MECHANISMS OF *Bacillus amyloliquefaciens* KPS46 ON ENHANCED GROWTH PROMOTION AND INDUCED SYSTEMIC RESISTANCE AGAINST BACTERIAL PUSTULE IN SOYBEAN

INTRODUCTION

Bacillus amyloliquefaciens strain KPS46, a plant growth promoting rhizobacteria/ bacteria (PGPR/ PGPB) originally isolated from a soybean plant at the agricultural research station of central Thailand, (Prathuangwong and Kasem, 2004; Prathuangwong *et al.,* 2005) can protect soybean and other crop plants against multiple plant pathogens by induced resistance and secretion of antimicrobial metabolites (Prathuangwong and Kasem, 2004; Prathuangwong *et al.,* 2004; Prathuangwong *et al.,* 2005; Buensanteai *et al.,* 2007a; Prathuangwong and Buensanteai, 2007; Buensanteai *et al.,* 2008). When applied as a seed treatment, it can also promote the growth and induced resistance of soybean (Buensanteai *et al.,* 2007b; Buensanteai *et al.,* 2008). Most of the research on KPS46 has focused on the mode of action of strain KPS46 as a biocontrol agent (Prathuangwong *et al.,* 2004; Prathuangwong *et al.,* 2005), the mechanism by which KPS46 promotes plant growth and induced reistance are not well understood.

In the particular case, the PGPR/PGPB could be enhanced plant growth and induced resistance by direct and indirect modes of action, but the specific mechanisms involved have not all been well-characterized and known. So far, bacillus group is well documented of enhanced growth promotion (Ryu *et al.,* 2003b) and induced systemic resistance (Ryu *et al.,* 2004b). Several strains belonging to the genus *Bacillus* and particularly to *B. subtilis* and the closely related *B. amyloliquefaciens* species were reported effective for the biocontrol of multiple plant diseases caused by several phytopathogens (Kloepper *et al.,* 2004a; Araujo *et al.,* 2005). The *Bacillus* genus is among the beneficial bacteria mostly exploited as biopesticides phytohormone and lipopeptide to control plant diseases and enhance plant growth

(Araujo *et al.,* 2005; Idriss *et al.,* 2007; Ongena *et al*, 2007). Their protective effects may rely on different mechanisms to directly antagonize pathogen growth and promote plant growth (Kloepper *et al.,* 2004b; Idriss *et al.,* 2007). In this context, *Bacillus sp.* produces a variety of secondary bioactive metabolite elicitors that could be involved in lipopeptides and phytohormone production (Araujo *et al.,* 2005; Buensanteai *et al.,* 2007b).

The phytohormone such as indole-3-acetic acid (IAA) is microbial secondary metabolite product, which allows certain bacteria, rhizobacteria, and fungi to direct a plant's physiology and biochemistry toward their own advantage (Martinez-Morales *et al.,* 2003) and have effects on plant growth and development process (Kang *et al.,* 2006; Idriss *et al.,* 2007) because IAA is the main auxin in plants, controlling many important physiological and biochemical processes including cell enlargement, cell division, tissue differentiation, and responses to light and gravity. PGPR/ PGPB's IAA producers have the potential to interfere with any of these processes by input of IAA into the plant's auxin complex that associates to plant growth and development process (Patten and Glick, 2002; Leveau and Lindow, 2005). In the other hand, lipopeptides produced by PGPR as member of genera *Bacillus*, *Pseudomonas*, and *Arthrobacter* such as surfactin, a family of lipopeptide group produced by bacillus group (Jacques *et al.,* 1999) is well known as powerful biosurfactant. Their surfactin has diverse biological of antimicroorganisms and induced resistance such as fungi, bacteria, and virus (Chen *et al.,* 2000; Pereira *et al.,* 2004; Ongena *et al.,* 2007; Buensanteai *et al.,* 2008) but not yet known about its direct and indirect mechanism of plant growth promotion and induced resistance. Besides auxin and lipopeptide group (surfactin), extracellular proteins are key components of defense mechanisms of many groups of PGPR/ PGPB. Bacillus group secretes high levels of extracellular proteins as secondary metabolites (Tjalsma *et al.,* 2004). Among the gene producing concerned from *B. subtilis* (Kunst *et al.,* 1997; Yoshida *et al.,* 2001) and *B. amyloliquefaciens* (Chen *et al.,* 2007), the number of extracellular proteins and the secretion machinery proteins that facilitate their extracellular production, remain not clearly publication (Oosthuizen *et al.,* 2002; Zavahir and Seneviratne, 2007). Apart from bacilli group PGPR/PGPB is well-characterized capacity to produce antipathogenic lipopeptides and extracellular proteins (Jock *et al.,* 2002; Araujo *et al.,* 2005), but is less known and fully understood about the beneficial action of the bacilli group at the molecular and biochemistry levels associated with plant growth promotion and induced resistance.

The strain KPS46 has been found to promote growth and induced resistance against various diseases in several plants (Prathuangwong and Kasem, 2004; Prathuangwong *et al.,* 2004, Prathuangwong *et al.,* 2006) and to be effective in controlling a wide range of bacterial and fungal plant pathogens: *Xanthomonas axonopodis* pv. *glycines*, *Collectotrichum truncatum, Fusarium solani, Rhizoctonia solani* and *Sclerotium rolfsii* on soybean (Kasem *et al.,* 2005; Prathuangwong and Kasem, 2001 and 2004; Prathuangwong and Kasem, 2003; Prathuangwong *et al.,* 2004b); *Bipolaris maydis* on corn (Prathuangwong *et al.,* 2004a; Prathuangwong *et al.,* 2005b); *Alternaria sp.* on sunflower (Prathuangwong *et al.,* 2004b); *Pseudomonas syringae* pv. *sesami* on sesame (Prathuangwong *et al.,* 2004b); *Pythium* sp. on teak (Prathuangwong *et al.,* 2004b); and *Alternaria* sp. and *Erwinia carotovora* subsp. *carotovora* on kale and cauliflower (Prathuangwong *et al.,* 2005c). Antibiotic production was implicated as one mechanism for pathogen biocontrol by KPS46 (Prathuangwong *et al.,* 2004). The strain also was demonstrated to induce resistance in soybean against bacterial pustule disease (Buensanteai and Pathuangwong, 2006; Prathuangwong and Buensanteai, 2006; Buensanteai *et al.,* 2007). In regards to the latter mode of action, there is no information as to what responses are triggered in soybean upon treatment with KPS46 or the signaling pathway activated by KPS46.

Despite the economic importance of soybean as crop worldwide, there are relatively few studies on the use of PGPR/ PGPB to enhance growth in soybean (Cattelan *et al.,* 1999; Dashti *et al.,* 2000) and, other than KPS46, only one other strain of *Bacillus* spp., *B. pumilus* INR7, has been reported to induce resistance in soybean (Kloepper *et al.,* 2004a; Ryu *et al.,* 2004a; Kloepper and Ryu, 2006). While there is some imformation regarding systemic responses in soybean to infection by plant pathogens (Park *et al.,* 2002; Bradley *et al.,* 2008), feeding by herbivorous insects (Bi et al, 1994; Felton *et al.,* 1994; Bi and Fleton, 1995; Graham and Graham, 1996; Underwood, 1998; Underwood *et al.,* 2002; Endo *et al.,* 2007) or to chemical or microbial elicitor inducers of resistance (Neupane and Norris 1991; Slaymaker and Keen, 2004; Waldmuller et al, 2004; Duzan *et al.,* 2005) the response of soybean to induction by PGPR has not been investigated; only an association of cell wall lignification of cell wall with induced resistance by strain INR7 in a unpublished study was mentioned (Kloepper *et al.,* 2004a). Studies on other plant models such as *Arabidopsis thaliana* (Pieterse *et al.,* 1996; van Wee *et al.,* 1999; Ryu *et al.,* 2003a, 2003b), tomato (Ramamoorthy *et al.,* 2002a; Silva *et al.,* 2004; Block et al., 2005), tobacco (Pan *et al.,* 1991; Achuo *et al.,* 2004), bean (Zdor and Anderson, 1992; Xue *et al.,* 1998), common bean (Alstrom *et al.,* 1999), cucumber (Wei *et al.,* 1991; Chen *et al.,* 2000; Ryu *et al.,* 2004b), sugar beet (Bargabus *et al.,* 2002), loblolly pine (Enabek and Carey, 2000), pearl millet (Niranjan *et al.,* 2003), pepper (Jetiyanon, 2007) have revealed some variations among plant species as to the signaling pathways involved in induced resistance to and the pathogen defense responses expressed in the plants (Pan *et al.,* 1991; van Loon *et al.,* 1998, 1999; Dorey *et al.,* 1999). Two pathways, however, are in common among these plant species (Sticher *et al.,* 1997; Pieterse *et al.,* 1998; van Wee *et al.,* 1999; Corath *et al.,* 2002; Dong *et al.,* 2005; Segarra *et al.,* 2007), one the SA-dependent pathway which leads to the expression of PR-proteins and oxidative enzymes and is commonly associated with infection by plant pathogens. The second pathway, is mediated by JA and does not trigger the expression of genes for PR - proteins. The pathogen defense mechanisms activated by the JA dependent pathway have not yet been elucidated. There is also considerable evidence that induced resistance involving the JA- or SA-dependent pathways may involve the sensitization of plants to pathogen, with pathogen infection resulting in a more rapid and elevated expression of responses associated with the SA-dependent pathway (Sticher et al.,1997; Corath *et al.,* 2002). Strains of PGPR in the genus Bacillus, such as *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a have been investigated as inducers of systemic resistance agent (Kloepper *et al.,* 2004a; Jitiyanon *et al.,* 2007). Induced resistance by some strains reported to involve JA dependent pathway while other strains induce SA-dependent responses, indicating that signaling pathways triggered by *Bacillus* spp. vary from strain to strain (Ryu *et al.,* 2003a; Kloepper *et al.,* 2004a). The pathway activated by KPS46, therefore, must be determined empirically.

The first main aim of this study was to determine whether or not strain KPS46 secretes signal molecules affected particulary plant growth elicitors. Two hypotheses were determined the elicitors produced by KPS46 in liquid culture such as extracellular proteins, lipopeptides and IAA could enhance plant growth on soybean plant when KPS46 applied to soybean seeds. Another hypothesis being to identify what proteins secreted by KPS46 that might be involved in biosynthesis and production of these signal molecules using proteomic approach analysis. Protein bioinformatics tools were also employed to identify the proteins and to understand the extracellular proteomes of KPS46. And second main aim of our goal in this study was to determine plant responses to treatment with KPS46, with an emphasis on processes occurring in soybean. One objective was to examine the time profile of biochemical responses in soybean following KPS46 treatment related to induced resistance. Preliminary results have been reported (Prathuangwong and Buensanteai, 2007). Another objective was to apply the proteomics approach to determine some of the proteins induced in soybean by the bacterium. The final objective was to determine the signaling pathway activated by KPS46. Because there does not yet exist a collection of soybean mutant lines affected in specific induced resistance-related genes, as available for *Arabidopsis thaliana* (Pieterse *et al.,* 2000; Rye *et al.,* 2004a; van Loon *et al.,* 2002), we used Arabidopsis as a model system to achieve this last objective.

OBJECTIVES

The objectives of this research are as follows.

1. To determine elicitors produced by KPS46 in liquid culture as extracellular proteins, IAA, lipopeptide and identify extracellular proteins using proteomics approach what proteins are secreted by KPS46 and its IAA^- mutant strain that might be involved in promoting growth.

