Chlorella vulgaris*[38] or with P-solubilizing *Bacillus licheniformis*,[39] so this diazotrophic leaf epiphyte would serve as a good model for a component of the phytosphere biocomplex. *R. aquatilis* showed similar biochemical characteristics to *P. myrsinacearum*. However, none of the specific PCR product corresponding to the segment of the *nifH* gene, which encodes a member of the nitrogenase family and is often used for detection of the nitrogenase gene cassette,[40] was obtained from this γ-proteobacteria.

V. 4-Hydroxycinnamate Decarboxylase Activity of Phylloplane Bacteria

Among higher plants rich in polyphenols, chlorogenic acid-accumulating plants include families such as Convolvulaceae, Compositae, and Umbelliferae, and these typically contain high concentrations of hydroxycinnamyl quinic acids in their leaves and/or roots. We investigated phylloplane bacteria from a representative chlorogenic acid-accumulating plant, *Polymnia sonchifolia*, a member of the Compositae family. In general, the surfaces of healthy, fresh leaves show a low population of phylloplane bacteria, but bacterial populations drastically increase after mechanical injury. Furthermore, these populations quickly disperse all over the leaflet surface along dew drops which develop on the phylloplane during the night. In our experiment, bacterial population on the phylloplane of *P. sonchifolia* was counted as that emerging on potato-dextrose agar plates. Approximately 200 living cells were present on 0.5 mm^2 fresh leaves, while hundreds of times greater bacterial populations were detected in damaged leaves.[41]

Some of the bacteria isolated from the damaged leaves and/or rhizoplane of chlorogenic acid-type plants can decarboxylate 4-hydroxylated *E*-cinnamic acids, partial units of cinnamylated quinic acids with 4-hydroxycinnamate decarboxylase (4-HCD). Specifically, one of the representative leaf epiphytes, *Klebsiella oxytoca*, had a substrate-inducible 4-HCD.[42] As a model of a damaged leaf of a 4-hydroxycinnamic acid-rich plant, 1 mM of 4-hydroxycinnamic acid-containing potato-dextrose agar plate was prepared, and inoculation of the wild *K. oxytoca* was done in a direction perpendic-

ular to previous inoculation of *Fusarium solani* on the agar plate. This led to clear inhibition of hyphal growth only at the region where bacterial colonies and fungal colonies intersected (arrows), and this unique hyphal growth inhibition was observed only in the media which contained 4-hydroxycinnamic acids (Fig. 2).

When we tested the susceptibility of both *K. oxytoca* and *F. solani*, the substrates shown in Fig. 2 were more toxic to the bacterium. In contrast, the decarboxylation products, 4-hydroxystyrenes, clearly inhibited the hyphal growth of eukaryotic organisms, *F. solani* and *Rhizoctonia solani*, but were not toxic to the bacterium.[42] Hence it was hypothesized that some of the phylloplane bacteria are capable of rapid detoxification of secondary metabolites that exude from damaged leaves. Furthermore, these bacteria are also capable of utilizing such detoxified metabolites as chemicals for microfloral regulation. It is, hence, concluded that some leaf epiphytes stabilize phyllosphere microflora and function to calm the injured part from which phytopathogens often invade the plant tissues.

The only quandary that exists for this hypothesis is the fact that *K. oxytoca*, which possesses chlorogenic acid-hydrolytic activity, accumulates 3,4-dihydroxystyrene *via E*-3,4-dihydroxycinnamic acid (*E*-caffeic acid) in the presence of chlorogenic acid.[43] If the induction of *de novo* synthesis of 4-HCD is indeed due to detoxification of 4-hydroxycinnamic acids for the 4-HCD-inducible *K. oxytoca*, it is perplexing if the organism possesses chlorogenic acid-hydrolytic ability. This phenomenon leads to accumulation of *E*-caffeic acids which are toxic to the bacterium itself. Considering the significance of GD activity in some phylloplane bacteria of hydrolyzable tannin-rich plants, it is suggested that 4-HCD inducing bacteria might have a specific significance in phyllopherous microecosystems of chlorogenic acid-rich plants. It has been reported that some bicarboxylic acids are utilized for ATP-production due to H^+ inflow through cytoplasmic membranes which results from H^+ quenching, non-oxidative decarboxylation of the substrate.[44] With respect to ATP production, such decarboxylative properties of the phylloplane bacteria warrant further discussion.

In the early logarithmic phase of the bacterial cell culture, the 4-HCD activity/O.D.$_{660\,nm}$ drastically increased, but after the mid-logarithmic phase the 4-HCD activity/O.D.$_{660\,nm}$ decreased and was maintained at reduced levels through the late logarithmic and stationary phases.[42,43] We conclude that this reduction in 4-HCD activity/O.D.$_{660\,nm}$ occurred because the 4-HCD-inducing substrates in the medium were quickly decomposed by the induced 4-HCD. Hence non-substrate analogs that were able to induce 4-HCD but never decomposed by 4-HCD itself were screened from analogous substances of the *E*-4-hydroxycinnamic acid. In consequence, 6-hydroxy-2-naphthoic acid and 7-hydroxycoumarin 3-carboxylic acid were found.[43] In particular, the former 4-HCD inducer did not exhibit activity as a competitive inhibitor of 4-HCD, and can therefore serve as a desirable 4-HCD inducer.

Since inducing properties became clear for 4-HCD, we attempted to clone the corresponding gene that encodes 4-HCD. Previously we regarded this gene as the same gene as the "pop off-flavor gene", *POF*1, from the wild yeast *Saccharomyces cerevisiae*,[45] but now it is known to be phenylacrylic acid (or simply phenolic acid) decarboxylase (PAD) genes (*PAD*s) from prokaryote. Chromosomal DNA from wild *K. oxytoca* was digested with H*ind*III, and with the resulting genomic fragments in a recombinant plasmid possessing an ampicillin-resistant gene region, a DNA library was made.[46] In order to detect the 4-HCD gene (*4-HCD*, probably a synonym of *PAD*) carrying recombinant DNA in transformed *Escherichia coli* strain JM109, a spore suspension of 4-hydroxystyrene-susceptible *Cladosporium herbarum* AHU9262 was sprayed on solid medium of 5 mM 4-hydroxycinnamic acid, 50 mg/l ampicillin, and heart infusion broth-containing potato-dextrose agar. Using this selection medium, we obtained approximately 2,000 transforming colonies from the library. In particular, three strains of transformants exhibited growth inhibition of fungal growth around the colonies. Despite the fact that the *E. coli* JM109 strain cannot decarboxylate 4-hydroxycinnamates by itself, these positive strains enabled this function to work.

The three transformants possessed either 9.6 kb or 16.6 kb (9.6 + 7.0 kb, an incompletely digested fragment which possessed an internal 9.6 kb H*ind*III site).[46] The full DNA sequence of the 9.6 kb fragment has recently been determined, and one open reading frame within the fragment was characterized as 504 bases of 4-HCD gene with nearly 50% agreement of the coding amino acid sequences with PADs reported from *Bacillus pumilus*,[47] *B. subtilis*,[48] and *Lactobacillus plantarum*,[49] but no similarity to PAD1 of *S. cerevisiae*.[50] Interestingly, these transformed strains possessing the 9.6 kb fragment lost their capability of indole production during exposure to 4-hydroxycinnamates. This observation is consistent with a known tendency for 4-hydroxystyrene producing bacteria also to fail in the production of indole.[51] Moreover, 4-HCD producing bacteria are of two types: one induces 4-HCD dependently on the substrate, and the other has constitutive 4-HCD. To date in our investigation, the latter group has relatively weak 4-HCD activity. Including a substrate-sensing system to induce 4-HCD *de novo* and its relationship with chlorogenic acid-hydrolytic activity, several unknown aspects remain unsolved and warrant further investigation.

