Abstract . 10

Review Paper

PRESENT STATUS AND CHALLENGES ON AGRICULTURAL MICROBE BIO-PROSPECTING IN INDONESIA

Endang Sukara

Indonesian Institute of Sciences (LIPI), Jakarta, Indonesia
endang.sukara@lipi.go.id

ABSTRACT

Food security is one of the essential needs which can never be ignored by any society, including in particular Indonesia, as the world population is expected to rise to around 10 billion marked by 2025. The problem is that the agriculture in developed and developing countries that follow the path of developed countries experiences creates a range of serious environmental degradation. An intensive agricultural production through the use of pesticides and herbicides, forest clearing and continued use of land resources for the agricultural purposes are not sustainable practices. In consequences, degradation of ecosystem, soil erosion, and low productivity of land, poor water quality and lost of biological diversity (including microbial and genetic resources) occurred at alarming state. Meanwhile, the efforts in addressing the above situation is traditionally focused on plant phenotypes (breeding, plant genetic engineering etc.) but the important role of microbial communities that interact with plants to influence plant health and productivity has been largely ignored. The interaction of microbes with plants and its relevant to soil fertility and productivity, the bio-control properties of microbes and challenges toward sustainable agriculture is highlighted.

INTRODUCTION

Food security is one of the essential needs which can never be ignored by any society, including in particular, Indonesia. The world population is expected to rise to around 10 billion mark by 2025. Most of the population explosion is witnessed in developing and under developed countries. It is speculated that global demand for cereals will increase from 1 billion tones to 2.7 billion tones and considering the losses in storage and processing, the real need may be about 3.4 billion tones. More people mean more food. Most of this additional demand needs to be met either by improving the crop yield or by preventing post-harvest losses. It is urgent need to intensify the agricultural practices, but at the same time must address its sustainability. Agricultural practices must be carried out without destroying the environment on which it depends. The development of such a global system for sustainable food production is one of the greatest challenges faced in the future.

Agriculture in developed and developing countries like Indonesia that follow the path of developed countries experiences already creates a range of serious environmental problems through the use of pesticides and herbicides etc. Meanwhile, agricultural production especially in many African, South American and Asian countries cannot be increased without further converting more areas into arable land, thus threatening the ecosystem and global biodiversity, which is already under stress from human action. Ecosystems are now in agony. It is characterized by erosion, low productivity, and poor water quality. This mainly caused by forest clearing, intensive agricultural practices, and continued use of land resources for purposes that are not sustainable. The biological diversity, including microbial and genetic resources losses occurred at alarming state (Kennedy and Smith, 1995). The pressure also worsened since the government policy in many developing nation including Indonesia introducing a new scheme in boosting bio-energy which also requires space of land to grow Jathropa and palm oil.

A major effort in maintaining global food sufficiency is traditionally focused on the development of new crop varieties with enhanced disease and pest resistance, greater drought and salt tolerance and better nutritional value through the introduction of desirable traits either by conventional breeding or plant biotechnology including genetic modification. However, these efforts only focused on plant phenotypes. What has been largely ignored is the important role of microbial communities that interact with plants to influence plant health and productivity. The impact of the microbial world on plants is evident: worldwide each year, microbial diseases cost crop producers billions of dollar. Similarly, the important role of microbial resources in soil fertility, as bio-control agent, as bio-remediator etc., has been known for decades. What is less appreciated, and less well understood, is the pervasive influence that other microbes have on plant health and growth; they enhance stress tolerance, provide disease resistance, aid nutrient availability and uptake. Although major advances in genomic technologies and *in situ* studies of beneficial plant–microbe interactions have produced a large amount of knowledge and given insights into the mechanisms of these interactions, their application in biotechnology and agriculture has yet to be exploited. A greater understanding of how plants and soil microbes live together and benefit each other can therefore provide new strategies to improve plant productivity, while helping to protect the environment and maintain global bio-diversity (Morrissey *at al*., 2004).