2. To describe the strain KPS46 can interact sensitization mechanisms with soybean plants to cause growth promotion and induced resistance when KPS46 is applied to seeds, plant signaling transduction pathways and proteome involved in growth metabolism and defense response are stimulated against *Xag* in soybean plant.

LITERATURE REVIEW

1. Importance of soybean in Thailand

Soybean (*Glycine max* L. Merr) is an important economic crop worldwide. The first record of soybean cultivation is from the north of china in the 11th century B.C. and spread into Japan, Korea and Southeast Asia between 200 B.C. and 300 A.D. respectively (Hartman *et al.,* 1999). Utilitization of soybean is provides as an important protein source in the diets of many Asian nations and extremely valuable food and industrial products. Soybean has good potential for production in the tropics zone, but the yields are lower than those in temperate regions, because of poor nodulation by nitrogen-fixing bacteria and the lack of adapted cultivar.

Soybean has been planted since 2503 B.E in Thailand. The important soybean growing area is mainly grown in the north and northeast regions of the country including Chiang Mai, Chiang Rai, Lum Pang, Nakhorn Sawan with three growing seasons for planting, during May to August, June to November, and December to April respectively. The recommended cultivars of soybean are SJ 4, CM60, KKU35 and Rachamongkol1 which are planted in different areas. However, soybean product in Thailand is not enough for both human consumption and use in animal feed materials. Total of soybean seed product show approximately 500,000 tons / year when the total demand of soybean seed materials is more than 600,000 tons. Diseases caused by several pathogens including fungi, bacteria and virus are mainly problem to soybean production (Prathuangwong, 1989, Prathuangwong *et al.,* 1996). Impotant diseases which significantly affected on both of quality and quantity of soybean produced such as rust, anthracnose, downy mildew, bacterial pustule, sudden death, and viral diseases which are negative effected on soybean commercial production in Thailand. The disease distribution and severity observed were difference based on the different climate of soybean cultivation regions. (Prathuangwong *et al.,* 1996).

2. Soybean bacterial pustule, causal pathogen, and disease control

Bacterial disease of soybean occuring worldwide and cause limited production during years of warm temperature and high moisture. The most common bacterial incited diseases are bacterial blight and bacterial pustule. The prevalence and severity of these diseases vary considerably from year to year which depend on the differences in weather patterns and cultivar of soybean. Bacterial pustule caused by *X. axonopodis* pv. *glycines* (*Xag*) (Sinclair, 1982; Parathuangwong, 1983; Vauterin, *et al.,* 1995) is a worldwide bacterial disease of soybean which reveals more severe infection in the moderate to high temperature areas. The disease occurs as important distributes in many soybean growing countries such as Argentina, Australia, Bolivia, Brazil, Cambodia, Canada, China, India, Japan, Malaysia, Nicaragua, Nigeria, Sudan, Taiwan, United states, (Sinclair and Dhingra, 1975) and Thailand (Prathuangwong *et. al.,* 1983). The diseases can be severe especially when the soybean is 30-40 days old (Prathuangwong, 1984) and affect both quality and quantity of soybean product.

The disease symptoms primarily occured on the foliage is minute pale green spots with elevated center on either or both leaf surfaces (Fig.1A; 1B). Thereafter, a small, raised, light- colored pustule forms in the center, usually in lesion on the under surface of the leaf, that some time may confuseed with the typical symptom of bacterial leaf blight caused by *Pseudomonas syringae* pv. *glycenea* (Sinclair, 1982). Moreover, the symptom of bacterial pustule has vary from minute specks to large, irregular, mottled brown areas that arise when smaller lesions coalesce. In later stages, dried, broken remnants of pustules may be seen on small brown necrotic areas surrounded by narrow yellowing haloes. Leaves then, become ragged when dead areas are torn away by the wind. Pustules are mainly formed by hypertrophy and also hyperplasia with surrounded by yellowing haloes (Sinclair, 1982; Prathuangwong, 1984). The symptoms may develop on stems and pods of susceptible varieties. The pustule pathogen commonly survives in soybean seeds and also in soybean crop debris depends on the environment, cultivars and virulence of the bacterial strain. Symptoms on resistant soybean varieties have occurred as small chlorotic spots but no well-defined pustules or light green chlorosis or fewer and smaller pustules. The seriously damaged leaves causing defoliation in susceptible varieties resulted in 15-50% yield losses, giving small, light and low capacity soybeans. (Prathuangwong and Amnuaykit, 1989).

Xag is a negative bacteria, rod shaped, does not form spores, has a single flagellum and a size of 0.2-0.9 x 0.57-2.59 µ (Fig. 2B). The optimum temperature for growth is 30-33°C. The unique characteristic of *Xag* are that it can produce catalase enzymes but not urease enzymes and is capable of digesting starch, gelatin, pectate and tween 80. This pathogen can produce hydrogen sulfide from peptone, cysteine and sodium thiosulfate to digest esculin and protein in milk and it is durable in 5% of salt medium. Characterization of colonies on media such as nutrient glucose agar, beef fusion agar and wakimoto's agar found them to be yellow, smooth rimmed (Fig. 2A), produce yellow pigment and dissolve in alcohol but not dissolve in water (Sinclear, 1982).

(A)

(B)

Figure 1 Bacterial pustule infection of susceptible soybean cultivar (Asksoy). In (A), bacterial pustule lesion with mature pustules on soybean leaf is shown (photo by Buensanteai). In (B), a close-up of arranged pustule symptom of *Xanthomonas axonopodis* pv. *glycines* is shown (photo by Buensanteai)

(A) (B)

Figure 2 *Xanthomonas axonopodis* pv. *glycines* causal agent soybean bacterial pustule diasese. In (A), Characterization of bacterial colonies on nutrient glucose agar media found them to be smooth rimmed, produce yellow pigment. In (B), *Xanthomonas axonopodis* pv. *glycines* is a negative bacteria, rod shaped, does not form spores, has a single flagellum and a size of 0.2-0.9 x 0.57-2.59 μ

3. Plant growth promoting rhizobacteria/ bacteria (PGPR/ PGPB)

The PGPR/ PGPB are free-living, isolated from the rhizosphere or plant leaf surface, when applied to seeds of several plants. It could enhance the growth of the plant or reduce the damage from several plant pathogens (Kloepper and Schroth, 1978; Kloepper *et al*., 1996; van Loon, 1997; van Loon *et al.,* 1998). It has been estimated that more than 300 million tonnes of nitrogen, potashsium and phosphate-chemical fertilizers have been used annually in order to increase crop yield (Glick *et al*., 1999). This result show, the potential negative effect of chemical fertilizers and pesticides on the global environment and the cost associated with production has led to research with the objective of replacing chemical fertilizers and pesticides with bacterial inoculants. Bacterial inoculants which help in plant growth are generally considered to be of two types a) symbiotic and b) free-living (Kloepper *et al*., 1996). Beneficial free-living bacteria referred to as PGPR/ PGPB are found in the rhizosphere of the roots of many different plants (Kloepper *et al*., 1996). Breakthrough research in the field of PGPR occurred in the mid 1970s with studies demonstrating the ability of *Pseudomonas* strains capable of controlling soil-borne pathogens to indirectly enhance plant growth and increase the yield of potato and radish plants (Kloepper and Schroth, 1981; Kloepper *et al*., 1980).

Rhizosphere bacteria/ bacteria promote plant growth and yield either directly or indirectly mechanisms (Kloepper *et al*., 1989; Glick, 1995; van Loon *et al.,* 1998). The direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick *et al*., 1997). The indirect mechanism occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Handelsman and Stabb, 1996; Nehl *et al.,* 1996; Cartieaux *et al.,* 2003). The direct growth promoting mechanisms are as follows i) nitrogen fixation ii) solubilization of phosphorus iii) sequestering of iron by production of siderophores iv) production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration (Kloepper *et al.,* 1989; Glick, 1995; Glick *et al.,* 1997) (Fig. 6). The indirect mechanisms by PGPR/ PGPB include i) antibiotic production ii) depletion of iron from the rhizosphere iii) synthesis of antifungal metabolites iv) production of fungal cell wall lysing enzymes v) competition for sites on roots and vi) induced resistance (Kloepper *et al.,* 1989; Kloepper *et al.,* 2004a; van Loon *et al.,* 1998; Glick *et al*., 1997).

4. Overview the mode of action of plant growth promoting rhizobacteria

Plant growth is influenced by a excess of abiotic and biotic factors. The region around the plant rhizosphere, is relatively rich in exudates nutrients because as much as 40% of plant photosynthates are lost from the roots (Lynch and Whipps, 1991). Therfore, the plant rhizosphere supports large microbial populations capable of exerting beneficial, neutral effects on plant growth and development. PGPR/ PGPB, first defined by Kloepper and Schroth (1978), include soil bacteria that colonize the roots of plants following inoculation onto seed and enhance plant growth. In the present PGPR/ PGPB are increasingly used as inoculants for biocontrol, biofertilization, phytostimulation, phytorenidiation, growth regulator and induced resistance mechanism. According to their mode of action, PGPR/ PGPB have been divided into two large classes, namely the Biocontrol- PGPR/ PGPB, which suppress plant pathogens, thereby benefiting the plant indirectly, and the PGPR/ PGPB that directly affect plant metabolism resulting in increased plant growth, seed emergence rotect thenselve from pathogen infection and improved crop yields (Bashan and Holguin, 1997). Generally, the interactions of plants and PGPR/ PGPB are based on an concentrated and precise exchange of signaling molecules between both partners.

4.1 Biofertilization

The most important biofertilizers are the nitrogen-fixing rhizobia that enhance the supply of nitrogen to the plant. Unlike other bacteria, these form a host-specific symbiosis with leguminous plants. The molecular interaction between the plants (providing the carbon and nitrogen source) and the microorganisms (providing the nitrogen supply) is highly complex and involves many factors (Steenhoudt and

Vanderleyden, 2000). For example, the enhanced rate of nitrogen-uptake stimulated by N-fixing Azospirillum is largely indirect and is linked to the impact of phytohormones on root development (Rodriguez and Fraga, 1999). Other PGPR/ PGPB biofertilizers influence the availability of phosphate by secreting phosphatases for mineralization of organic phosphorus, or by releasing organic acids for the solubilization of inorganic phosphates (Bloemberg and Lugtenberg, 2001). The release of siderophores that chelate iron and make it available to the plant root is another example of biofertilization (Walling, 2001).

4.2 Biological control

PGPR/ PGPB used for plant disease protection and biocontrol either affect the status of the plant via an induced systemic resistance (ISR) and/or a systemic acquired resistance (SAR) (Klopepper *et al., 1980,* 1988; Rasmussem *et al*., 1991; Ryals *et al*., 1996; van Loon *et al.,* 1998; Walling, 2001; Cartieaux *et al.,* 2003), or secrete anti-microbial metabolites, such as surfactin, fengycin, bacillomycin, pyoluteorin, phenazines and phoroglucinols, or surface-active cyclic lipopeptides that contribute to the control of soilborne plant pathogens (Walling, 2001; Ongena *et al.,* 2007). Bacterial determinants that are claimed or proven to produce ISR include siderophores and the O-antigen of lipopolysaccharides and salicylate, which also mediates SAR. Often ISR and SAR are activated imultaneously, although both are linked to independent pathways (Walling, 2001; Cartieaux *et al.,* 2003).