VI. Rhizo-Biocomplex Study, Using Improved Soft Gel Medium Developed for Their Physiological Properties and Behaviors in the Rhizosphere

In the rhizosphere, it is likely that micro-environ-

ments vary dependent on root age and root activity. These environmental variations might therefore affect rhizosphereous microfloral components. Microfloral communities which develop on the rhizoplane thus form certain mosaic patterns, as commonly observed in nature,[52] and the rhizospherous microbial communities can maintain this high diversity as well-balanced microflora.[53] For such a rhizospherous ecosystem as mentioned above, we coined the term "rhizo-biocomplex".[54] The rhizo-biocomplex, which is active in element circulation, probably possesses a complicated signal network or food web among the biocomplex composers.

Current research has revealed that many non-leguminous plants also act upon rhizospherous microorganisms *via* secondary metabolic chemicals which are exuded from their roots, compounds which affect (activate or inhibit) the rhizospherous biocomplex. These bioactive chemicals, in which quorum sensing signal molecule mimics,[55–57] quorum sensing signal disturbance,[58] phytosiderophores,[59–61] and soil-bound phosphate solubilizing principles[61,62] are involved, are highlighted in a chemical interaction between plants and microbes.

Hence we set out to identify some easy and convenient tools that would be useful to investigate microorganisms which constitute the rhizoplane microflora. In particular, we were interested in identifying those of nitrogen-fixing bacteria. In our strategy, we used a soft gel medium for observation and evaluation of rhizoplane nitrogen-fixing bacteria, a convenient method for field sampling of nitrogen-fixing bacteria that was first developed by Dobereiner and her coworkers in 1980.[63,64] Dobereiner originally used agar or agarose as gel matrix at a low concentration (0.2%). However, in our studies, we found some inconvenience in this type of gel matrix due to the reduced transparency, inflexible gel structure, and rapidly solidifying nature of this gel composition.

To overcome these inconveniences, we replaced agarose with 0.3% gellan gum,[65] a material originating from polysaccharides produced by *Pseudomonas elodea* ATCC31461 as a bacterial extracellular polymeric substance (EPS).[66,67] A nitrogen-free medium (Winogradsky's salt medium), with 1% glucose as sole carbon source, was initially used with this solidifying gel matrix (0.3%).[65] When this gellan gum is used as the gel matrix, nitrogen-fixing bacteria form a highly transparent colony and/or have the motility to disperse in the soft gel media, and thus become clearly visible. Recently, strain ATCC31461 was shifted from the genus *Pseudomonas* to a member of the genus *Sphingomonas*, family Sphingomonaceae, so this gellan gum-producing bacterium was renamed *Sphingomonas elodea* ATCC31461.[68] Because *Sphingomonas* spp. are frequently isolated as functional rhizobacteria from stressed soil-tolerant plants (see section 7), this fact suggests that gellan gum used for gel matrix in N-free soft gel medium has further advantage in rhizobacterial culture.

In order to investigate and identify functional root-associating nitrogen-fixers, we selected a tropical area in Southeast Asia for our research plots. This area was selected according to our observation and abiding conviction that it represents a region where plants and microbes have created characteristic interrelationships. In fact, functional rhizobacteria were first screened in correlation with their distribution within highly stressed soils, and we have isolated several PGPR-like bacteria from the rhizoplane of stressed soil-tolerant plants.

In field investigations, particularly those that are collaborative works with local researchers, it is necessary to prepare study tools that will ease the process of investigation of functional rhizospherous microfloral communities. For this purpose, the improved nitrogen-free, soft gel medium solidified with gellan gum was found to be a powerful tool for hunting for functional rhizobacteria. Gellan gum has three characteristics which were advantageous for our study: i) the medium can remain in a liquid state for several hours subsequent to autoclaving during the process of cooling down to room temperature,[65] ii) it is able to maintain live cultures of bacteria for relatively long periods of time at room temperature,[65] and iii) gellan gum does not prevent direct PCR reactions for bacterial cell grown in the soft gel medium.[69] This last advantage of gellan gum is of particular importance because the soft gel medium that has trapped oligotrophic and/or diazotrophic, rhizospherous microflora allows us to employ several techniques, such as DNA array, DGGE (denaturing gel gradient electrophoresis),[70] and real time PCR, for analysis of factual microfloral development under oligotrophic conditions.

Our initial target was set to identify free nitrogen-fixing bacteria which inhabit the rhizosphere and/or rhizoplane, because the rhizosphere and rhizoplane are often nitrogen-deficient due to severe competition for nitrogen uptake among plants, saprophytes, and rhizosphere microorganisms. Using this gellan gum-base N-free soft gel medium, we were able accordingly to culture some rhizobacteria. We found that bacterial motility and behaviors were highly affected by mix-culture partners.

In our studies, a non-interfering mix-culture of two bacteria was clearly demonstrated, using two type strains of free-living nitrogen fixing bacterium, *Klebsiella pneumoniae* IFO3318 and *Beijerinckia indica* subsp. *indica* IFO3744.[65] The former α-proteobacteria was a facultatively anaerobic, free-living nitrogen fixing bacterium, while the latter α-proteobacteria was an aerobic nitrogen-fixer. A high population density of *K. pneumoniae* cells resulted in the formation of a micro-aerobic bacterium-like layer in the soft gel medium to an approximate depth of 10 mm from the surface. On the other hand, *B. indica* subsp. *indica* showed aerobic colonization throughout the upper surface of the medium. When these two bacteria were

mixed together and co-inoculated in the soft gel medium, both maintained their characteristic patterns of colony emergence. It was therefore concluded that *K. pneumoniae* and *B. indica* subsp. *indica* rarely affect each other during colony development.

Due to their oligopolistic nutrient availability and/or antibiotic production, many nitrogen-fixers often eliminate not only minor competitors but also true predominant rhizobacteria. In such interfering cases, we observed "true" predominant rhizobacteria, when each of a series of diluted inoculants was added to the soft gel medium and the inoculant was at a threshold or a more diluted level. Therefore, this soft gel method is applicable to the detection of predominant microorganisms that are often regarded as unculturable microfloral composers.