PLANT MICROBE INTERACTION

The interactions of rhizosphere microbes with plants depend on the establishment of intimate associations between the two partners. Research on some of these interactions, such as those between symbiotic rhizobia and legumes, has demonstrated that this intimate cooperation between plant and bacteria displays a high level of host specificity. Different plant species, and even different cultivars of the same plant species, establish distinct microbial populations in their rhizospheres when grown in the same soil. The formation of these communities depends, at least in part, on the activation of specific gene expression in the microbe in response to chemical signals secreted from the plant. A pertinent example is the induction of nodulation genes in receptive rhizobia, which are triggered by the production and secretion of particular flavonoids by the plant. In the case of the rhizobia–legume interaction, the plant also responds to bacterial signals, and it is likely that this type of chemical cross-talk is typical of other microbe–plant interactions. Other examples of plant-derived signals that influence microbial gene expression include phenolics exuded from plant wounds, which induce expression of virulence genes in pathogenic *Agrobacterium* spp., and compounds that mimic the quorum sensing signals used by bacteria to regulate gene expression (Loh *et al*, 2002; Newton & Fray, 2004). In general, however, there is only very few knowledge of signalling interactions between beneficial microbes and plants. Understanding how microbes respond to plant signals in terms of growth and gene expression, and the role that plant signalling has in determining interaction specificity or driving population selection is central to reaping the benefits of plant–microbe interactions.

Before the advent of genomic technologies, scientists had only limited options to investigate these interactions in detail, particularly with a view to their commercial exploitation. This situation has changed considerably, now that the genomes of more than ten plant associated bacteria have been sequenced, and the sequencing of another 35 relevant genomes is already under way (Puhler *et al*, 2004). A detailed investigation of the molecular basis of pathogenic, symbiotic and associative plant–microbe interactions, both at the levels of comparative and of functional genomics is now possible. So far, most data have come from comparative analyses with a particular emphasis on mutuality and pathogenic interactions. Although comparative genomics focuses on genetic potential rather than gene expression, these studies do raise some interesting questions about the distinction between pathogenic and non-pathogenic bacteria. For instance, investigations have already yielded a surprising insight regarding type-III protein secretion systems. These are ordinarily associated with pathogenicity in bacteria and are possibly also involved in non-pathogenic associations (Puhler *et al*, 2004). In the future, transcriptome profiling and functional genomics are likely to produce more information about microbial responses to plant signals and the contribution of specific gene products to the establishment of an interaction with the host. An important question is whether plant metabolites exuded from roots induce microbial type-III protein secretion systems, and, if so, how? *Pseudomonas fluorescens,* for instance, is chemotactic towards components of root exudates (de Weert *et al*, 2002), but the global effect of these exudates on gene expression in the bacterium is still unknown. By profiling the complete genetic response to root exudates, it will be possible to assemble a full picture of how gene expression, and thereby function, is modulated in the bacterium after perception of plant signals. It will facilitate studies of how different bacterial species and subspecies respond to particular plants and how a bacterium responds differentially to signals from different plant species or varieties. This, in turn, will lead to a better understanding of the basis of host specificity and host selection during microbe–plant interactions. It may also be possible to describe general principles of plant–microbe communication that define or distinguish associative, mutual and pathogenic interactions of microbes with plants. In addition to the economic effects of plant disease, microbial interaction with plants can also have serious and direct consequences for human health.