4.3 Phytostimulation

The PGPR/ PGPB that are able to affect plant physiology and biochemistry directly often synthesize and exudate plant hormones or compounds that imitate their activating of mode of action because of structural similarities. Some PGPR/ PGPB produce auxin (indole-3-acetic acid), cytokinin(s), gibberellin(s) (Bloemberg and Lugtenberg, 2001; Buensanteai *et al.,* 2007b), octadecanoids and compounds that stimulate the action of jasmonate. Others produce salicylate (Walling, 2001) or control the biosynthesis of ethylene via an ACC deaminase (1-aminocyclopropylcarboxylic acid) that reduces the available pool of ACC for ethylene biosynthesis by the plant (Pieterse *et al.,* 2002). Because ethylene often acts either synergistically or antagonistically with other plant hormones, manipulation of this pathway has an impact on many response complexes, for example, ISR, SAR, or salicylic acid- and jasmonic acid-linked gene expressions (Pieterse *et al.,* 2002; van Loon *et al.,* 2000). The phenomenon of phytostimulation profits particularly from manipulating the complex and balanced network of plant hormones or hormone-like compounds that directly and indirect influence growth or stimulate root formation. For example, *B. amyloliquefaciens* KPS46 produce auxins that stimulate root development, which can lead to improved crop yield (Buensanteai *et al.,* 2007b).

4.4 Volatile signals

The signaling transduction network between host plants and microbes has been extensively studied over the past 20 years, no volatile signal specifically acting between the PGPR/ PGPB and the plant had been reported until recently. To a large extent this might be because of the complexity of the matrix ‘soil’ in which the interactions take place. This is different from the gas phase where there are methods to identify volatiles that are exchanged between plants, or plants and organisms from other trophic levels. In addition to gaseous ethylene, and particularly the volatile methyl esters of the phytohormones jasmonic acid and salicylic acid, certain unsaturated aldehydes and terpenoids were shown to induce gene expression associated with growth enhancement and induced resistance in plants (Ryu *et al.,* 2003a; Ryu *et al.,* 2003b).

4.5 Plant growth promotion

The physiological effect of PGPR/ PGPB on plant growth depends ultimately on the amount of hormone and several nutrient that is available to the plant, which is based on the interaction between the plant and the bacterium (Patten and Glick, 1996). Three general types of possible association between the plant and the bacterium are important in order to exert a positive effect. These are a) transfer of IAA genes directly into the host genome b) infection of internal regions of the plant and secretion of IAA into the surrounding tissue and c) colonization of the external surface and secretion of IAA as an exogenous source to plants. In the case of beneficial rhizobacteria the effect is primarily thought to be advantageous when the bacteria are colonizing the external surface of the plant (Del Gallo and Fendrik, 1994). Early work showed that PGPR/ PGPB such as *Azotobacter paspali* secreted IAA into culture media and significantly increased the dry weight of leaves and roots of several plant species following root treatment (Barea and Brown, 1974). *Azospirillum brasilense*, which had the ability to produce plant growth-promoting substances such as indole acetic acid, indole lactic acid, gibberellin and cytokinin when applied to pearl millet, increased the number of lateral roots which were densely covered by root hairs (Tien *et al.,* 1979). Experiments with pure plant hormones showed that gibberellin increased production of lateral roots and cytokinins stimulated root hair formation. A combination of all three phytohormones was found to produce changes in the root morphology of pearl millet similar to those produced when inoculated with *A. brasilense* (Tien *et al.,* 1979). When *Pseudomonas putida* GR12-2, a nitrogen fixing strain, was applied to seeds of canola, it increased root length in sterile growth pouches (Lifshitz *et al.,* 1986). Barbieri *et al*. (1986) found that inoculation of wheat seedlings with *A. brasilense* increased the number and length of lateral roots. In 1989 Fallik and Okon showed that when maize seedlings were inoculated with *A. brasilense,* root surface area was significantly increased. The ability of *P. fluorescens* M.3.1 to produce auxins in the presence of maize root exudates in the culture medium indicated that the strain can convert exudates into secondary metabolites which can play an important role in plant development and increase maketable yield. (Benizri *et al.,* 1998).

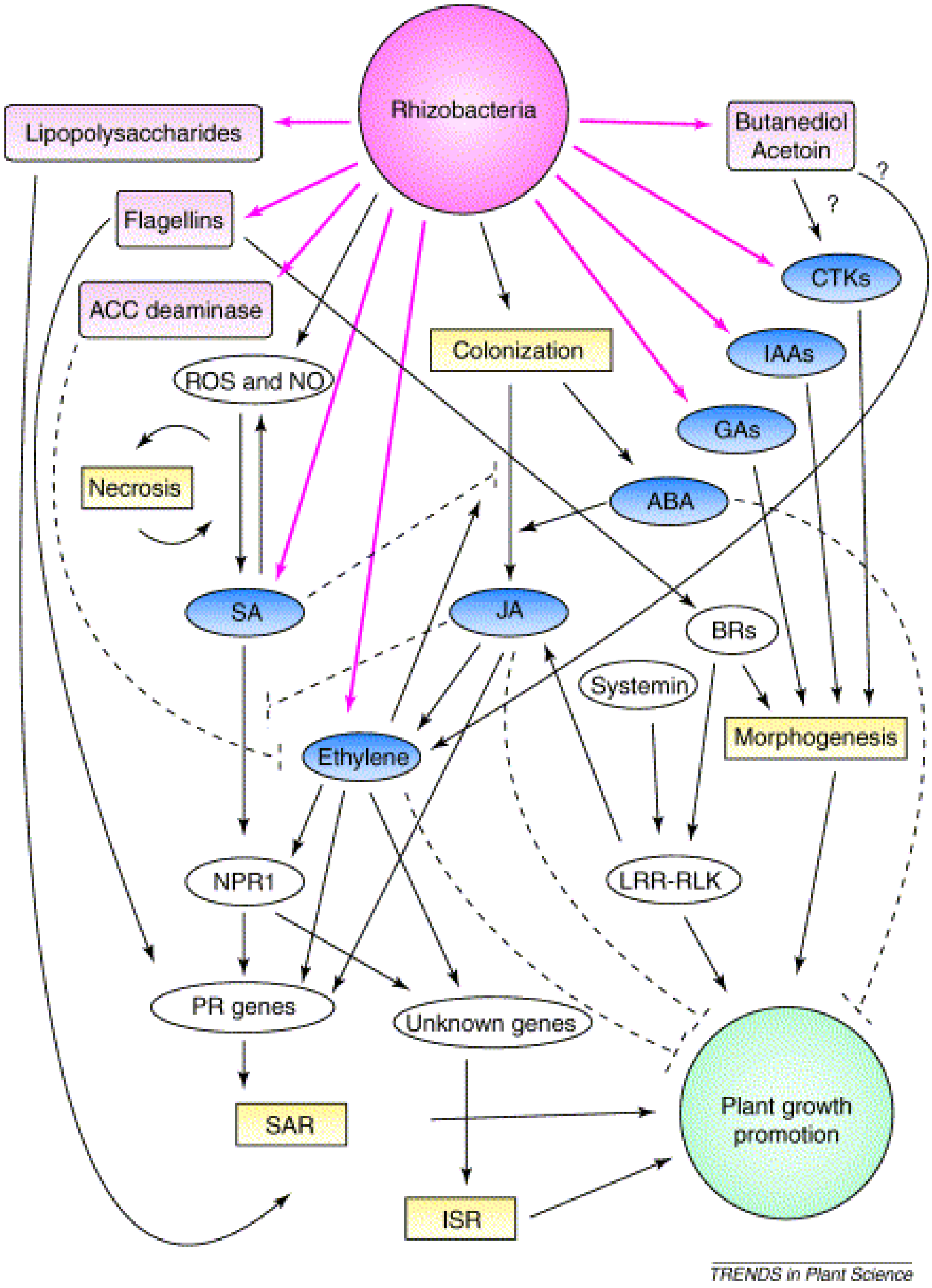


Figure 3 Network of signaling cascades involved in plant growth promotion and induced systemic resistance by rhizobacteria (Ping and Boland, 2004)

4.6 Induced systemic resistance

Systemic resistance has been induced by prior inoculation with PGPR/ PGPB, nonpathogenic microorganisms, biological control agents, microbial metabolites and chemical compounds (van Loon, 1997). The *Bacillus* group is well known through previous documents and publications for enhanced growth promotion and induced systemic resistance (Kloepper *et al.,* 2004a; Ryu *et al.,* 2003a; Ryu *et al.,* 2004a). The all results of previous reports are summarized showing *Bacillus* spp. including *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* elicit significant reductions in the incidence or severity of various diseases on a diversity of hosts (Bakker *et al.,* 2003, 2007; Kloepper *et al.,* 2004b; Prathuangwong and Buensanteai, 2007; Buensanteai *et al.,* 2007a). Elicitation of ISR by these strains has been demonstrated in greenhouse or field trials on tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, Arabidopsis, cucumber, loblolly pine, and two tropical crops. The main mechanisms of disease control and disease protection were traditionally believed to be those that act direct and indirect on phytopathogens such as fixation of atmospheric nitrogen that is transferred to the plant (Christian-Weneger, 1992), production of siderophores that chelate iron and make it available to the plant root, solubilization of phosphorus and iron (De Freitas *et al.,* 1997), release of volatile compounds (Ryu *et al.,* 2003b), and synthesis of various phytohormones (Araujo *et al.,* 2005; Boiero *et al.,* 2007), production of lytic enzymes (Bagyan and Setlow, 2002), producing antibiotics (Araujo *et al.,* 2005), increase in nitrate uptake (Mantelin and Touraine, 2003.), and competition for resources and space. However, more recent data have led to a new understanding of the way in which *Bacillus* spp. interact with plants: this PGPR/ PGPB produces changes in plant metabolism, thus increasing growth and enhancing resistance to biotic and abiotic stresses (Zehnder *et al.,* 2007).

Our previous report found that the *B. amyloliquefaciens* KPS46 is a plant-associated bacterium which stimulates plant growth and produces secondary metabolites that suppress several plant pathogens, the strain has been identified as a potential biocontrol agent by induced systemic resistance and secretion of elicitors to inhibit phytopathogens in various plant disease systems (Prathuangwong and Kasem, 2004; Prathuangwong *et al.,* 2004) that is well-studied in our laboratory (Prathuangwong and Buensanteai, 2007; Prathuangwong and Kasem, 2004). It could be used as a plant growth promoter and protect soybean plants from multiple diseases including bacterial pustule disease caused by *X. axonopodis* pv. *glycines* (Prathuangwong and Kasem, 2004; Prathuangwong and Buensanteai, 2007; Buensanteai *et al.,* 2007a). The strain has also been shown to suppress various plant diseases caused by soilborne and airborne pathogens (Prathuangwong and Kasem, 2004; Prathuangwong *et al.,* 2004). Variability in plant growth promotion and disease suppression by KPS46 of this beneficial bacterium has been reported in greenhouse and field trials conducted with soybean, corn, sunflower, sesame, teak, mangium, and vegetables (Prathuangwong *et al.,* 2004). Most of the research has focused on the mode of action of the strain KPS46 biocontrol agent and has found it to exhibit direct antagonism as well as antibiotic production (Prathuangwong and Kasem, 2004), with little study and not fully understood on induced systemic resistance and growth enhancement mechanisms. Throughout, the mode of action of available *B. amyloliquefaciens* KPS46 in induced resistance of soybean against bacterial pustule disease that we could identify at the protein activity level, an increase in the activity of peroxidaes, 1,3-β-glucanases, phenylalanine ammonia lyase and phenol compounds in soybean leaves treated with the PGPR/ PGPB biocontrol agent *B. amyloliquefaciens* KPS46 (Prathuangwong and Buensanteai, 2007; Buensanteai *et al.,* 2007a).