Using 0.5% saccharose as the sole carbon source for this soft gel medium, rhizospherous bacteria were practically screened from some naturally growing young dipterocarps (involving the genera *Shorea* and *Hopea*) that were sampled in the Carita Experimental Forest, West Java, Indonesia. Rhizoplane bacteria that were trapped in the soft gel medium were screened on agar plates of *Azotobacter* medium. Their activities as plant growth-promoting rhizobacteria (PGPR) were tested toward *S. selanica* and some other dipterocarpous seedlings, and we obtained several oligotrophic bacteria functional as PGPR. Due to the collective experiments mentioned above, we conclude that the soft gel medium is applicable for primary screening of functional rhizobacteria.[71)]

VII. Functions of *Sphingomonas* spp. Isolated from Rhizoplane of Acidic Soil-Tolerant Plants in Southeast Asia

Kim *et al.* have used PCR detection and colony counting methods to investigate the population size of *Sphingomonas* spp. Their data indicated that the colonization of *Sphingomonas* spp. is relatively high in young leaves, ears, and floral buds, particularly in Gramineae plants.[72)] These phylloplane-attaching *Sphingomonas* spp. are highly tolerant of direct sunlight, a tolerance that is most likely due to their adaptability to the phylloplane environment which results from the formation of biofilm-like resting cells. These resting cells produce capsular substances and/or extracellular polymeric substances (EPS) that function to shelter the bacterial cells against UV light.[73,74)] In fact, many *Sphingomonas* spp. have abilities to produce EPSs, representative of which is *S. elodea* ATCC31461 (also a synonym of *S. paucimobilis* ATCC31461).[68,75)]

For investigation of microfloral components in the rhizoplane of acid-tolerant plants inhabiting acid-sulfate soil in Central and South Kalimantans, Indonesia, we frequently isolated *Sphingomonas* spp. Using a DNA array plate that we designed, sphingomonads inhabiting the rhizoplane of local rice varieties cultivated on acid-sulfate soil paddocks in South Kalimantan displayed unique behaviors. *Sphingomonas* is a genus of the α-subclass proteobacteria, which displays the unique character of an outer membrane that is mainly composed of sphingolipids. This layer functions to wrap the bacterial cell wall,[76,77)] and occurs instead of lipopolysaccharides and/or capsular polysaccharides that exist for a large group of Gram-negative rods.[78)] Probably in association with this property, the genus *Sphingomonas* involves many biofilm-forming bacteria,[79,80)] xenobiotic-degrading bacteria, including hardly decomposable polysaccharides,[76,77)] and polyaromatics and polychlorophenols.[79,81–83)]

One of the most characteristic typical of the genus *Sphingomonas* is that it produces EPS, as mentioned in section 6. This is representative of a rhizoplane sphingomonad from *Artocarpus champeden* cuttings that were grown on peat soil in Central Kalimantan. In a collaborative study, we found that peat soil is better for growth of *A. champeden* than sandy soil, in contrast with many tropical trees.[84)] After 3 months in a nursery station at the University of Palangka Raya, the young *A. champeden* seedlings grew well, to over 2 meters, in peat soil pots, while they were dwarfed at approximately 30 cm in sandy soil. Due to these differences, we were interested to compare root system and rhizoplane microfloral compositions. Unexpectedly, *A. champeden* grown on peat soil exhibited a relatively poor root system and less mycorrhizal development than that grown on sandy soil. However, investigation of rhizobacteria revealed that *A. champeden* grown on peat soil contained a powerful EPS-producing *Sphingomonas* sp. in association with it.

In Winogradsky's mineral salt mixture, many free-living nitrogen fixers, including *Klebsiella pneumoniae* and *Beijerinckia indica* subsp. *indica,* are capable of turning the medium into an acidic composition ($<$ pH 3.0) due to the production of organic acids in the presence of sugar containing nitrogen-free medium. Unlike these representative free-living nitrogen fixing bacteria, *Sphingomonas* spp. found as rhizobacteria of acidic soil-tolerant plants often had the ability to adjust the pH of N-free cultured medium into neutral regions. Even in acidic medium that was adjusted to a pH of 4.0 or less with sulfuric acid, some rhizospherous *Sphingomonas* spp. increased the pH of the medium to neutral regions (*ca.* 5.5–6.0) within 4 weeks.

In *Xyris complanata*, *Sphingomonas* spp. and *Frateuria* spp. were specifically isolated from the rhizosphere and rhizoplane respectively. Although *Frateuria* spp. were able to grow efficiently in nutrient broth media, they exhibited poor growth in Winogradsky's mineral salt mixture. In contrast, *Sphingomonas* spp. were oligotrophic and able to grow well in Winogradsky's mineral salt mixture, but they failed to grow in nutrient broth medium. When PCR applications were done to the isolates for specific detection of the *nifH* region and a separate acetylene reduction assay was performed, three

Sphingomonas isolates showed positive responses in these tests, while none of *Frateuria* spp. showed any nitrogen-fixing ability in the acetylene reduction assay.

Since the peat soil in Central Kalimantan showed an extraordinary low pH (pH 3.0–3.7), it was examined whether *Sphingomonas* spp. and *Frateuria* spp. exhibited an adaptation and/or tolerance to such acidic conditions. In a modified Winogradsky's mineral salt mixture liquid medium adjusting pH to 3.0, *Sphingomonas* spp. grew well, while *Frateuria* spp. did not. The soil conditions where *X. complanata* had established a pure community was nutrient-poor and acidic (pH 3.1–3.3), but the rhizoplane pH of the plants was closer to a neutral region.[4)] The rhizoplane comprises a limited space where available organic matter is most likely to be relatively rich, but *Frateuria* spp. are auxotrophic and therefore require excessive nutrients. Considering the properties and behaviors of *Sphingomonas* spp., it is reasonable to consider that *Sphingomonas* spp. are an important director to develop the rhizospherous microfloral community of *X. complanata*. Although *Frateuria* sp. has shown none of the functional behaviors, this bacterium is also speculated to have a role(s) because of its dominant occupation in the rhizoplane niche and growth promotion with the assistance of *Sphingomonas* spp.

In a co-inoculation assay, some *Sphingomonas* spp. showed growth promotion activity toward *Frateuria* sp. that was impregnated in an agar plate comprised of modified Winogradsky's mineral mixture plus 0.5% saccharose. Thus, *Frateuria* sp. was highly dependent on the nitrogen-fixing *Sphingomonas* spp. as to pH adjustment and nutrient supply. For one of the predominant *Frateuria* spp., there was no indication that any of them functioned as PGPRs in *X. complanata* in our investigation. In contrast, it is clear that *Sphingomonas* sp. contributes to increases in microfloral diversity in the rhizoplane. It is also known that a *Sphingomonas* sp. isolated from certain activated sludge enabled an unculturable bacterium to become culturable in a mixed culture.[85)]

A *Sphingomonas* sp., isolated from *Melastoma malabathricum* and tentatively identified as *S. rosa*, was tested for growth promotion activity toward *M. malabathricum* seedlings planted in a vermiculite bed imbibed with N-free Hoagland's no. 2 medium that had been acidified to pH 3.0 with a 2 M H_2SO_4 solution and subsequently autoclaved. Five seedlings of *M. malabathricum*, briefly rinsed with 70% ethanol and followed by a soak in sterilized water, were planted in the vermiculite bed. *S. rosa* EC-K013 was generated on modified Winogradsky's solid medium, and a bacterial cell suspension consisting of 1×10^5 cells/ml was used as the inoculum for the vermiculite bed. After 3 weeks, *S. rosa*-inoculated seedlings exhibited growth promotion. After 8 weeks, the harvested *S. rosa*-inoculated seedlings not only showed a growth promotion effect in their aerial parts, but enhanced growth was also detected in their root systems.[86)] This sphingomonad also enhanced the microfloral diversity of the root system of the test seedlings and allowed the emergence of a root-associating fungus and two oligotrophic nitrogen-fixing bacteria. In a phylogenetic study using ITS1 and ITS4 primers,[87)] this fungus was thought to be a probable new genera close to the genus *Nectria*. On the other hand, one of the oligotrophic nitrogen-fixers was identified to be *Mesorhizobium* sp.