The effects of microbes on plant physiology itself have received less attention, although it is known that many food crops naturally produce toxic metabolites; potatoes and tomatoes, for example, can accumulate high levels of toxic steroidal alkaloids (Friedman, 2002; Korpan *et al*, 2004). The possible role of microbes in inducing toxin production, or in modifying metabolites or metabolic pathways within the plant, remains largely unexplored. There are clear precedents for this premise, for instance the production of secondary metabolites by plants, such as phytoalexins, in response to pathogenic attack and fungal modification of plant saponins (Morrissey & Osbourn, 1999; Bouarab *et al*, 2002). Although it is clearly a complex issue, the combination of plant and microbial functional genomics with metabolome analysis provides a route to start addressing these questions. Future biotechnological developments in the agricultural sector—whether based on gene modification technology or on traditional breeding—should recognize the importance of plant–microbe associations. The 'traditional' plant biotechnology sector is based around plant breeding and the selection of varieties with desired traits, and it pays little attention to plant–microbial ecology. But the expression of desirable traits, such as disease resistance, or drought and salt tolerance, could equally be driven by interactions between a particular plant variety and the colonizing microbial flora. Conversely, particular plant genotypes may attract a microbial flora with undesirable traits. Understanding the genetic basis of plant–microbe interactions in the context of how the plant selects its microbial population in the soil may allow 'conditioning' of the rhizosphere to promote desirable traits in the plant, which is, in fact, the basis of natural disease-suppressive soils. Similarly, it is also an attractive proposition to influence or modulate the microbes that interact with the plant to generate improved productivity or health for the latter. Furthermore, bacteria could be genetically engineered to confer increased disease resistance or growth promotion that is only activated when the bacterium is associated with its host plant. From the perspective of developing nations, these are exciting strategies that may help to increase yields while avoiding some of the costs and environmental problems that come with the use of fertilizers, pesticides, herbicides and fungicides. However, most of the microbial biodiversity in soil remains unexplored and much work remains to be done to first identify and then characterize microorganisms that could be used in such applications. Furthermore, such approaches require a detailed knowledge of the molecular signalling that takes place between plants and microbes to drive expression of desirable traits and suppress unwanted effects in a controlled manner. Exploiting plants and microbes by using such an integrated approach requires a systems biology strategy to understand the degree and complexity of plant–microbe interactions through the application of modern '-omics' technologies.

Modern agriculture has gone through similar phases in recent history. The first agricultural revolution in the eighteenth century introduced crop rotation to take advantage of and manipulate microbial populations in the soil, although at that time it was not known why this benefited plant health and growth. The second revolution, which began in the 1960s and is sometimes described as the 'green revolution', was based on improved plant breeding techniques and the development of hybrid varieties; it now includes genetic engineering of plants but also a heavy reliance on the use of chemicals. We may now be at the cusp of a third stage, which will combine both approaches in a more holistic and elegant strategy. Applying knowledge about beneficial plant–microbe interactions in the rhizosphere to plant-breeding and genetic-engineering technologies may allow us to increase food production while reducing stress on the environment and on global biodiversity.

SOIL FERTILITY AND BIOCONTROL OF PLANT PATHOGENS

Biological control of plant pathogens is currently accepted as a key practice in sustainable agriculture because it is based on the management of a natural resource, i.e. certain rhizosphere organisms, common components of ecosystems, known to develop antagonistic activities against harmful organisms (bacteria, fungi, nematodes etc.). Arbuscular mycorrhizal (AM) associations have been shown to reduce damage caused by soil-borne plant pathogens. Although few AM isolates have been tested in this regard, some appear to be more effective than others. Furthermore, the degree of protection varies with the pathogen involved and can be modified by soil and other environmental conditions. This prophylactic ability of AM fungi could be exploited in cooperation with other rhizospheric microbial angatonists to improve plant growth and health. Despite past achievements on the application of AM in plant protection, further research is needed for a better understanding of both the ecophysiological parameters contributing to effectiveness and of the mechanisms involved. Current research based on molecular, immunological and histochemical techniques is providing new insights into these mechanisms (Azcón-Aguilar and Barea, 1977).