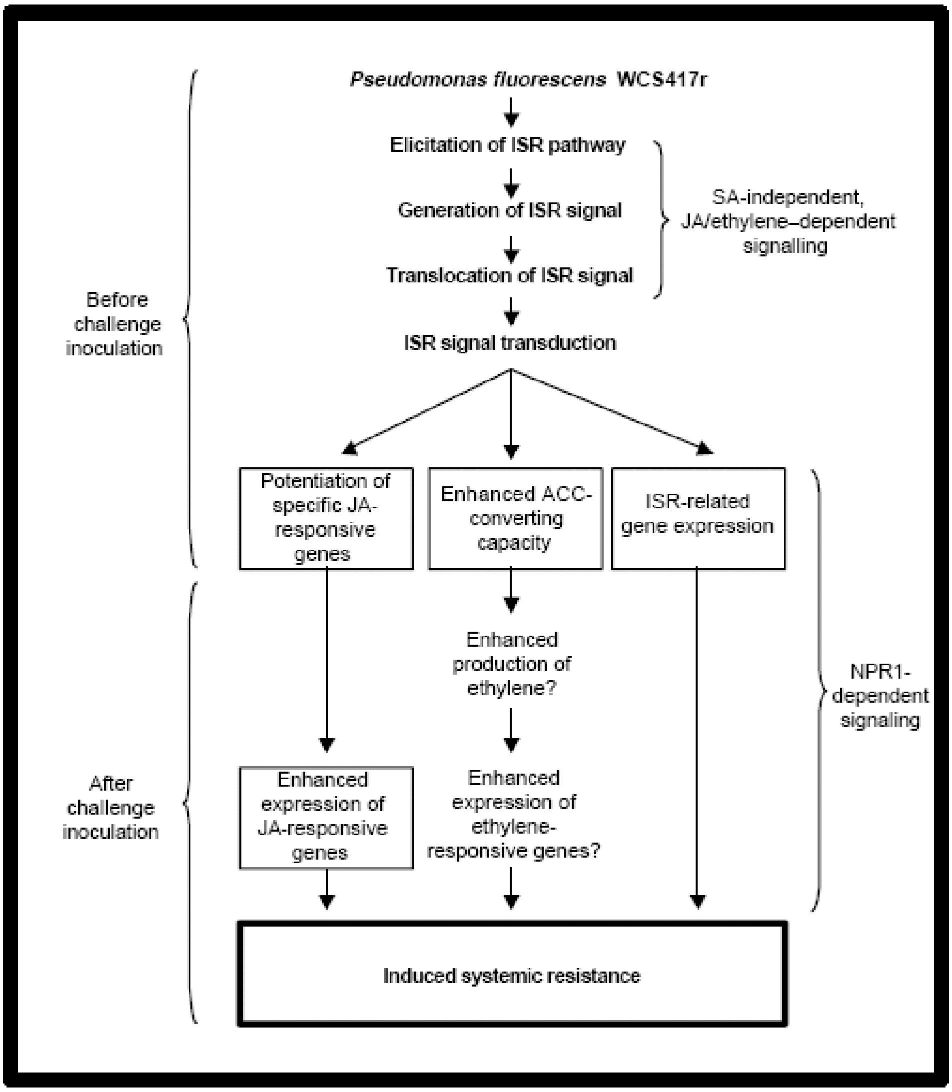


Figure 4 Working model explaining the possible involvement of JA and ethylene in *Pseudomonas fluorescens* WCS417r-mediated ISR in Arabidopsis (Pieterse *et al.,* 2001)

5. Overview of plant defense

All plants perform active defense mechanisms to protect themselves against several pathogen infection. To survive, the host plant is required to respond in an appropriate manner to these external stresses. In order to response, the plant needs certain defense mechanisms that would help it either to prevent the stress from causing any damage or to heal the damage that has already occurred. The plant defense mechanisms include both structural defenses and biochemical mechanisms (Hammerschmidt, 1999). Of these defense mechanisms, most of the responses need to be activated through a signal (Xang *et al.,* 1997). The signaling transduction network might originate from the pathogen itself. It can however also be produced by the damaged plant after pathogen attack (van Loon *et al.,* 1998; van Loon, 2000).

5.1 Structural defense mechanisms

The structural defense mechanisms include waxes that are deposited on the leaves (Hammerschmidt and Kue, 1995; Hammerschmidt, 1999). These waxes prevent the leaves from getting wet and thus prevent a suitable place for the germination of fungi. Hairs on the leaf will also have a similar effect. The structure of stomata also plays an important role in that any structural modification would make penetration by pathogens more difficult (Agrios, 1988). The thickness of the cuticle, as well as the thickness and strength of the epidermal cells will make penetration by pathogens more difficult (Barthlott and Neinhuis, 1997). These mechanisms are present in most plants (Hammerschmidt and Kue, 1995).

Induced structural defense mechanisms can be divided into two classes, namely histological and cellular defense mechanisms. Histological defense mechanisms include the deposition of gum or the production of cork, which will prevent the spread of the pathogen throughout the plant (Agrios, 1988). In the other hand, cellular defense mechanisms include the fortification of cell walls (El-Gendy *et al.,* 2001). One type of cell wall fortification that occurs rapidly in response to fungal invasion is the formation of papillae. They are thought to physically block fungal penetration of the host cells (Bayles *et al.,* 1990). Rapid callose deposition in cell walls is also frequently associated with sites of pathogen penetration. The blockage of plasmodesmata with callose is an essential component of the defense response required to impede cell-to-cell movement of viruses (Beffa *et al.,* 1996). An additional but probably slower mechanism that renders cell walls more resistant is the localized increase of their lignin content (Mulosevic and Slusarenko, 1996). The most compelling evidence for the role of lignification in resistance has been provided by Moerschbacher *et al.* (1990). Furthermore, the cross-linking of cell wall proteins can be induced by hydrogen peroxide (H_2O_2) by activating glutatione peroxidases as well as plant cell wall phenolics that contribute to prevent penetration of fungal hyphae (Grant and Loake, 2000). These induced structural mechanisms are only employed after pathogen infection and thus prevents the pathogen from spreading throughout the plant.

5.2. Biochemical defense mechanisms

Various physiological changes occur in plants after the recognition of the pathogen. These physiological changes occur in both resistant and susceptible plants. The main difference between resistant and susceptible plants is the response time after the recognition event (Hammerschmidt and Schultz, 1996; Maleck *et al.,* 2000; Lam *et al.,* 2001). Therefore, if a plant can respond faster to an invading pathogen, it seems that resistance would be acquired, but a delay in responding to an attacking pathogen would lead to infection. Moreover, the accumulation of ROS and phenolics forms part of these changes (Schmelzer *et al.,* 1993). Some of these ROS are involved in the cross-linking of cell wall polymers to strengthen it against penetration by pathogens (Thordal-Christensen *et al.,* 1997). They include amongst others super oxide anions (O_2^-), which can be converted to H_2O_2 via superoxide dismutase (SOD), singlet oxygen and the hydroxyl radical (OH^-) (Apel and Hirt, 2004). The above-mentioned leads to cell destruction. Hydrogen peroxide might also be responsible for the activation of other defense pathways in the HR and induced resistance (Levine *et al.,* 1994). The accumulation of phytoalexins is also associated with the HR and induced resistance (Rustérucci *et al.,* 1996). Phytoalexins are novel phenolics that accumulate after infection has occurred and have antimicrobial functions (Hain *et al.,* 1993). The antimicrobial activity of the phytoalexins allows the plant to defend itself against penetration by a pathogen (Schmelzer *et al.,* 1993). The production of PR-proteins that accumulate in the extracellular regions and vacuoles of plant cells is strongly related to the HR (Bertini *et al.,* 2003).

Most economic crops have the ability to protect themselves from several plant pathogen infection via activating defense response and developed biochemical defense response as innate immune mechanisms complex of protection of both above- and under-ground of plant parts. This type of resistance is referred to as induced systemic resistance (ISR), in order to distinguish it from the systemic acquired resistance (SAR) developed after primary infection by a pathogen (van Loon *et al.,* 1997; van Wee *et al.,* 2000). Throughout, ISR of plants against pathogens is a widespread phenomenon that has been intensively investigated with respect to the underlying signaling transduction pathways as well as to its potential use in plant protection and plant diseases control. Elicited by a local infection, plants respond with a signaling transduction cascade complex that leads to the systemic expression of a broad spectrum and long-lasting disease resistance that is efficient against plant pathogenic fungi, bacteria and viruses (van Loon, 1997; Bakker *et al.,* 2007). As well, ISR is activated by elicitors through changing the physiological and biochemical reactions of the host leading to the synthesis of defense chemicals against the challenge phytopathogens (Ahn *et al.,* 2002; Dorey *et al.,* 1999). These defense substances in the elicitors treated plants may play either a direct or an indirect role in the suppression of pathogen development (van Loon and van Strien, 1999). Moreover, resistance mechanism to pathogens appears by a delay in symptom development, a reduction of disease severity, and an inhibition of the pathogen growth. Two major pathways are involved in the induction of systemic plant resistance as SAR and ISR. The defense proteins are importance regulators signal transduction during plant responses to pathogen attack as well as to various environmental stresses (Yang *et al.,* 1997; Wang *et al.,* 2002). The spectrum of effectiveness of WCS417r-mediated ISR and pathogen-induced SAR overlaps but is also partly divergent (Pieterse *et al.,* 2002). Moreover, the level of induced protection can be enhanced further when both

ISR and SAR are activated simultaneously, indicating an additive effect of both type of resistances (van Wees *et al.,* 2000). If SAR and ISR are phenotypically similar (a broad-spectrum systemic resistance), they differ by their molecular mechanism signature. The onset of SAR is unavoidably accompanied by a local and systemic increase in the endogenous level of salicylic acid (SA) (Vernooij *et al.,* 1994; Pieterse *et al.,* 1998). In contrast, ISR is independent of SA (Pieterse *et al*. 1996). Two types of molecular tools made it possible to precisely define the role of SA during the SAR: i) transgenic plants degrading SA (Gaffney *et al*. 1993) and ii) mutants unable to induce the biosynthesis of SA after an attack by a pathogen (Pieterse *et al.,* 1998). Using these mutant plants, it was also possible to show that the WCS417r-mediated ISR in *Arabidopsis* is independent of the accumulation of SA (van Loon *et al.,* 1998). On the other hand, the use of *jar1-1* and *etr1-1*, two mutants altered in the response to jasmonic acid (JA) and ethylene (ET) respectively, indicated that WCS417r-mediated ISR requires an intact signaling pathway to these two phytohormones (Pieterse *et al.,* 1998; Pieterse *et al*. 2000).