From the rhizoplane of spinach seedlings, we isolated *S. yanoikuyae* EC-S001 and found that it is a root-associating bacterium that regulates its cell division with a relatively high threshold level of Mg^{2+}.[88)] The threshold level for normal cell growth was calculated to be 0.10 mM, the concentration of which is higher than that in potato-dextrose broth medium. *S. yanoikuyae* EC-S001 shows characteristic uniform dispersal and attachment throughout the root epidermal surface of spinach seedlings, regardless of strong aggregation in spinach leaf extract medium. Hence we speculate that the population size of EC-S001 in the rhizoplane and/or rhizosphere is highly regulated by the plant root in response to the concentration of Mg^{2+}.[88)]

Thus, our collective data and observations on rhizospherous *Sphingomonas* spp. suggest that certain members of the genus *Sphingomonas* adjust the rhizosphere

Table 1. Phytoepiphytic *Sphingomonas* spp. and Their Function in Their Specific Niches

Isolation source (isolated part)	Soil type	Function and ability
Oryza sativa (rhizoplane)	Acid sulfate soil	Nitrogen fixation, rhizosphere neutralization
Melastoma malabathricum (rhizoplane)	Acid sulfate soil	Nitrogen fixation, fungal growth promotion
Combretocarpus rotundatus (slime on aerial root)	Tropical peat, with leaching	Slime-forming promotion
Screlia sumatrensis (stem marrow)	Tropical swampy peat	Stimulation of nitrogen fixation
Artocarpus champeden (rhizoplane)	Tropical peat, with leaching	Organic matter-sink formation
Xyris complanata (rhizosphere)	Tropical peat, without top soil	Nitrogen fixation, rhizobacterial growth promotion

to create a more adaptable condition for plants that inhabit stressed soils (Table 1). The specific details are currently under preparation for a manuscript to submitted elsewhere.

VIII. Interaction between Rhizobacteria and Root *via* Root Exudates: Case Studies of Some Rhizoplane Bacteria from White Lupin and Chenopodiaceae

When grown under phosphate-deficient conditions, white lupin (*Lupinus albus*) forms cluster roots that produce organic acids and mucilaginous polymers that function to acidify rhizospherous soil and solubilize soil-bound phosphate.[89] It is also known that the presence of soil microorganisms is necessary for cluster root development.[90] On the other hand, when white lupin is exposed to the stress of nitrogen-deficiency, it specifically releases 2′-hydroxylupalbigenin (**8**) from the root. This diprenylated isoflavone is known to be a *Nod* gene inducer and also a chemoattractant toward *Rhizobium lupini*, the symbiotic nodulation bacterium for white lupin.[91] It has been found that *Nod* gene inducers, mainly flavonoidal compounds, are exuded from most leguminous plants.[92,93] *Nod* genes are important, active genes that regulate production of Nod factor, a low molecular fragment of lipooligochitosaccharide produced by the bacterium.[94] When leguminous host plants received this chemical signal at the root surface, the root hairs curl and branch, and mitosis actively occurs in the cortex under the root hairs to allow nodulation. Thus *Nod* genes determine the host-specificity of the symbiotic nodulation bacteria. Aside from this, it is possible to regard *Nod* gene inducers as SOS signals that can attract any potent, functional rhizobacteria to the host plants.

For 7-day-old seedlings of white lupin grown in nutrient-free pots, only after they were exposed to 200 mg/l $CaHPO_4$-containing ion-exchanged water, they also started to release compound **8** from the roots. Hence we concluded that rhizospherous microfloral composers acidifying their microhabitats in P-deficient medium and showing positive chemotaxis toward compound **8** would serve as an indication of rhizo-functional microbes. Accordingly, non-symbiotic but PGPR-like rhizobacteria were screened from the rhizosphere of young white lupin. For a preliminary screen among 25 microbes separated from white lupin seedlings that were grown on river sand pots, 10 isolates acidified a pH 5.5 Hoagland's no. 2-based soft gel medium to pH 3.0 or less. Meanwhile, 4 out of the 25 microbial isolates showed positive chemotactic responses toward 5 μg of compound **8**, of which 3 isolates (*Herbaspirillum* sp., *Arthrobacter* sp., and one unidentified) had the ability to acidify a medium of 1/2 Hoagland's S plus 100 mg/l insoluble $AlPO_4$ powders (pH 5.5).

For a preliminary investigation, those four strains which exhibited a chemotactic response to compound **8** were each inoculated as 10^4–10^5 cells to imbibed, surface-sterilized white lupin seeds. These lupin seeds were allowed to germinate and were subsequently grown on a vermiculite bed with watering regimes consisting only of ion-exchanged water for 6 weeks. Control plants showed severe stunting and yellowing due to nitrogen deficiency, whereas the two groups of seedlings that were inoculated with *Herbaspirillum* sp. and the unidentified microbe respectively maintained their deep-green leaf color after 6 weeks. Futhermore, these plants did not exhibit any stunting nor nitrogen deficiency symptoms. Thus our preliminary data support the notion that white lupin utilizes diprenylated isoflavone **8** which is exuded from the root to attract not only nodulation bacteria, but also some free-living and rhizofunctional microorganisms.

An additional finding we report here is specific colonization that was associated with effective antibiotic production of a rhizobacterium on the root of Chenopodiaceae plants. An antagonistic rhizobacterium, SB-K88, produced a tetramic acid moiety-containing macrolactam antibiotic, xanthobaccin A (**9**), the stereochemistry of which is still ambiguous at this time.[95,96] SB-K88 was isolated by Homma *et al.* from the fibrous roots of sugar beet grown in a field heavily infested with rhizomania.[97] This rhizobacterium was recently identified as *Lysobacter* sp. by means of 16S rRNA gene sequence (accession no. AB190258) and transmission electron microscopic observation.[98] The best performance for yield of **9** was recorded in PS medium (potato extract, sucrose, $CaNO_3$, and NaH_2PO_4) by Nakayama,[95] in which 130 mg was produced from 15-liter of bacterial culture fluid as the best performance. However, reproducibility of the antibiotic yield was not observed and its productivity often stayed at lower levels (less than 2 mg/l).

When SB-K88 was inoculated into the rhizosphere of sugar beet (*Beta vulgaris*, Chenopodiaceae) seedlings, however, antibiotic production per bacterial cell population was calculated to be $1 \times 10^{4-5}$ fold-higher in the rhizosphere than in the liquid media.[95] Scanning electron microscopic observation of SB-K88 colonization was done in a gnotobiotic system of sugar beet seedlings that were grown in gellan gum-base soft gel medium of Hoagland's S medium. Subsequently, characteristic perpendicular attachments of the rod-shaped SB-K88 cells on the rhizoplane were observed.[98] Hence, we concluded that this perpendicular cell attachment and the successive development of a polysaccharide-like polymeric envelop, which resulted in the formation of a biofilm-like architecture, resulted in effective antibiotic production by SB-K88 (Fig. 3). We concluded furthermore that this phenomenon is highly associated with quorum sensing signal mimics from sugar beet seedlings.