In sustainable, low-input cropping systems the natural roles of microorganisms in maintaining soil fertility and biocontrol of plant pathogens may be more important than in conventional agriculture where their significance has been marginalized by high inputs of agrochemicals. Better understanding of the interactions between arbuscular mycorrhizal fungi and other microorganisms is necessary for the development of sustainable management of soil fertility and crop production. Many studies of the influence of mycorrhizal colonization on associated bacterial communities have been conducted; however, the mechanisms of interaction are still poorly understood. Novel approaches including PCR-based methods, stable isotope profiling, and molecular markers have begun to shed light on the activity, identity and spatiotemporal location of bacteria in the mycorrhizosphere (Johansson, *et. al., 2004).*

Recent study by Khan (2006) and Jing et. al., (2007) reported that rhizobacteria adapted to heavy metal pollution would have an important vehicle in developing new strategy for bioremediation processes. Their study clearly shows that mycorrhiza could be used to improve phytoremediation of heavy metal contaminated soil.

PLANT DISEASES

Fungal plant diseases are one of the major concerns to agricultural production. It has been estimated that total losses as a consequence of plant diseases reach 25% of the yield in western countries and almost 50% in developing countries. Of this, one third is due to fungal infections (Bowyer, 1999). So there is a pressing need to control fungal diseases that reduce the crop yield so as to ensure a steady and constant food supply to ever increasing world population. Conventional practice to overcome this problem has been the use of chemical fungicides which have adverse environmental effects causing health hazards to humans and other non-target organisms, including beneficial life forms.

Chitinases are reported to play a protective role against fungal pathogens (Boller, 1985). Besides its ability to attack the fungal cell wall directly, chitinases release oligo-N-acetyl glucosamines that function as elicitors for the activation of defense-related responses in plant cells (Ren and West, 1992). Studies on chitinolytic microorganisms have yielded a large increase in knowledge regarding their role in inhibition of growth of fungal plant pathogens. Moreover, extensive studies are required on the maximum utilization of chitinous wastes for production of chitinases and biomass (Gohel *et. al.*, 2006).

Screening of thermophilic microorganisms capable of producing chitinase was carried out from several geothermal areas in West Java

Indonesia (Rahayu *et. al.*, 2004). More recent work is to use a molecular method for isolating chitinases from uncultivated microorganisms. Genes encoding chitinases may be particularly interesting examples of non-essential genes in uncultured bacteria since previous work has suggested that the evolution of these enzymes has been impacted by lateral gene transfer (Gracia-Vallve *et al.*, 1999). Many types of cultured bacteria and archaea are known to degrade chitin but the identity of uncultured bacteria degrading chitin in nature is unknown. Chitinase genes cloned directly from uncultured marine microorganisms suggested the presence of a large pool of uncultured chitin degrading bacteria in aquatic systems. Information on bacterial chitinase genes is largely restricted to cultured γ-proteobacteria or gram positive bacteria. Since γ-proteobacteria are widespread in the ocean (Giovannoni *et al.*, 2000). To access chitinase genes in uncultured β-proteobacteria and in other bacteria, it may be possible to use a PCR based approach with oligonucleotide primers patterned after conserved amino acid residues or after conserved nucleotide sequences of chitinase genes in cultured bacteria (Suitil and Kirchman, 1998). Molecular methods are needed to study chitinase producers without the isolation of bacteria in pure cultures. Methods that use nucleic acid probes and PCR primers cannot be designed solely with cultured bacteria because nucleotide sequences of chitinase genes from cultured bacteria so far characterized are very different suggesting that the chitinase sequences from uncultured bacteria will differ from the culturable ones (Cottrell *et al.*, 1999).

BIOACTIVE AGENTS

Plants constitute an excellent ecosystem for microorganisms. The environmental conditions offered differ considerably between the highly variable aerial plant part and the more stable root system. Microbes interact with plant tissues and cells with different degrees of dependence. The most interesting from the microbial ecology point of view, however, are specific interactions developed by plant-beneficial (either non-symbiotic or symbiotic) and pathogenic microorganisms. Endophytes, microorganisms that reside in the tissues of living plants, are relatively unstudied and potential sources of novel natural products for exploitation in medicine, agriculture, and industry. It is noteworthy that, of the nearly 300,000 plant species that exist on the earth, each individual plant is host to one or more endophytes. Only a few these plants have ever been completely studied relative to their endophytic biology. Consequently, the opportunity to find new and interesting endophytic microorganisms among myriads of plants in different settings and ecosystems is great (Strobel and Daisy, 2003).