5.2.1 Salicylic acid (SA) signaling pathway

The first leads to the expression of pathogenesis-related proteins, is salicylic acid (SA)-dependent and was initially described as being pathogen inducible (van Loon and van Strien, 1999; Pozo *et al.,* 2004). SA can be synthesized via two routes, firstly through the phenylalanine biosynthetic pathway by PAL from phenylalanine (Mauch-Mani and Slusarenko, 1996). Alternatively, it can be synthesized through isochorismate synthase (ICS) and isochorismate pyrovate lyase (IPL) from chorismate (Wildermuth *et al.,* 2001) (Fig. 3). Evidence confirming the key role of SA in defense came from analyzing transgenic plants expressing the *nahG* gene, which encodes for an enzyme salicylic acid hydroxylase that inactivates SA by converting it to catechol (Delaney *et al.,* 1994). Plants with the NahG phenotype showed to be more susceptible to normally avirulent pathogens (van Wees and Glazebrook, 2003). The NahG phenotype was found to be a direct result of reduced SA levels, since functional analogs of SA, namely INA (2, 6-dichloroisonicotinic acid) and BTH (benzothiadiazole) was able to restore resistance in the plants (Lawton *et al.,* 1996). PAL–inhibited plants also showed to be more susceptible to avirulent strains of pathogens, while resistance was restored by treating plants with SA or INA (Mauch-Mani and Slusarenko, 1996). Salicylic acid signaling seems to be mediated through at least two mechanisms, one dependent on NPR1 (Cao *et al.,* 1998) and one independent of NPR1 (Kachroo *et al.,* 2000). Signaling through NPR1 has been the aim of various studies. It was found that NPR1 contains at least four ankyrin repeats, which are also found on proteins that are involved in very diverse biological functions and also in protein-protein interactions (Bork, 1993). Some of the effects of SA are that it binds to certain effector proteins. Four of these effector proteins have been identified and includes a salicylic acid binding protein (SABP) catalase (Chen *et al.,* 1993), ascorbate peroxidase (Durner and Klessig, 1995), carbonic anhydrase (SABP3; Slaymaker *et al.,* 2002) and SABP2 (Kumar and Klessig, 2003).

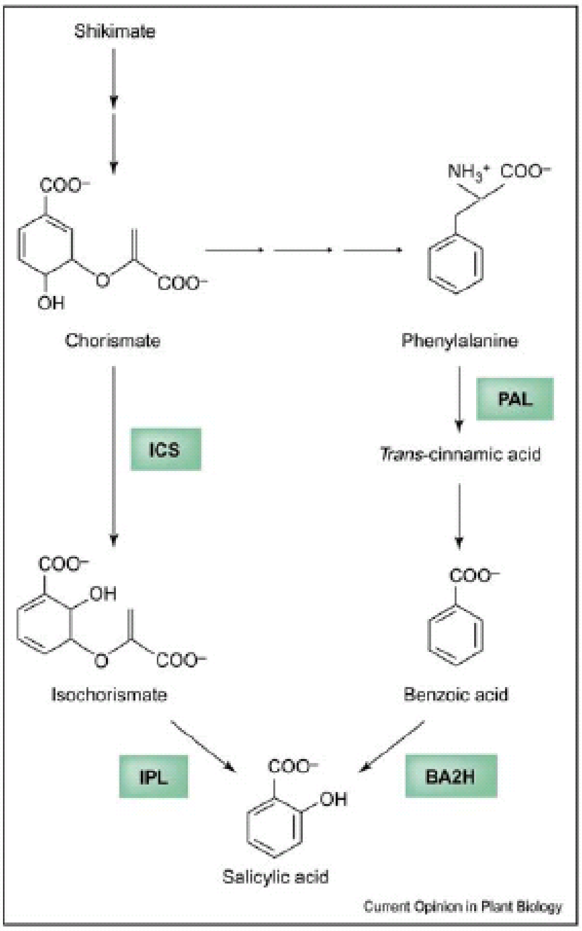


Figure 5 In plants, SA can be synthesized from two different pathways, the phenylpropanoid pathway with benzoic acid as an intermediate, as well as the newly identified isochorismate pathway. Isochorismate synthase (ICS), Isochorismate pyruvate lyase (IPL), Phenylalanine ammonia lyase (PAL), Benzoic-acid-2-hydroxylase (BA2H) are the important enzymes for SA biosynthesis (Shah, 2003)

5.2.2 Jasmonic acid (JA)/Ethylene (ET) signaling pathway

The second was initially described in plants colonized by nonpathogenic rhizobacteria, is jasmonic acid (JA)/ethylene (ET)-dependent and induces a primed state which enhances defense gene expression in the plant upon subsequent pathogen attack (Sticher *et al.,* 1997; Pozo *et al.,* 2002). Besides, JA has also been implicated as a signal in plant defense response (Hammond-Kosack and Jones, 1996). Jasmonic acid is derived from linolenic acid by a lipoxygenase (LOX)–mediated oxygenated process (Fig. 4) (Hamberg and Gardner, 1992). Exogenous application of JA was found to induce expression of several defense related genes, including osmotin (PR-5) (Xu *et al.,* 1994) as well as different enzymes involved in defense reactions such as PAL (Gundlach *et al.,* 1992) and LOX (Bell *et al.,* 1995). The fact that PAL is a key enzyme in the biosynthesis of SA demonstrates the interaction between these two signals in plant defense. One example of this antagonistic relationship between SA and JA was demonstrated by Rao *et al*. (2000) in showing how these two signaling pathways interacted in controlling the magnitude of ozone-induced HR-like cell death. The role that JA plays in protecting plants from pathogens and especially insects are deemed critical (Huang *et al.,* 2004). Previous studies on cell cultures that were treated with fungal elicitors, showed an increase in JA biosynthesis, demonstrating the role of JA in plant–fungal interactions (Gundlach *et al.,* 1992).

The plant hormone, ethylene, is involved in many aspects of the plant life cycle, including germination (Goeschl *et al.,* 1966), leaf senescence (Grbic and Bleecker, 1995), nodule development (Oldroyd *et al.,* 2001) and plant defense (Hoffman *et al.,* 1999). Ethylene is formed from methionine via S-adenosyl-L-methionine and the cyclic, nonprotein amino acid 1-aminocyclopropane-1-carboxylic acid (Miyazaki and Yang, 1987).

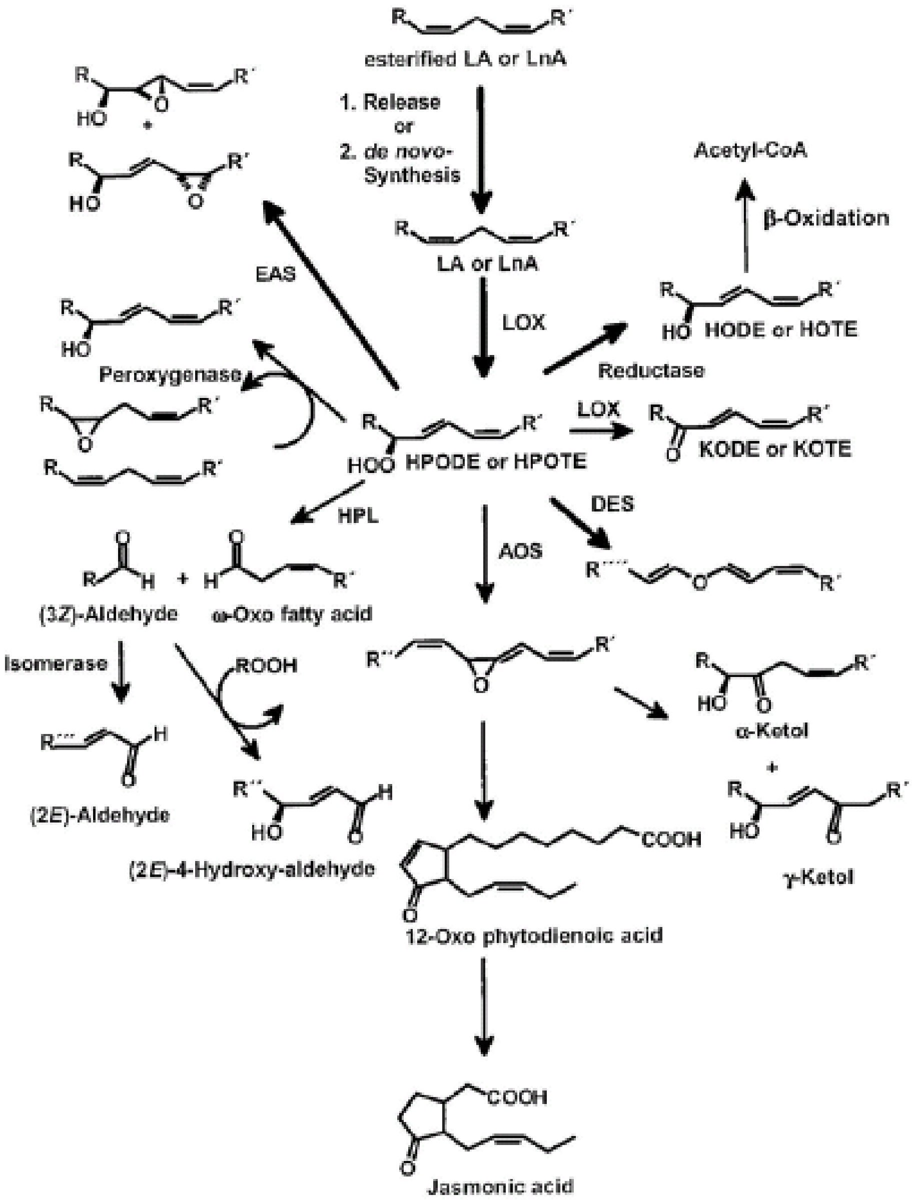


Figure 6 JA originates from linolenic acid. Through the LOX pathway and three times β-oxidation reaction, JA is produced

6. Proteomic technology

Proteomic technology, two-dimensional polyacrylamide gel electrophoresis (2D–PAGE) is one of the most powerful proteomics tools for the separation and quantification of proteins. There have been a number of recent advances in 2D methodologies, including improved sample application and use of immobilized pH gradient (IPG) strips for isoelectric focusing (IEF), allowing more proteins to be arrayed in micropreparative quantities. Among several proteomics tools, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF–MS) and liquid chromatography mass spectrometry (LC–MS) are sensitive methods for accurately characterizing protein expression profiles (Fig. 8). Furthermore, the proteomics has become an important tool in the study of plant biology and their plant-microbes interaction (Kim *et al.,* 2004) because these tools can be used both to visualize and compare complex mixtures of proteins and to gain a large amount of information about the individual proteins involved in specific plant both biological responses with biotic (Kim *et al.,* 2004) and abiotic strss (Shen *et al.,* 2003). In the last decade, proteomics has succeeded in identifying approximately 400 proteins associated with the development and functioning of both mycorrhizal and rhizobial symbioses (Segarra *et al.,* 2007). However, little is known and not fully understood about the induction of protein or gene expression changes in the plant by *Bacillus* spp. and plant associated interaction.

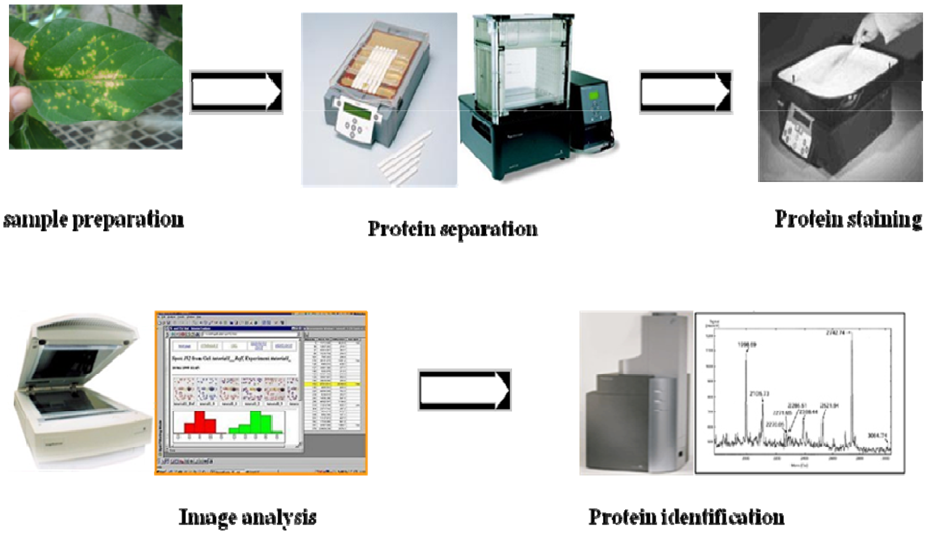


Figure 7 Model of proteomic technology, two-dimensional polyacrylamide gel electrophoresis (2D–PAGE) is one of the most powerful proteomics tools for the separation and quantification of proteins. There have been a number of recent advances in 2D methodologies, including improved sample application and use of immobilized pH gradient (IPG) strips for isoelectric focusing (IEF), allowing more proteins to be arrayed in micropreparative quantities. Among several proteomics tools, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI–TOF–MS) and liquid chromatography mass spectrometry (LC–MS) are sensitive methods for accurately characterizing protein expression profiles.