When the concentration of the endogenous quorum sensing signal molecule (*N*-acyl L-homoserine lactone, AHL) exceeds the threshold level in a medium, the

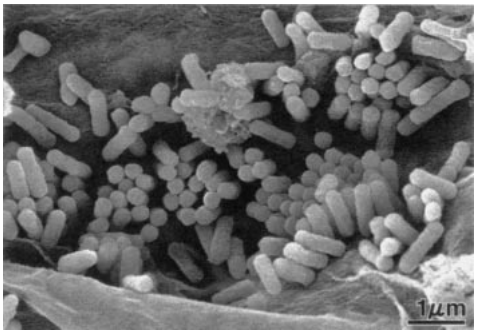


Fig. 3. SEM Observation of Gnotobiotic Rhizoplane of Sugar Beet Seedling Inoculated with *Lysobacter* sp. SB-K88.

Sugar beet seedlings were grown from surface sterilized seeds that were grown in 1/2 Hoagland's S medium solidified with 0.3% gellan gum. The gnotobiote was nearly indistinguishable from control seedlings. This clearly shows that SB-K88 is not phytopathogenic, even in the gnotobiotic condition. The mature bacterial cells perpendicularly attached on the rhizoplane most likely produced polymeric substances that functioned to form a biofilm-like community.

bacterial community begins to form a biofilm.[99] Specialized bacteria within the biofilm are characteristically able to exhibit certain abilities, such as xenobiotic degradation, EPS production, and antibiotic production. It has been found recently that the roots of some plants release agonists of quorum sensing signal molecules and regulate rhizospherous bacteria. Such AHL-agonistic compounds, designated quorum sensing signal mimics, stop random cell division of the rhizospherous bacteria and induce biofilm formation.

In recent times, studies related to the attractive topic of quorum sensing signals of rhizosphere bacteria are becoming more prevalent.[100] For example, root exudates of two plants (pea and crown vetch) inhibited AHL-induced violacetin synthesis and also induced bacterial swarming, both of which responses are regarded as responses of AHL reporters for *Chromobacterium violaceum* CV026.[55] The discovery of quorum-sensing signal mimics from plant roots[55,56] indicates that plants possess reliable systems that function positively to regulate the population size, physiological behaviors, and function of rhizobacteria to levels that are greater than we ever believed previously. Now we are focusing on rhizoplane bacteria and quorum-sensing signal mimics of Chenopodiaceae plants that are known as representative non-arbuscular mycorrhizal plants. Such quorum sensing signal mimics from the plant root would be a powerful tool in rhizosphere regulation technology.

IX. Concluding Remarks

Considering the importance of the communication web in rhizospherous microcosms, all of the studies reviewed here are based on chemical substances in order to understand the natural ecosystem with respect to chemical ecology. The plant surface, which is characterized by specific metabolites of the host plant, creates specific and unique micro-scale habitats for epiphytes. Particularly in the rhizosphere and rhizoplane, the microfloral community does not consist of single species. Rather, several genera and species of microbes exist in this space and collectively form the rhizobiocomplex. Currently, rhizosphere regulation is regarded as one of the important agro-technologies that might enable us to reduce the impact of agricultural crop production on the global environment.[8,101,102] More importantly, this technology might be applicable to management of highly stressed soils and might shift low productive land to highly productive and highly sustainable arable land.

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References

1) Hirano, S. S., and Upper, C. D., Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae*—a pathogen, ice nucleus, and epiphyte. *Microbiol. Mol. Biol. Rev.*, **64**, 624–653 (2000).
2) Denison, R. F., Bledsoe, C., Kahn, M., O'Gara, F., Simms, E. L., and Thomashow, L. S., Cooperation in the rhizosphere and the "free rider" problem. *Ecology*, **84**, 838–845 (2003).
3) Hashidoko, Y., Tahara, S., and Mizutani, J., New complex isoflavones in the roots of yellow lupin (*Lupinus luteus* L., cv. Barpine). *Agric. Biol. Chem.*, **50**, 1797–1807 (1986).
4) Hashidoko, Y., Hasegawa, T., Purnomo, E., Tada, M., Limin, S. H., Osaki, M., and Tahara, S., Neutral rhizoplane pH of local rice and some predominant tree species in South and Central Kalimantans: a possible strategy of plant adaptation to acidic-soil. *Tropics*, **14**, 139–147 (2005).
5) Yadav, R. K. P., Halley, J. M., Karamanoli, K., Constantinidou, H. I., and Vokou, D., Bacterial populations on the leaves of Mediterranean plants: quantitative features and testing of distribution models. *Environ. Exp. Bot.*, **52**, 63–77 (2004).
6) Lindow, S. E., and Brandl, M. T., Microbiology of the phyllosphere. *Appl. Environ. Microbiol.*, **69**, 1875–1883 (2003).
7) Lindemann, J., and Upper, C. D., Aerial dispersal of epiphytic bacteria over bean plants. *Appl. Environ.*