Recently, a novel fungal genus that produces extremely bioactive volatile organic compounds (VOCs) is found. This fungal isolate was initially discovered as an endophyte in *Cinnamomum zeylanicum* in a botanical garden in Honduras. This endophytic fungus, *Muscodor albus*, produces a mixture of VOCs that are lethal to a wide variety of plant and human pathogenic fungi and bacteria. It is also effective against nematodes and certain insects. The mixture of VOCs has been analyzed using GC/MS and consists primarily of various alcohols, acids, esters, ketones, and lipids. Final verification of the identity of the VOCs was carried out by using artificial mixtures of the putatively identified compounds and showing that the artificial mixture possessed the identical retention times and mass spectral qualities as those of the fungal derived substances. Artificial mixtures of the VOCs nicely mimicked the biological effects of the fungal VOCs when tested against a wide range of fungal and bacterial pathogens. Potential applications for "mycofumigation" by *M. albus* are currently being investigated and include uses for treating various plant parts, and human wastes. Another promising option includes its use to replace methyl bromide fumigation as a means to control soil-borne plant diseases (Strobel, 2006). Similar microbe also successfully isolated from Indonesian unidentified vine, generally used by the indigenous people of the Tesso Nilo region in Sumatra to treat snakebites (Atmosukarto *et. al.*, 2005). This unique organism produces a number of VOC's not previously observed in other *M. albus* isolates including tetrohydofuran, 2-methyl furan; 2-butanone; aciphyllene, and large amounts of an unusual azulene derivative. Noticeably absent from the VOC mixture was 1-butanol, 3-methyl-. Scanning electron micrographs of the organism showed a unique fishnet-like deposit of what appears to be a biopolymer covering the hyphae. The ITS-5.8S rDNA partial sequence data showed 99% identity to the original *M. albus* strain cz-620. In addition, an artificial mixture of some of the VOC's produced by this new isolate generally mimicked the inhibitory as well as lethal effects of the fungal VOC's on the test microorganisms. One of the most sensitive test fungi was *Stachybotrys chartarum*, an organism associated with the "toxic mold" syndrome of buildings. Fungi belonging to the *Muscodor* genus regularly appear in tropical rainforests throughout the world and these isolates appear to have chemical, biological, and structural characteristics that make them potentially useful in medicine, agricultural and industrial applications (Atmosukarto *et. al.*, 2005).

A highly potent allelopathic factor, lepidimoide, was initially extracted from mucilage of germinated cress seeds. This compound is a shoot growth promoter and inhibits root growth (stimulatory or inhibitory effects). This should be an importance to the future agriculture. An endophytic fungal strain AHU9748 belonging to the coelomycetes, closely related to the genus *Colletotrichum*, isolated from *Coleus galeatus*, demonstrate its ability to produce oligosaccharide having similar properties to lepidimoide on thin layer chromatography. The physico-chemical data from ESI-MS, NMR spectra and other analyses also showed the purified product to be identical to lepidimoide (Tanaka *et. al.*, 2002).

RECENTS DEVELOPMENT

Low pH and aluminum tolerance of *Bradyrhizobium* strain isolated from acid soils in Indonesia has been studied (Takashi *et. al.*, 1999). The strain is evaluated for its effectiveness on various cultivar of soybean. In addition, the technique for the production of *Bradyrhizobium* biomass is also successfully developed. A pressure cooker fermenter is introduced for this process. It is now possible to use of such system, for the production of biomass economically event at soy bean production center as the process do not require a special laboratory. In addition, two stages fermentation system is also developed. For this purpose, cassava starch may be use as a sole source of energy for the production of *Bradyrhizobium* biomass.