MATERIALS AND METHODS

1. Bacterial strains and culture conditions

Bacterial strains used in soybean experiments included *B. amyloliquefaciens* strain KPS46 and two strains of *X. axonopodis* pv. *glycines* (*Xag*): Thailand strain 2-2, from the collection of the third author, and Nebraska strain 20036a, provided by A. K. Vidaver, University of Nebraska-Lincoln. In addition, *P. syringae* pv. *tomato* (Pst) strain DC3000, provided by J. Alfano, University of Nebraska-Lincoln. The bacterial strains were stored in nutrient broth with 10% glycerol at -80 °C and revived by streaking onto nutrient glucose agar. Cell suspensions of KPS46 and the *Xag* strains were produced by culturing each strain in nutrient glucose broth (NGB) at 28 ± 2 °C for 48 h with shaking at 180 rpm. Cells were collected and washed twice by centrifugation (13,000 rpm; 20 min) and resuspension in sterile distilled water (SDW). Distilled water, rather than phosphate buffer, was used for suspending cells to avoid induction of resistance in plants by phosphates. Cell concentrations were determined turbidimetrically and adjusted to optical density of 0.2 at 600 nm, corresponding to 1×10^8 CFU ml^{-1}. Similar methods were used to produce cells suspensions of Pst DC3000 except that the strain was cultured in tryptic soy broth (Sigma Laboratories, USA) for 2 days at 28ºC (Bisgrove *et al.,* 1994) and 0.85% NaCl solution supplemented with Tween 20 (250 µg/ml) was used as the suspension medium.

Soybean (*Glycine max* L.) susceptible cultivar to *Xag* pathogen including Spencer (gift from Prof. G. Hartman, University of Illinois, USA) and SJ4 (Thai cultivar) were used in all experiments. The soybean seed surface sterilized by treatment with 95% ethanol (v/v) for 20 s, followed by soaking in 20% bleach (v/v) for 20 min. Seeds were washed with sterile distilled water 5 times in order to remove excess bleach. The seeds were then air dried by placing them in the laminar flow hood for 24 h. To produce soybean, 2 viable seed were planted in 30 cm-diameter pots containing a steam-pasteurized potting medium (Sharpsburg silt clay loam, vermiculite, and sand mixed in equal volumes), and the seedlings were grown in a greenhouse for 2 weeks. The pots were watered daily with a nutrient solution (20-10-20 Peat-Lite Special, Scotts-Sierra Horticultural Co., Marysville, OH). In all experiments, pots of soybean plants were kept in a greenhouse with a 12-h photoperiod (25 ^{o}C and 60–75% relative humidity during the light period, 15 ^{o}C and >93% relative humidity during the dark period). Procedures for growing plants and regulating greenhouse conditions were described previously (Kilic-Ekici and Yuen, 2003).

2. Bioassay

The first series of experiments was conducted to KPS46 culture fluid fractions and extracted extracellular factors for enhancement of soybean growth. Seeds of soybean cv. Spencer were surface disinfested by treatment with 95% ethanol (v/v) for 2 min, followed by soaking in 20% commercial bleach (v/v) for 20 min. The seeds were then washed with sterile distilled water 5 times in order to remove the bleach. Before planting, 30 g of soybean seeds were mixed thoroughly with 5 ml of a liquid treatment. The treatment included a whole culture of KPS46 in NGB, cell-free fluid from a KPS46 culture in NGB, suspension of live cells collected from a KPS46 culture, and a suspension of heat-killed cells. The cell concentration in the whole culture and cell suspensions were adjusted to $1x10^{8}$ cfu ml^{-1} on the basis of absorbance, while culture fluid was diluted by the same dilution factor as the whole culture. Other treatments included suspensions of extracellular proteins (250 ug/ml), lipopeptides (50 ug/ml) and IAA (50 ug/ml) extracted from KPS46 culture fluids (Buensanteai *et al.,* 2007b; Buensanteai *et al.,* 2008). In one experiment conducted under gnotobiotic conditions, treated seeds were placed onto 0.5X MS medium (Murashige and Skoog basal salt mixture, Cat M5524, Sigma, St. Louis) in square plates (gnotobiotic condition). There was four replicate plates per treatment with three seeds per plate. The plates were incubated on edge at a 65 degree angle to allow root growth along the agar surface and unimpeded growth of the tops of the seedlings after the seed had germinated. The plates were maintained in a growth chamber with a photoperiod of 16 h of light, 8 h of darkness, light intensity of 200 umol m^{2} s^{-1}, and constant temperature of 24 ^{o}C. At 7 days after germination, seedlings were harvested for growth parameter measurement (root and shoot lengths; fresh and dry weights; and number of lateral root) to confirm that the bacterial treatment was effective in plant growth promotion. The experiment was performed three times. For the greenhouse experiment, soybean seeds treated with KPS46 culture fractions and extracts were planted in pots (30 cm diameter) containing a steam-pasteurized potting medium of Sharpsburg silt clay loam, vermiculite, and sand mixed in equal volumes. There was 10 replication pots per treatment with two seeds per pot. The pots were watered daily with a nutrient solution (20-10-20 Peat-Lite Special, Scotts-Sierra Horticultural Co., Marysville, OH) and kept in a greenhouse with a 12-h photoperiod (25 ^{o}C and 60–75% relative humidity during the light period, 15 ^{o}C and >93% relative humidity during the dark period). At 14 days after seedling emergence, seedlings were harvested for measurements of root and shoot lengths and fresh and dry weights. The experiment was conducted three times.

In other experiment is inoculation of soybean with bacterial strains. Experiments were conducted at Kasetsart University, Bangkok Campus, and University of Nebraska-Lincoln. Bacterial pustule-susceptible soybean cultivars SJ4 and Spencer (seeds provided by G. Hartman, University of Illinois) were used in the Thailand and Nebraska experiments, respectively. Seed were surface disinfested before experiments by treatment with 95% ethanol (v/v) for 20 s, followed by soaking in 20% bleach (v/v) for 20 min. Seeds were washed with sterile distilled water 5 times to remove the bleach and then air dried in a laminar flow hood for 24 h. Disinfected seed were treated with strain KPS46 by mixing 30 g seed with 15 ml of bacterial cell suspension for 5 min. Seed treated by distilled water served as the control. Treated seed were planted in 30-cm-diameter pots (two per pot) containing steam-pasteurized potting media (silt clay loam soil, vermiculite, and sand mixed in equal volumes). The pots were kept in a greenhouse with a 12-h photoperiod (25 ^{o}C and 60–75% relative humidity during the light period, 15 ^{o}C and >93% relative humidity during the dark period) and watered daily with a nutrient solution (20-10-20 Peat-Lite Special, Scotts-Sierra Horticultural Co., Marysville, OH). Soybean plants were grown for approximately 2 weeks to the two-fully-expanded-leaf stage. At this time, plants of cv. SJ4 were challenge inoculated with *Xag* strain 2-2 and those of ‘Spencer’ were inoculated with strain 20036a. The pathogen cell suspensions were sprayed onto plants using hand-held spray bottles. Plants treated with distilled water served as the no-pathogen control. Put the pathogen treated plants in a moisture chamber. At various intervals after pathogen inoculation, pots of plants were sacrificed for collection of leaf samples used in biochemical and proteomic analyses described below. Remaining pots of plants were maintained in the greenhouse for 15 days. Disease severity (percent leaf area exhibiting lesions and chlorosis) was estimated for each leaf using the method described by Prathuangwong *et al.* (1993). Measurements from all leaves in each pot were averaged prior to statistical analysis. The experiment was performed three times and the data obtained was analyzed using SAS version 9.1.

3. Extraction and analysis of KPS46 metabolites

The cell free filtrate, indole-3-acetic acid (IAA), lipopeptides and extracellular proteins of the strain KPS46 were conducted in mechanism of growth promotion. These elicitors were extracted from bacterial supernatant by the slight modification method described by Araujo *et al.* (2005); Buensanteai *et al.* (2007b) and Buensanteai *et al.* (2008). The culture was centrifuged at 13,000 rpm at 4 ^{o}C for 20 min and the supernatant retained for further testing.

3.1 Cell free filtrate

The supernatant of stationary phase of KPS46 growth of prevented proteolytic digestion (48-h old culture) was collected by centrifugation at 12,000 rpm at 4 ^{o}C for 10 min followed by filtration through a 0.2 nm nitrocellulose filter. The bacterial cell pellet was washed three times in sterile saline (0.85% NaCl). The cells were resuspended in sterile distilled water and the density of the suspension was adjusted to 10^8 CFU ml^{-1} based on optical density (OD of 0.2 at 600 nm). A portion of the cell suspension was used in live form while another portion was heated in a water bath at 100°C for 30 min to kill the cells prior to use in experiments.

3.2 Auxin phytohormone

Production of plant growth regulator, IAA, by KPS46 was assayed based on the method described by Patten and Glick (2002) with slight modifications. Supernatants from bacterial cultures of the strains KPS46 grown in NGB for 24, 48, 72, 96 and 120 h, and in DF salt minimal medium for 48 h. The 20 ml single bacterial colony was transferred to 500 ml of NGB and DF salt minimal medium amended with 0, 125, 250, 500 2,500 and 5,000 mM of tryptophan obtained from a filter-sterilized 2-mg/ml stock L-tryptophan prepared in warm water, incubated at 28 ± 2°C for 48 h on a shaker incubator, the method slightly modification described by Patten and Glick (2002). Bacterial cultures were resuspended in sterile distilled water and adjusted to 1×10^8 CFU/ml by 0.2 OD at 600 nm. Fluid from KPS46 cultures were made cell free by centrifugation at 8,000 rpm for 20 min at 4°C and filtration. For the colorimetric Salkowski assay of IAA analysis, 1 ml of culture fluid was mixed with 4 ml of Salkowski's reagent in the ratio of 1:4 and incubated at room temperature for 20 min. The absorbance was measured at 535 nm using a spectrophotometer (Spectronic 20D, Rochester, NY, USA) (Crozier *et al.,* 1988). The quantity of indoles was determined by comparison with a standard curve using IAA in the concentration range of 0-50 ug/ml. Concentration of IAA also was determined by reverse phase-HPLC. IAA extraction, KPS46 culture fluid was extracted three times with ethyl acetate after adjusting the pH to 2.8 and then evaporated at 40 °C for 20 min, use for IAA treatment. The 60 ul aliquots were injected into an Alltech, type Econosphere C185U column (250 by 4.6 mm) equipped with a differential UV detector absorbing at 280 nm. The isocratic solvent used for reverse-phase chromatography was acetonitrile-glacial acetic acid (1%) in water (10:90). The flow rate was adjusted to 1 ml/min. Peak retention times were compared with those of chemically synthesized IAA standards and quantified by comparison of peak areas.