Microbiol., **50**, 1229–1232 (1985).
8) Walker, T. S., Bais, H. P., Grotewold, E., and Vivanco, J. M., Root exudation and rhizosphere biology. *Plant Physiol.*, **132**, 44–51 (2003).
9) Farrar, J., Hawes, M., Jones, D., and Lindow, S., How roots control the flux of carbon to the rhizosphere. *Ecology*, **84**, 827–837 (2003).
10) Longman, K. A., and Jenik, J., "Tropical Forest and Its Environment" 2nd ed., Longman Scientific and Technical, New York (1987).
11) Savka, M. A., Dessaux, Y., Oger, P., and Rossbach, S., Engineering bacterial competitiveness and persistence in the phytosphere. *Mol. Plant-Microbe Interact.*, **15**, 866–874 (2002).
12) Wagner, G. J., Secreting glandular trichomes: more than just hairs. *Plant Physiol.*, **96**, 675–679 (1991).
13) Hashidoko, Y., Bioconversion of plant secondary metabolites by predominant epiphytic microorganisms, and allelopathic effect of these bioconverted chemicals (in Japanese). *Proceedings of 1st Vegetation Management Workshop, Tsukuba, 31 Oct., 1997*, pp. 58–64 (1997).
14) Harborne, J. B., "Introduction to Ecological Biochemistry", Academic Press, London, pp. 186–210 (2002).
15) Shibatani, M., Hashidoko, Y., and Tahara, S., A major fungitoxin of *Pachira aquatica* and its accumulation in the outer bark. *J. Chem. Ecol.*, **25**, 347–353 (1999).
16) Shibatani, M., Hashidoko, Y., and Tahara, S., Accumulation of isohemigossypolone and its related compounds in inner bark and heartwood of diseased *Pachira aquatica*. *Biosci. Biotechnol. Biochem.*, **63**, 1777–1780 (1999).
17) Kistner, C., and Parniske, M., Evolution of signal transduction in intracellular symbiosis. *Trends Plant Sci.*, **7**, 511–518 (2002).
18) Hashidoko, Y., Tahara, S., and Mizutani, J., Antimicrobial sesquiterpene from damaged *Rosa rugosa* leaves. *Phytochemistry*, **28**, 425–430 (1989).
19) Hashidoko, Y., Iwaya, N., Tahara, S., and Mizutani, J., Absolute configuration of rugosal A, an endoperoxy carotanoid sesquiterpene. *Agric. Biol. Chem.*, **53**, 2505–2507 (1989).
20) Hashidoko, Y., Tahara, S., and Mizutani, J., Carota-1,4-dienaldehyde, a sesquiterpene from *Rosa rugosa*. *Phytochemistry*, **29**, 867–872 (1990).
21) Hashidoko, Y., Tahara, S., and Mizutani, J., Identification of an intermediate in autoxidation of carota-1,4-dien-14-al into rugosal A. *J. Chem. Soc., Perkin Trans. 1*, 211–214 (1991).
22) Hashidoko, Y., Tahara, S., and Mizutani, J., Autoxidation study of carotane sesquiterpenes possessing a non-conjugated 1,4-diene system. *J. Chem. Soc., Perkin Trans. 1*, 2351–2356 (1993).
23) Hashidoko, Y., Tahara, S., and Mizutani, J., Carotanoids and an acoranoid from *Rosa rugosa* leaves. *Phytochemistry*, **30**, 3729–3739 (1991).
24) Hashidoko, Y., Tahara, S., and Mizutani, J., Novel bisabolanoids in *Rosa rugosa* leaves. *Zeitschrift fur Naturforschung*, **46c**, 349–356 (1991).
25) Hashidoko, Y., The phytochemistry of *Rosa rugosa*. *Phytochemistry*, **43**, 535–549 (1996).
26) Hashidoko, Y., Tahara, S., and Mizutani, J., Rugosal A and related carotane sesquiterpenes in the glandular trichome exudate of *Rosa rugosa*. *Phytochemistry*, **31**, 779–782 (1992).
27) Hashidoko, Y., and Urashima, M., Efficient preparation of browning-free glandular trichomes from the surface of leaves of *Rosa rugosa* Thunb. *Plant Cell Physiol.*, **36**, 127–132 (1995).
28) Hashidoko, Y., Endoh, K., Kudo, T., and Tahara, S., Capability of wild *Rosa rugosa* and its varieties and hybrids to produce sesquiterpene components in the leaf glandular trichomes. *Biosci. Biotechnol. Biochem.*, **65**, 2037–2043 (2001).
29) Hashidoko, Y., Endoh, K., Kudo, T., and Tahara, S., (+)-4-*epi*-α-Bisabolol as a major constituent in the glandular trichome exudates of two *Rosa rugosa* hybrids, Martin Frobisher and Vanguard. *Biosci. Biotechnol. Biochem.*, **64**, 907–910 (2000).
30) Villegas, L. F., Marcalo, A., Martin, J., Fernandez, I. D., Maldonado, H., Vaisberg, A. J., and Hammond, G. B., (+)-*epi*-α-Bisabolol is the wound-healing principle of *Peperomia galioides*: investigation of the *in vivo* wound-healing activity of related terpenoids. *J. Nat. Prod.*, **64**, 1357–1359 (2001).
31) Itokawa, H., Watanabe, K., Morita, H., Mihashi, S., and Iitaka, Y., A novel sesquiterpene peroxide from *Alpinia japonica* (Thunb.) Miq. *Chem. Pharm. Bull.*, **33**, 2023–2027 (1985).
32) Zeida, M., Wieser, M., Yoshida, T., Sugio, T., and Nagasawa, T., Purification and characterization of gallic acid decarboxylase from *Pantoea agglomerans* T71. *Appl. Environ. Microbiol.*, **64**, 4743–4747 (1998).
33) Hashidoko, Y., Itoh, E., Yokota, K., Yoshida, T., and Tahara, S., Characterization of five phyllosphere bacteria isolated from *Rosa rugosa* leaves, and their phenotypic and metabolic properties. *Biosci. Biotechnol. Biochem.*, **66**, 2474–2478 (2002).
34) Brenner, D. J., Section 5, Facultatively anaerobic gram-negative rods. In "Bergey's Manual of Systematic Bacteriology" 8th ed. Vol. 1, eds. Krieg, N. R., and Holt, J. G., Williams and Wilkins, Baltimore, pp. 408–600 (1984).
35) Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T., and Williams, S. T., Group 5, Facultatively anaerobic gram-negative rods. In "Bergey's Manual of Determinative Bacteriology" 9th ed., Williams and Wilkins, Baltimore, pp. 175–289 (1994).
36) Jordan, D. C., Rhizobiaceae Gonn 1938, 321AL. *Phyllobacterium* (ex Knosel 1962) nom. rev. In "Bergey's Manual of Systematic Bacteriology" 8th ed. Vol. 1, eds. Krieg, N. R., and Holt, J. G., Williams and Wilkins, Baltimore, pp. 254–256 (1984).
37) Mergaert, J., Cnockaert, M. C., and Swings, J., *Phyllobacterium myrsinacearum* (subjective synonym *Phyllobacterium rubiacearum*) emend. *Int. J. Sys. Evol. Microbiol.*, **52**, 1821–1823 (2002).
38) Gonzalez, B. L. E., Lebsky, V. K., Hernandez, J. P., Bustillos, J. J., and Bashan, Y., Changes in the metabolism of the microalga *Chlorella vulgaris* when coimmobilized in alginate with nitrogen-fixing *Phyllobacterium myrsinacearum*. *Can. J. Microbiol.*, **46**, 653–659 (2000).
39) Rojas, A., Holguin, G., Glick, B. R., and Bashan, Y., Synergism between *Phyllobacterium* sp. (N_2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a