It also important to note, that *Bradyrhizobium* may also be inserted directly into the soy bean seed without affecting germination. This technique could significantly improve soy bean growth and its productivity. It is predicted that *Bradyrhizobium* may colonize root of soy bean quite early during seed germination process. This technique saw a tremendous result as it could effectively forming root nodules, maintain the effectiveness of *Bradyrhizobium* in helping the soy bean plant to fix nitrogen. In addition to *Bradyrhizobium*, *Azospirillum*, independent nitrogen fixing bacteria also studied and together with *Pseudomonas* sp. and *Bradyrhizobium* could be inserted into soy bean seed (Sukiman & New, 1990). The relationship between root colonization and the effect of inoculation on growth and productivity of soy bean continued. The experiment of injection of a mixture of microbial biomass to soy bean seed could improve the production of soy bean in limited trial at Musi Rawas with the production of between 3.8 and 4.0 Ton per Ha.

Intensive study also carried out on the identification of acetic acid bacteria from Indonesian sources. Study is focused on the genus of *Gluconobacter* (Yamada *et. al.*, 1999 and 2000; Katsura *et. al.*, 2001; Lisdiyanti *et. al.*, 2002;). From their study, it is firmed that Indonesia rich in microbial diversity. Many new species is found and some genus including *Kozakia* and *Asiana* are proposed. The later is mainly isolated from flower and recently reported present in the mosquito gut. This sought by world scientific community to have an importance and significant asset for the future strategy in combating malaria.

Isolation and screening of endophytic microbes from various plant resources in Indonesia is intensively carried out since early 2000 (Michiko *et. al.*, 1999; Atmosukarto *et. al.*, 2005, Strobel, 2006). A large collection of endophytic microbes is now available at Research Center for Biotechnology - Indonesian Institute of Sciences (LIPI) as materials for further study. Early screening show a significant result toward the development of biocontrol agent and for the improving quality of soil and plant growth and productivity. In addition, an intensive exploration also carried out on fungi and actinomycetes. The number of fungi and actinomycetes deposited at Research Center for Biology and Research Center for Biotechnology LIPI now approaching 6,000 culture. Intensive taxonomic study is being carried out. Early screening on almost 1,000 cultures by Kiohako and Chugai Pharmaceutical Company under the collaboration with LIPI and NITE (National Institute for Technology Evaluation of Japan) show promising results. Meanwhile, study on around 1,800 culture carried out by Research Center for Biotechnology found there is a change to develop anti viral agents as some 16 culture showing to produce anti helicase properties, an enzymes responsible for the proliferation of virus.

CONCLUSION

Indonesia has wide range of ecological habitats from Puncak Jayawijaya (covered by iced) to a deep Weber seas laid in the tropical belt between Asia and Australia. It should be a perfect habitat for diverse microbial resources with abundance of novel taxa of culturable and unculturable microorganisms of great potential value. Microorganisms of Indonesian origin should be a major source of genetic information to solve many problems in agriculture, industry, plant, animal and human health and several other biotechnological applications. The vast majority of the microbial diversity of Indonesia, however, is unexplored. It is important to Indonesia to formulate strategy in harnessing the value of microbial resources to improve the efficient and cost effectiveness in producing a diverse array of novel value-added products and tools, increase food production, reduce dependency of agriculture on chemicals, lowering the cost of raw materials, all in an environmentally friendly manner to provide solution to natural resources depletion, environmental, agricultural, food, forestry and public health towards poverty eradication and improved livelihoods of the people. It is a priority to Indonesia to continue mapping and sequencing of animal/plant/ microbial genomes to elucidate gene function and regulation and to facilitate the discovery of new gens\es as a prelude to gene modification. It is also important to determine biochemical and genetic control mechanisms of metabolic pathways in animal, plants, and microbes that may lead to products with novel food, pharmaceutical, and industrial uses.