3.3 Surfactin lipopeptides

Extracellular lipopeptides were extracted from KPS46 culture supernatant by acidifying the fluid to pH 2.0 with concentrated HCl and allowing the formation of a precipitate at 4°C overnight. The precipitate was collected by centrifugation (12,000 rpm, 4°C, 15 min), washed three times with distilled water, and dried by vacuum lyophilization. The dried lipopeptide was extracted three times with 100 % methanol for 3 h. The methanol was removed with the aid of an EYELA rotary evaporator under reduced pressure. The crude lipoipeptide was obtained as a brown-colored material using an EYELA rotary evaporator. The crude lipoipeptide was dissolved in butanol and was loaded onto a column of reverse-phase HPLC system. The lipopeptide-enriched extract was obtained from 20 ml of the corresponding cell-free supernatant after purification on a ISOLUTE C-18 CE type cartridge (International Sorbent Technology Ltd., Hengoed, UK) following the slighly modification method described by Jacques *et al.* (1999) and Araujo *et al.* (2005). The system was operated at a flow rate of 2.0 ml/min with a solvent of 90% methanol as the mobile phase. HPLC spectra were detected by a UV monitor at 210 nm. We identified in this work sufactin-type lipopeptides: by the basis of its typical retention times, compared with those of surfactin purified standards (Sigma S3523). Lipopeptides were resolubilized in $NaHCO_3$ 0·1 mol l^{-1}, use for lipopeptides treatment.

3.4 Extracellular proteomes

Extracellular proteins were extracted from the culture fluid of late-exponential phase cultures of KPS46 to prevent proteolytic digestion. The supernatant was filtrated through a 0.2 nm nitrocellulose filter and then 20 ml 50% trichloroacetic acid (Sigma T6399) was added to 500 ml of filtrate, mixed well and placed on ice for 30 min. The aggregated proteins were precipitated by centrifugation at 12,000 rpm at 4 ^{o}C for 15 min, washed three times in cold 70% ethanol (-20 °C), dried and dissolved in IEF (isoelectric focusing) sample buffer consisting of 8 M urea (Sigma U6504), 2 M thiourea (Sigma T7875), 2% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate, Sigma C9426), 2% Triton X-100 (Sigma T8532), 50 mM DTT (dithiothreitol, Sigma D9163), and 0.5% ampholytes (Bio-Rad 163-1152) (Buensanteai *et al.,* 2008). Total protein content of the samples was quantified by the Bradford assay (Bradford, 1976), use for extracellular proteins treatment.

4. Mutagenesis of KPS46 and selection of mutant strains

Cells were obtained from a culture of strain KPS46 in NGB at stationary phase, 4 h after the optical density (λ= 600 nm) of cultures stopped increasing. The cells were washed and suspended in 0.05 M potassium phosphate buffer, pH 6.8, to $1x10^8$ cfu ml^{-1}. Volumes (200 ul) of this suspension were exposed to UV irradiation for various durations in glass petri dishes at a constant distance from the UV source (λ = 254 nm, 10 erg/mm^2; BII Illuminator, USA). The samples were frequently agitated during exposure. Aliquots (100 ul) were removed from the irradiated samples at different time intervals, and spreaded onto nutrient glucose agar plates. Colonies arising from surviving cells were selected following 48 h of incubation in the dark condition (Whistler *et al.,* 2000; Saxena *et al.,* 2002).

More than 2,490 irradiated strains were screened for loss of or decrease in indole production. Fluid from cultures of these strains in NGB was tested for indole concentration via the Salkowski assay as described previously and analysis of KPS46 metabolites: auxin phytohormone. These strains in which indole production was decreased by more than 10% compared with wildtype KPS46 were considered to be indole-reduced mutants and were retained for further study. Select indole-reduced mutants were compared further to wildtype KPS46 with respect to excretion of IAA, extracellular proteins and lipopetides. For IAA and extracellular protein excretion, KPS46 and representative UV irradiated strains were grown at 28 ± 2^{o}C for 48 h in NGB. Culture fluid was extracted and assayed for IAA using reverse-phase HLPC as described above. Relative extracellular protein excretion was gauged on the basis of chitosanase activity (Pelletier and Sygusch, 1990) in the culture fluid assayed by quantitative estimation of the reducing sugar produced with chitosan as the substrate. Briefly, a reaction mixture consisting of 0.5 ml culture fluid and 0.5 ml of 1% colloidal chitosan in 1 ml McIlvaine buffer (100 mmol/L citric acid, 200 mmol/L sodium phosphate, pH 7.0) was incubated for 30 min at 56 °C in a shaking water bath. The reaction was stopped in boiling water for 10 min. The amount of reducing sugar released was measured using dinitrosalicylic acid reagent, and the absorbance was measured at 540 nm. One unit of chitosanase activity was defined as the amount of enzyme that liberated 1 μmol of reducing sugar per min at the same conditions using GlcN as the standard (Pelletier and Sygusch, 1990; Rivas *et al.,* 2000). In the other experiment, swarming motility across a solid surface was used to access relative lipopeptide production (Kearns and Losick, 2003). This trait was evaluated by seeding cell suspensions of stationary-phase cultures (50 μl; 1 x 10^8 cfu ml^{-1}) onto the centers of motility test plates (NGB with 1.5% agar; and LB with 0.3, 0.7, or 1.5% agar). Plates were incubated at 37°C, and the diameters of halos due to bacterial migration were measured 24 to 48 h postinoculation, and swarm cell differentiation was analyzed (Senesi *et al.,* 2002; Kearns and Losick, 2003).

In addition, an experiment was conducted to determine whether or not a UV mutant strain N19G1 derived from KPS46 can enhance plant growth. As in the gnotobiotic soybean growth experiment described above, 30 g of soybean seeds were mixed thoroughly with 15 ml of cell suspensions of wild type strain KPS46 and mutant strain N19G1 for 5 min. and the seed germinated and seedlings grown in plates with 0.5X MS medium. There were 10 replication plates per treatment with three seeds per plate. Biomass, number of lateral root, root and shoot length were determined 7 days after seed germination. The experiment was conducted three times.

5. Identification and quantification of metabolites producted by wild type and mutant strains

The concentration of IAA produced by KPS46 wild type and UV mutant, excreted in the culture fluid was assayed using the method described by Patten and Glick (2002) with slight modifications. Fluid from KPS46 cultures were made cell free by centrifugation at 8,000 rpm for 20 min at 4ºC and filtration. The quantity of indoles following the Salkowski assay was determined by comparison with a standard curve using IAA in the concentration range of 0-50 ug/ml. Concentrations of IAA also were determined by reverse phase-HPLC. After culture fluids were acidified to pH 2.8 and extracted three times with ethyl acetate, 60 ul aliquots were injected into an Alltech, type Econosphere C185U column (250 by 4.6 mm) equipped with a differential UV detector absorbing at 280 nm. The isocratic solvent used for reverse-phase chromatography was acetonitrile-glacial acetic acid (1%) in water (10:90). The flow rate was adjusted to 1 ml/min. Peak retention times were compared with those of chemically synthesized IAA standards and quantified by comparison of peak areas.

The motility assay**,** swimming motility was evaluated by stationary-phase of bacterial cell inoculation of KPS46 wild type and representative UV mutant onto the centers of swim motility plates. Plates contained 1.5% NGA and varied 0.3%, 0.7%, 1.5% (wt/vol) LBA. The swarm cell differentiation was analyzed as slightly modification method (Kearns and Losick, 2003). Cell were grown to stationary-phase at 28 ± 2 ^{0}C NGB and resuspended to the appropriate OD in sterile distilled water. The each medium containing difference agar concentration were dried for 1 h in a laminar flow hood, centrally inoculated with 10^8 cfu ml^{-1} in 50 µl, dried for another 10 min and incubated at 37°, and the diameters of halos due to bacterial migration were measured up to 24 to 48 h postinoculation. The swarm cell differentiation was analyzed as previously described (Senesi *et al.,* 2002; Kearns and Losick, 2003). The lipopeptide-producing bacterium, KPS46, was cultured in NGB at 28 ± 2 ^{0}C with constant shaking at 180 rpm for 2 days in dark condition. Extracellular lipopeptide were extracted from KPS46 culture supernatant and the HPLC system was conducted following the method described by Ongena *et al.* (2007) and Araujo *et al.* (2005). Surfactin lipopeptides were detected and identified its typical retention times and function in enhanced plant growth with seed treatment assay as earlier described.

6. Proteomic analysis of extracellular proteins of KPS46

This experiment was to determine some of the specific components of the extracellular proteome of KPS46 that may have some relationship to IAA biosynthesis, lipopeptide production and plant growth promotion. To reduce the range of proteins to those more closely involved in these processes, extracellular proteins of KPS46 were compared with those excreted by mutant strain, which was reduced in IAA production, swarming motility, chitosanase activity (a group of extracellular protein), and growth promotion ability. Phenylmethylsulphonyl fluoride (Sigma) was added to late-exponential phase cultures of KPS46 and N19G1 to a final concentration of 5 mM to prevent proteolytic digestion. Proteins were then extracted from the culture fluids as described above. Total protein content of the samples (extracellular protein) was quantified by the Bradford assay (Bradford, 1976). The extracellular protein samples were used directly to passively rehydrate isoelectrically focused on an Electro Immobiline Dry Strip pH 3–10 and pH 4-7 (11 cm; Bio-Rad, USA) by applying 185 µl of each sample (equivalent to 250 µg of protein). Extracellular preparations dissolved in the 10X of IEF sample solution was applied to the first dimension. IPGs were focused for 15 h at 400V followed by 1 h at 600V using a Multiphor II (Amersham Pharmacia). After placing IPG strip in equilibration buffer A [50 mM Tris/HCl, pH 6.8, containing 8 M urea, 30 % (v/v) glycerol, 2.5% SDS (sodium dodecyl sulfate, Sigma-Aldrich 436143) and 0.25% DTT] for 15 min, then placed them into buffer B [50 mM Tris/HCl, pH 6.8, 8 M urea, 30% (v/v) glycerol, 2.5% SDS, 0.25% DTT and 4.5% iodoacetamide (Sigma, 1149)] for 15 min. The isoelectric focusing gels were embedded in gels (0.25 M Tris/HCl, pH 6.8, 0.25% SDS, 1% agarose onto 14% SDS polyacrylamide) for confirmed extracellular proteins of KPS46 and its UV mutant strain N19G1. The proteins were resolved in the second dimension by a constant current of 100 volts until the bromphenol blue marker entered the stacking gel, followed by 140 volts until the blue dye reached the bottom of the gel (Antelmann *et al.,* 2003; Lai *et al.,* 2003; Duy *et al.,* 2007). The 2D gels were visualized by staining with colloidal coomassie blue G-250 [17% ammonium sulphate, 34% methanol, 3.6% orthophosphoric acid, 0.1% coomassie G-250 (Sigma B0770)] (Voigt *et al.,* 2006). The gels were fixed in destaining solution (80% ethanol and 20% acetic acid mixture) and washed with 70% ethanol. The gels were analyzed using ImageMaster software for protein spots visible exclusively or at higher intensity in the gel containing extracellular proteins from wildtype strain KPS46 as compared to the UV mutant strain N19G1. Proteins showing more than 2-fold increase in expression of wildtype compared with mutant were selected for identification. Amino acid sequences of the protein spots were identified by mass spectrometry analysis, as slightly modification methods described by Voigt *et al.* (2006) and Domon and Aebersold (2006). The sequence data were compared with the NCBI and SwissProt databases using local MASCOT to identify possible extracellular protein names and functions.