semiarid mangrove rhizosphere. *FEMS Microbiol. Ecol.*, **35**, 181–187 (2001).
40) Ohkuma, M., Noda, S., Usami, R., Horikoshi, K., and Kudo, T., Diversity of nitrogen fixation genes in the symbiotic intestinal microflora of the termite *Reticulitermes speratus*. *Appl. Environ. Microbiol.*, **62**, 2747–2752 (1996).
41) Hashidoko, Y., Urashima, M., and Yoshida, T., Predominant epiphytic bacteria on damaged *Polymnia sonchifolia* leaves, and their metabolic properties on phenolics of plant origin. *Biosci. Biotechnol. Biochem.*, **58**, 1894–1896 (1994).
42) Hashidoko, Y., Urashima, M., Yoshida, T., and Mizutani, J., Decarboxylative conversion of hydroxycinnamic acids by *Klebsiella oxytoca* and *Erwinia uredovora*, epiphytic bacteria of *Polymnia sonchifolia* leaf, possibly associated with formation of microflora on the damaged leaves. *Biosci. Biotechnol. Biochem.*, **57**, 215–219 (1993).
43) Hashidoko, Y., Tanaka, T., and Tahara, S., Induction of 4-hydroxycinnamate decarboxylase in *Klebsiella oxytoca* cells exposed to substrate and non-substrate 4-hydroxycinnamate analogs. *Biosci. Biotechnol. Biochem.*, **65**, 2604–2612 (2001).
44) Marty-Teysset, C., Posthuma, C., Lolkema, J. S., Schmitt, P., Divies, C., and Konings, W. N., Proton motive force generation by citrolactic fermentation in *Leuconostoc mesenteroides*. *J. Bacteriol.*, **178**, 2178–2185 (1996).
45) Goodey, A. R., and Tubb, R. S., Genetic and biochemical analysis of the ability of *Saccharomyces cerevisiae* to decarboxylate cinnamic acids. *J. General Microbiol.*, **128**, 2615–2620 (1982).
46) Hashidoko, Y., Urashima, M., and Mizutani, J., Cloning of a DNA fragment carrying the 4-hydroxycinnamate decarboxylase (*Pof* K) gene from *Klebsiella oxytoca*, and its constitutive expression in *Escherichia coli* JM109 cells. *Biosci. Biotechnol. Biochem.*, **58**, 217–218 (1994).
47) Zago, A., Degrassi, G., and Bruschi, C. V., Cloning, sequencing, and expression in *Escherichia coli* of the *Bacillus pumilus* gene for ferulic acid decarboxylase. *Appl. Environ. Microbiol.*, **61**, 4484–4486 (1995).
48) Cavin, J.-F., Dartois, V., and Divies, C., Gene cloning, transcriptional analysis, purification, and characterization of phenolic acid decarboxylase from *Bacillus subtilis*. *Appl. Environ. Microbiol.*, **64**, 1466–1471 (1998).
49) Cavin, J.-F., Barthelmebs, L., and Divies, C., Molecular characterization of an inducible *p*-coumaric acid decarboxylase from *Lactobacillus plantarum*: gene cloning, transcriptional analysis, overexpression in *Escherichia coli*, purification, and characterization. *Appl. Environ. Microbiol.*, **63**, 1939–1944 (1997).
50) Clausen, M., Lamb, C. J., Megnet, R., and Doerner, P. W., *PAD1* encodes phenylacrylic acid decarboxylase which confers resistance to cinnamic acid in *Saccharomyces cerevisiae*. *Gene*, **142**, 107–112 (1994).
51) Lindsay, R. F., and Priest, F. G., Decarboxylation of substituted cinnamic acids by Enterobacteria: the influence on beer flavour. *J. Appl. Bacteriol.*, **39**, 181–187 (1975).
52) Nowak, M. A., and Sigmund, K., Bacterial game dynamics. *Nature*, **418**, 138–139 (2002).
53) Kerr, B., Riley, M. A., Feldman, M. W., and Bohannan, J. M., Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature*, **418**, 171–174 (2002).
54) Hashidoko, Y., and Tahara, S., Rhizo-biocomplex: an important concept to regulate rhizospherous environment for sustainable crop production. In "Proceedings of International Conference on 'Sustainable Crop Production in Stress Environments: Management and Genetic Options', JNKVV, Jabalpur, India, February 9–12, 2005", eds. Tomar, V. S., and Behl, R. S., in press.
55) Teplitski, M., Robinson, J. B., and Bauer, W. D., Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol. Plant Microbe Interact.*, **13**, 637–648 (2000).
56) Bauer, W. D., and Teplitski, M., Can plants manipulate bacterial quorum sensing? *Aust. J. Plant Physiol.*, **28**, 913–921 (2001).
57) Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anolles, G., Rolfe, B. G., and Bauer, W. D., Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 1444–1449 (2003).
58) Huber, B., Eberl, L., Feucht, W., and Polster, J., Influence of polyphenols on bacterial biofilm formation and quorum-sensing. *Z. Naturforsch.*, **58c**, 879–884 (2003).
59) Takagi, S., Nomoto, K., and Takemoto, T., Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plants. *J. Plant Nutr.*, **7**, 469–477 (1984).
60) Singh, K., Sasakuma, T., Bughio, N., Takahashi, M., Nakanishi, H., Yoshimura, E., Nishizawa, N. K., and Mori, S., Ability of ancestral wheat species to secrete mugineic acid family phytosiderophores in response to iron deficiency. *J. Plant Nutr.*, **23**, 1973–1981 (2000).
61) Otani, T., Ae, N., and Tanaka, H., Phosphorus (P) uptake mechanisms of crops grown in soils with low P status: II. Significance of organic acids in root exudates of pigeonpea. *Soil Sci. Plant Nutr.*, **42**, 553–560 (1996).
62) Strom, L., Root exudation of organic acids: importance to nutrient availability and the calcifuge and calcicole behaviour of plants. *Oikos*, **80**, 459–466 (1997).
63) Dobereiner, J., Forage grasses and grain crops. In "Methods for Evaluation Biological Nitrogen Fixation", ed. Bergersen, F. J., John Wiley and Sons, Chichester, pp. 535–555 (1980).
64) Dobereiner, J., Isolation and identification of aerobic nitrogen-fixing bacteria from soil and plants. In "Methods in Applied Soil Microbiology and Biochemistry", eds. Alef, K., and Nannipieri, P., Academic Press, London, pp. 134–141 (1995).
65) Hashidoko, Y., Tada, M., Osaki, M., and Tahara, S., Soft gel medium solidified with gellan gum for preliminary screening for root-associating, free-living nitrogen-fixing bacteria inhabiting the rhizoplane of plants. *Biosci. Biotechnol. Biochem.*, **66**, 2259–2263 (2002).
66) Kang, K. S., Veeder, G. T., Mirrasoul, P. J., Kaneko,

T., and Cottrell, I. W., Agar-like polysaccharide produced by *Pseudomonas* species: production and basic properties. *Appl. Environ. Microbiol.*, **43**, 1086–1091 (1982).

67) Kang, K. S., and Veeder, G. T., U.S. Patent 4377636 (Mar. 22, 1983).

68) Harding, N. E., Patel, Y. N., and Coleman, R. J., Organization of genes required for gellan polysaccharide biosynthesis in *Sphingomonas elodea* ATCC 31461. *J. Indust. Microbiol. Biotechnol.*, **31**, 70–82 (2004).

69) Rath, P. M., and Schmidt, D., Gellan gum as a suitable gelling agent in microbial media for PCR applications. *J. Med. Microbial.*, **50**, 108–109 (2001).

70) Yang, C.-H., Crowley, D. E., Borneman, J., and Keen, N. T., Microbial phyllosphere populations are more complex than previously realized. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 3889–3894 (2001).

71) Sitepu, I. R., Aryanto, Ogita, N., Osaki, M., Santoso, E., Tahara, S., and Hashidoko, Y., Screening and further investigation of plant growth promoting rhizobacteria from dipterocarpous youngsters. *Tropics*, submitted.

72) Kim, H., Kunito, T., Senoo, K., Kawahara, K., Murakami, K., and Oyaizu, H., High population of *Sphingomonas* species on plant surface. *J. Appl. Microbiol.*, **85**, 731–736 (1998).

73) Morris, C. E., Monier, J. M., and Jacques, M. A., A technique to quantify the population size and composition of the biofilm component in communities of bacteria in the phyllosphere. *Appl. Environ. Microbiol.*, **64**, 4789–4795 (1998).

74) Elasri, M. O., and Miller, R. V., Study of the response of a biofilm bacterial community to UV radiation. *Appl. Environ. Microbiol.*, **65**, 2025–2031 (1999).

75) West, T. P., and Fullenkamp, N. A., Ability of casamino acids to support gellan production by *Sphingomonas paucimobilis* ATCC 31461. *Microbios*, **102**, 89–101 (2000).

76) Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T., and Yamamoto, H., Proposals of *Shingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas. Microbiol. Immunol.*, **34**, 99–119 (1990).

77) Hashimoto, W., Momma, K., Miki, H., Mishima, Y., Kobayashi, E., Miyake, O., Kawai, S., NanKai, H., Mikami, B., and Murata, K., Enzymatic and genetic bases of assimilation, depolymerization, and transport of heteropolysaccharides in bacteria. *J. Biosci. Bioengin.*, **87**, 123–136 (1999).

78) Logan, N. A., "Bacterial Systematics", Blackwell Scientific Publications, Oxford, pp. 13–34 (1994).

79) Coughlin, M. F., Kinkle, B. K., and Bishop, P. L., Degradation of acid orange 7 in an aerobic biofilm. *Chemosphere*, **46**, 11–19 (2002).

80) Koskinen, R., Ali-Vehmas, T., Kaempfer, P., Laurikkala, M., Tsitko, I., Kostyal, E., Atroshi, F., and Salkinoja-Salonen, M., Characterization of *Sphingomonas* isolates from Finnish and Swedish drinking water distribution systems. *J. Appl. Microbiol.*, **89**, 687–696 (2000).

81) Moore, E., Genetic and serological evidence for the recognition of four pentachlorophenol-degrading bacterial strains as a species of the genus *Sphingomonas*. *Syst. Appl. Microbiol.*, **18**, 539–548 (1995).

82) Wittmann, C., Zeng, A. P., and Deckwer, W. D., Physiological characterization and cultivation strategies of the pentachlorophenol-degrading bacteria *Sphingomonas chlorophenolica* RA2 and *Mycobacterium chlorophenolicum* PCP-1. *J. Indust. Microbiol. Biotechnol.*, **21**, 315–321 (1998).

83) Sorensen, S. R., Ronen, Z., and Aamand, J., Isolation from agricultural soil and characterization of a *Sphingomonas* sp. able to mineralize the phenylurea herbicide isoproturon. *Appl. Environ. Microbiol.*, **67**, 5403–5409 (2001).

84) Hashidoko, Y., Kitso, Sampang, Osaki, M., Melling, L., and Tahara, S., Utilization of epiphytic and endophytic bacteria for reforestation sustainable, hydrophilous agriculture management of wet, abandoned tropical peat land to protect peat soil and swampy forest from fire. Proceedings of International Workshop on Human Dimension of Tropical Peatland under Global Environmental Changes, December 8–9, 2004, Bogor, Indonesia, eds. Wijaya, H., and Osaki, M., in press.

85) Tanaka, Y., Hanada, S., Manome, A., Tsuchida, T., Kurane, R., Nakamura, K., and Kamagata, Y., *Catellibacterium nectariphilum* gen. nov., sp. nov., which requires a diffusible compound from a strain related to the genus *Sphingomonas* for vigorous growth. *Int. J. Sys. Evol. Microbiol.*, **54**, 955–959 (2004).

86) Osaki, M., Wasaki, J., Hashidoko, Y., Shinano, T., Purnomo, E., Hasegawa, T., Yamagishi, M., Watanabe, T., Yamamura, T., Honma, S., and Tahara, S., Novel technology of rhizosphere regulation to grow plants in adverse soil- a review. *Plant Soil*, submitted.

87) Gardes, M., and Bruns, T. D., ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. *Mol. Ecol.*, **2**, 113–118 (1993).

88) Hoo, H., Hashidoko, Y., Islam, M. T., and Tahara, S., Requirement of a relatively high threshold level of Mg^{2+} for cell growth of a rhizoplane bacterium, *Sphingomonas yanoikuyae* EC-S001. *Appl. Environ. Microbiol.*, **70**, 5214–5221 (2004).

89) Neumann, G., Massonneau, A., Langlade, N., Dinkelaker, B., Hengeler, C., Romheld, V., and Martinoia, E., Physiological aspects of cluster root function and development in phosphorus-deficient white lupin (*Lupinus albus* L.). *Ann. Bot.*, **85**, 909–919 (2000).

90) Marschner, P., Neumann, G., Kania, A., Weiskopf, L., and Lieberei, R., Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (*Lupinus albus* L.). *Plant Soil*, **246**, 167–174 (2002).

91) Gagnon, H., Grandmaison, J., and Ibrahim, R. K., Phytochemical and immunocytochemical evidence for the accumulation of 2′-hydroxyluparbigenin in lupin nodules and bacteroids. *Mol. Plant Microbe Interact.*, **8**, 131–137 (1995).

92) Peter, N. K., Frost, J. W., and Long, S. R., A plant

flavone, luteolin, induces expression of *Rhizobium meliloti* nodulation genes. *Science*, **233**, 977–980 (1986).

93) Phillips, D. A., Flavonoids: plant signals to soil microbes. In "Phenolic Metabolism in Plants", eds. Stafford, H. A., and Ibrahim, R. K., Plenum Press, New York, pp. 201–231 (1992).

94) Fisher, R. F., and Long, S. R., *Rhizobium*-plant signal exchange. *Nature*, **357**, 655–660 (1992).

95) Nakayama, T., Homma, Y., Hashidoko, Y., Mizutani, J., and Tahara, S., Possible role of xanthobaccins produced by *Stenotrophomonas* sp. strain SB-K88 in suppression of sugar beet damping-off disease. *Appl. Environ. Microbiol.*, **65**, 4334–4339 (1999).

96) Hashidoko, Y., Nakayama, T., Homma, Y., and Tahara, S., Structure elucidation of xanthobaccin A, a new antibiotic produced from *Stenotrophomonas* sp. strain SB-K88. *Tetrahedron Lett.*, **40**, 2957–2960 (1999).

97) Homma, Y., Uchino, H., Kanazawa, K., Nakayama, T., and Sayama, M., Suppression of sugar beet damping-off and production on antagonistic substances by strains of rhizobacteria. *Ann. Phytopathol. Soc. Jpn.*, **59**, 282 (1993).

98) Islam, M. T., Hashidoko, Y., Deora, A., Ito, T., and Tahara, S., Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is linked to plant colonization and antibiosis against soilborne Peronosporomycetes. *Appl. Environ. Microbiol.*, **71**, 3786–3796 (2005).

99) Bloemberg, G. V., and Lugtenberg, B. J. J., Bacterial biofilms on plants: relevance and phenotypic aspects. In "Microbial Biofilms", eds. Ghannoum, M., and O'Toole, G. A., ASM Press, Washington, DC, pp. 141–159 (2004).

100) Brelles-Marino, G., and Bedmar, E. J., Detection, purification and characterization of quorum-sensing signal molecules in plant-associated bacteria. *J. Biotechnol.*, **91**, 197–209 (2001).

101) Rao, T. P., Yano, K., Iijima, M., Yamauchi, A., and Tatsumi, J., Regulation of rhizosphere acidification by photosynthetic activity in cowpea (*Vigna unguiculata* L. Walp.) seedlings. *Ann. Bot.*, **89**, 213–220 (2002).

102) Yamamura, T., Dateki, H., Wasaki, J., Shinano, T., and Osaki, M., Possibility of rhizosphere regulation using acid phosphatase and organic acid for recycling phosphorus in sewage sludge. *Soil Sci. Plant Nutr.*, **50**, 77–83 (2004).