REFFERENCES

Atmosukarto, I., U. Castillo, W. M. Hess, J. Sears and G. Strobel. 2005. Isolation and characterization of *Muscodor albus* I-41.3s, a volatile antibiotic producing fungus. Plant Science, 169 (5): 854-861.

Azcón-Aguilar, C. and J. M. Barea. 1997. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. Mycorrhiza, 6 (6): 457-464.

Boller, T. 1985. Introduction of hydrolase as a defense reaction against pathogens. In: Cellular and molecular biology of plant stress (JL Key, T Kosuge) pp. 247-262, Alan R Liss, New York.

Bouarab K, Melton R, Peart J, Baulcombe D, Osbourn A (2002) A saponin-detoxifying enzyme mediates suppression of plant defences. *Nature* 418: 889–892.

Bowyer, P. 1999. Plant disease caused by fungi: phytopathogenicity In: Molecular Fungal Biology, (RP Oliver, M Schweizer), Cambridge University Press, Cambridge.

Byarugaba, D. K. 2004. Harnessing microbial resources for increased agricultural productivity and improved livelihoods. Uganda Journal of Agricultural Sciences, 9: 227-234.

Cottrell, M.T., J. A. Moore, and D. L. Kirchman. 1999. Chitinases from uncultured marine microorganisms. Appl. Environ. Microbiol. 65:2553-2557.

de Weert S, Vermeiren H, Mulders IH, Kuiper I, Hendrickx N, Bloemberg GV, Vanderleyden J, De Mot R, Lugtenberg BJ (2002) Flagelladriven chemotaxis towards exudates components is an important trait for tomato root colonization by *Pseudomonas fluorescens*. Mol Plant Microbe Interact 15:1173–1180

Friedman M (2002) Tomato glycoalkaloids: role in the plant and in the diet. *J Agric Food Chem* 50: 5751–5780

Garcia-Vallve S., J. Palau, A. Romeo. 1999. Horizontal gene transfer in glycosyl hydrolases inferred from codon usage in Escherichia coli and Bacillus subtilis Ml. Biol. Evol. 16: 1125-1134.

Giovannoni, S. And M. Rappe. 2000. Evolution, diversity and molecular ecology of marine prokaryotes, Microbial ecology of the oceans (DL Kirchman) pp. 47-84. John Wiley and Sons, Inc., N.Y. New York.

Gohel V. , A. Singh, M. Vimal, P. Ashwini and H. S. Chhatpar. 2006. Bioprospecting and antifungal potential of chitinolytic microorganisms . African Journal of Biotechnology, 5 (2): 54-72

Jing, Y., Z. He, and X. Yang. 2007. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. Journal of Zhejiang University Science B. 8(3): 192-207.

Johansson, J. F., Paul, L. R., Finlay, R. D. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiology Ecology 48 (1), 1–13.

Katsura, K., H. Kawasaki, W. Potacharoen, S. Saono, T. Seki, Y. Yamada, T. Uchimura and K. Komagata. 2001. Asaia siamensis sp. Nov., an acetic acid bacterium in the alpha-Proteobacteria. International Journal of Systematic and Evolutionary Microbiology, 51: 559-563.

Kennedy A. C. and Smith K. L. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil 170(1): 75-78

Kirchman, D. L. and J. White. 1999. Hydrolysis and mineralization of chitin in the Delaware Estuary. Aquat. Microb. Ecol. 18: 1773-1779.

Khan, A. 2006. Mycorrhizo remediation – an enhanced form of phytoremediation. 2006. Journal of Zhejiang University Science B. 7(7): 503-514.

Korpan YI, Nazarenko EA, Skryshevskaya IV, Martelet C, Jaffrezic-Renault N, El'skaya AV (2004) Potato glycoalkaloids: true safety or false sense of security? Trends Biotechnol 22: 147–151

Lisdiyanti, P., H. Kawasaki, Y. Widyastuti, S. Saono, T. Seki, Y. Yamada, T. Uchimura and K. Komagata. 2002. Kozakia baliensis gen. nov., a novel acetic acid bacterium in the alpha-Proteobacteria. International Journal of Systematic and Evolutionary Microbiology, 52: 813-818.

Loh J, Pierson EA, Pierson LS III, Stacey G, Chatterjee A (2002) Quorum sensing in plantassociated bacteria. Curr Opin Plant Biol 5: 285–290

Michiko, T., H. Sukiman, M. Takebayashi, K. Saito, M. Suto, T. K. Prana, M. S. Prana, F. Tomita. 1999. Isolation, screening and phylogenetic identification of endophytes from plants in Hokkaido Japan and Java Indonesia. Microbes and environments. 14(4): 237-241.

Morrissey, J. P., J.M. Dow, G. L. Mark and F. O'Gara. 2004. Are microbes at the root of a solution to world food production? EMBIO Report, Vol. 5 (10): 922-926.

Morrissey JP, Osbourn AE (1999) Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol Mol Biol Rev 63: 708–724

Newton JA, Fray RG (2004) Integration of environmental and host-derived signals with quorum sensing during plant– microbe interactions. Cell Microbiol 6: 213–224

Puhler A, Arlat M, Becker A, Gottfert M, Morrissey JP, O'Gara F (2004) What can bacterial genome research teach us about bacteria–plant interactions? Curr Opin Plant Biol 7: 137–147

Rahayu, S., F. Tanuwidjaja, Y. Rukayadi, A. Suwanto, M. T. Suhartono, J. Hwang and Y. R. PPyun. 2004. Study of thermostable chitinase enzymes from Indonesian Bacillus K29-14. J. Microbiol. Biotechnol. 14(4): 647-652.

Ren, Y. and C. A. West. 1992.Elicitation of diterpene biosynthesis in rice (Oryza sativa L.) by chitin. Plant Physiol. 99:1169-1178.

Strobel, G. 2006. *Muscodor albus* and its biological promise. Journal of Industrial Microbiology and Biotechnology, 33 (7): 514-522.

Strobel, G. and B. Daisy. 2003. Bioprospecting for Microbial Endophytes and Their Natural Products. Microbiology And Molecular Biology Reviews, 67 (4): 491–502.

Suitil, A.L., and D. L. Kirchman. 1998. A chitin binding domain in marine bacterial chitinase and other microbial chitinases: Implication for ecology and evolution of 1,4-âglycanases. Microbiol. 144: 1299-1308.

Sukiman, H. I. and P. B. New. 1990. Relationship between root colonization and initial adsorption of Azospirillum to plant roots. Microbial Ecology, 20 (1): 65-67.

Tanaka, M. , M. Yoshimura, M. Suto, A. Yokota, K. Asano, E. Sukara and F. Tomita. 2002. Production of Lepidimoide by an Endophytic Fungus from Polysaccharide Extracted from *Abelmoschus* sp.: Identification of the Product and the Organism Producing It. Journal of Bioscience and Bioengineering, 93 (6): 531-536

Takashi, O., I. Yuichi, I. S. Sukiman, H. Karsono, D. Ariani and S. Saono. 1999. Low pH and aluminum tolerance of Bradyrhizobium strains isolated from acid soils in Indonesia. Soil Science and Plant Nutrition. 45(4): 978-992.

Yamada, Y., K. Katsura, H. Kawasaki, Y. Wydiastuti, S. Saono, T. Seki, T. Uchimura and K. Komagata. 2000. Asaia bogoriensis gen. nov., sp. Nov., an unusual acetic acid bacterium in alpha-Proteobacteria. International Journal of Systematic and Evolutionary Microbilogy, 50: 823-829.

Yamada, Y. R. Hosono, P. Lyastuti, S. Saono, T. Uchimura, and K. Komagata. 1999. Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus Gluconobacter. The Journal of General and Applied Microbiology, 45(1): 23-28.