7. Biochemical analysis of soybean response to treatment with KPS46 and inoculation with *Xag*

Leaves from soybean plants treated as seed with KPS46 or distilled water were sampled just prior to challenge inoculation with the pathogen and at daily intervals for up to 7 days post pathogen inoculation for determination of enzyme activities, phenolic compounds accumulation, SA and JA stimulation. Peroxidase activity was measured in both cultivars while β-1,3- glucanase activity and phenolic compounds were assessed only in 'SJ4'. The SA and JA were evaluated in only Spencer cultivar.

7.1 Evaluation defense enzyme activity

7.1.1 Soybean total protein extraction

There were three replicate samples per treatment for each sampling date, with each sample containing approximately 0.1 g fresh weight of leaf tissue. Samples were ground in a mortar and pestle containing extraction buffer (0.1 M Tris–HCl buffer, pH 7, 0.1 M KCl , 1 mM PMSF (phenylmethanesulfonyl fluoride : Fluka 78830), 10 ml/L Triton X-100, 30 g/L PVPP (polyvinylpyrrolidone K30 : Fluka 81422). The soybean homoginization extracts by centrifuged at 12,000 rpm at 4 ^{o}C for 10 min, kept the plant protein samples on ice until the enzyme activity assays. Total protein concentration in the extracts was measured with a modified assay described by Bradford (1976). One ml of Bradford reagent was added to 0.1 ml of extract and absorbance of the mixture was read at 595 nm after a reaction time of 2 min. Sample protein content was determined from a standard curve generated with bovine serum albumin (BSA).

7.1.2 Evaluation peroxidase activity

Peroxidase activity was determined by a spectrophotometric method described in Hammerschmidt et al. (1982) and Ramamoorth et al. (2002b). The reaction mixture consisted of 10 µl leaf extact and a substrate solution containing 125 µl guaiacol and 153 µl hydrogen peroxide in 50 ml of 10 mM sodium phosphate buffer, pH 6.0. The reaction mixture was incubated in a water bath at 30 ^{0}C and absorbance readings at 460 nm were taken every 30 seconds for 15 and one half minutes. Enzyme activity was expressed as peroxidase activity units min^{-1} mg^{-1} protein.

7.1.3 Evaluation *β*-1,3-glucanase activity

Activity level for *β*-1,3-glucanase was assayed by the laminarin dinitrosalicylic acid method (Pan *et al.,* 1991; Buensanteai *et al.,* 2007a). The reaction mixture consisted of 62.5 µl of 4% laminarin and 62.5 µl of soybean leaf extract. The reaction was carried out at 40°C for 10 min and then stopped by adding 375 µl of dinitrosalicylic acid and heating for 5 min in boiling water. Absorbance then was measured at 500 nm. Enzyme activity was expressed as µg glucose released min^{-1} mg^{-1} protein.

7.2 Evaluation phenolic compounds

Leaves collected from cultivar SJ4 also were analyzed for phenolic content (Zieslin and Ben–Zaken, 1993). Leaf samples (1 g) were homogenized in 10 mL of 80% methanol and agitated for 15 min at 70 ^{0}C. One ml of the methanolic extract was added to 5 ml of distilled water and 250 ml of Folin-Ciocalteau reagent (1 N) and the mixture was kept at 25^{0}C. The absorbance of the mixture was measured using a spectrophotometer at 725 nm. Catechol was used as the standard. The phenolic content was expressed as µg catechol mg^{-1}protein.

7.3 Soybean salicylic acid and jasmonic acid quatification

In a separate planting of cultivar Spencer, concentrations of salicylic acid (SA) and jasmonic acid (JA) in the leaves were measured as described previously (Gaffney *et al.,* 1973; Sibylle *et al.,* 1998; de Meyer *et al.,* 1999; Engelberth *et al.,* 2003; Segarra *et al.,* 2007). Twelve leaves (1 g of fresh tissue) at the same developmental stage were sampled from four replicate pots (3 leaves from each of 4 replicate pots] per treatment at daily intervals for 15 days starting 6 h after inoculation with *Xag* 20036a and subsequently. Leaf tissue (about 1gram fresh weight) was ground in a mortar and pestle with liquid nitrogen and then extracted with methanol/water/acetic acid (90:9:1 by vol.). Fluid from the extract was centrifuged and filtered through a 0.45 um membrane before analysis by reverse phase-HPLC. Fifty ul aliquots of the fluid were injected into an Alltech, type Econosphere C185U column (250 by 4.6 mm) equipped with a differential UV detector absorbing at 280 nm. The isocratic solvent used for reverse-phase chromatography was acetonitrile-glacial acetic acid (1%) in water (10:90). The flow rate was adjusted to 1 ml/min. Peak retention times were compared with those of chemically synthesized SA and JA standards and quantified by comparison of peak areas.

8. Proteomic analysis of soybean response to KPS46 and *Xag* inoculation

The soybean samples (cv. Spencer) were collected 7 days after planting and 7 days after *Xag* strain 20036a pathogen challenge inoculation (21 days after planting) for determined protein complexs involved in sensitization and induced resistance mechanisms respectively, three repetitions per treatment were used for each protein analyze. Soybean samples stored immediately at –70°C until extraction. Protein assays were performed on soybean leaf extracts. Soybean leaf total protein was extracted by phenol extraction followed by methanolic ammonium acetate precipitation (Hurkman and Tanaka, 1996a, 1996b). Resuspend final pellet in an appropriate (minimum) volume of IEF Sample Buffer and store at -20 ^{o}C and total protein content of the samples were quantify by the Bradford assay (Bradford, 1976).

The above samples were used directly to passively rehydrate isoelectrically foucused on an Electro Immobiline Dry Strip pH 3–10 (11 cm; Bio-Rad, USA) by applying 180 ul of each sample (equivalent to 250 ug of protein), soybean total protein mixture dissolved in the 10X of IEF sample solution was applied to the first dimension. Cover the IPG strips with the appropriate volume of mineral oil. IPGs were focused for 15 h at 400V followed by 1 h at 600V using a Multiphor II (Amersham Pharmacia). The IPG strips were equilibrated for 15 min with the appropriate volume of DTT equilibration buffer [50 mM Tris/HCl, pH 6.8, containing 8 M urea, 30 % (v/v) glycerol, 2.5% SDS and 0.25% DTT] and then iodoacetamide equilibration buffer [50 mM Tris/HCl, pH 6.8, 8 M urea, 30% (v/v) glycerol, 2.5% SDS, 0.25% DTT and 4.5% iodoacetic acid] and subsequently place onto 12% polyacrylamide gel and quickly fill the well with molten Overlay Agarose. The isoelectric focusing gels were embedded in 0.25 M Tris/HCl, pH 6.8, 0.25% SDS, 1% agarose onto 14% SDS polyacrylamide gels (20x20x0.1 cm), and place the second dimension gels into the gel box and the proteins were resolved in the second dimension by a constant current of 100 volts until the bromphenol blue marker entered the stacking gel, followed by 140 volts until the blue dye reached the bottom of the gel (Kim *et al.,* 2004). The 2D–PAGE gels were visualized by staining with colloidal Coomassie blue G-250 (17% ammonium sulphate, 34% methanol, 3.6% orthophosphoric acid, 0.1% Coomassie G-250). The gels were fixed in destaining solution (80% ethanol and 20% acetic acid mixture) and washed by 70% ethanol (Herbert *et al*., 1999; Gorg *et al.,* 2002). The gel images use for computer analysis for the extracellular protein spots of soyben treated by the strain KPS46 and treated by negative control detection, matching and quantification were performed with the Phoretix 2D Advanced Software Version 2003.02 (Phoretix, Newcastle upon Tyne, UK). Average values of protein spot volume intensities and their corresponding standard deviations were calculated to characterize expression changes of proteins. Protein spot intensity was defined as the normalized spot volume which is the ratio of the single spot volume to the total spots volumes on a 2D gel. Normally only proteins showing more than 2-fold increase or decrease in expressions were considered to be identified. The spots were identified by mass spectrometry analysis, as modified from described by and Domon and Aebersold (2006). Database searching, peptide mass fingerprint (PMF) data were generated from raw MALDI-TOF-MS data using the SNAP algorithm (Bruker) with a signal/noise threshold. The PMF data were used to screen the NCBInr database (http://129.93.41.90/mascot/cgi/master_results.pl?file=../ data/20080219/F011009.dat) and using local MASCOT (MASCOT 2.1.0, Matrix Science).

9. Analysis of signaling pathway activated in *Arabidopsis* by KPS46

9.1 Arabidopsis lines and pathogen inoculation

An experiment was conducted using *Arabidopsis thaliana* Hynn. ecotype Columbia (Col-0) and mutant and transgenic lines derived from this parent. The SA hydroxylase gene transgenic NahG line (which does not express PR proteins) was obtained from P. Staswick, University Nebraska-Lincoln. The JA-insensitive mutant line jar1-1 and coi 1-35, ethylene insensitive mutant etr1-1, and auxin insensitive mutant aux1-1 were obtained from the Ohio State University Stock Center, Columbus (http://www.arabidopsis.org/abrc/catalog/mutant_seed_8.html). Seeds were surface disinfested with 70% ethanol for 2 min followed by treatment with 1% sodium hypochlorite for 20 min prior to planting in a pots of pasteurized soil mix (Speedling Inc., Bushnell, FL, USA). The pots were kept at 23 ± 3°C under fluorescent lights. Two-week-old seedlings were transplanted individually into 10-cm square pots.

For each Arabidopsis line, 25 plants were primed with KPS46 two weeks after transplantation and challenge inoculated with Pst DC3000 one week after KPS46-supplemented drencing soil mix. Plants were inoculated with the pathogen by spraying a bacterial cell suspension until all the leaves were covered with fine droplets. The inoculated plants were kept in a dew chamber for 16 h at 25ºC and 100% relative humidity, and then transferred to a growth chamber. Five days after inoculation, disease severity was assessed by determining the percentage of leaves per plant with typical yellowing or water-soaking symptoms (ramdom selection for 10 plants per treatment) (Pieterse *et al.,* 1996).

9.2 Evaluation of histochemistry: hydrogen peroxide production

The effects of KPS46 and Pst DC300 pathogen challenge on sensitization and, thereby, Arabidopsis plant responses such as oxidative burst deposition were determined. KPS46-treated leaves were sprayed or infiltrated with virulent DC3000 1 week days after KPS46 treatment. Histochemical detection of hydrogen peroxide were performed as described previously (Doke *et al.,* 1996; Wohlgemuth et al. 2002), with minor modification. To visualize accumulation of hydrogen peroxide, leaves were stained with 0.1% (wt/vol) diaminobenzidine (DAB). Then, the leaves were cleared with 96% (vol/vol) ethanol and preserved in 50% (vol/vol) ethanol. DAB staining indicates hydrogen peroxide as red-brown precipitate under the light microscope.

9. Statistic analysis

Each experiment was repeated at least three times, and each mean of data by obtained was analyzed to ANOVA using SAS version 9.1. Mean separation of all experiment was accomplished by Duncan's Multiple Range Test, and all test of significant were conducted at $p \leq 0.01$.

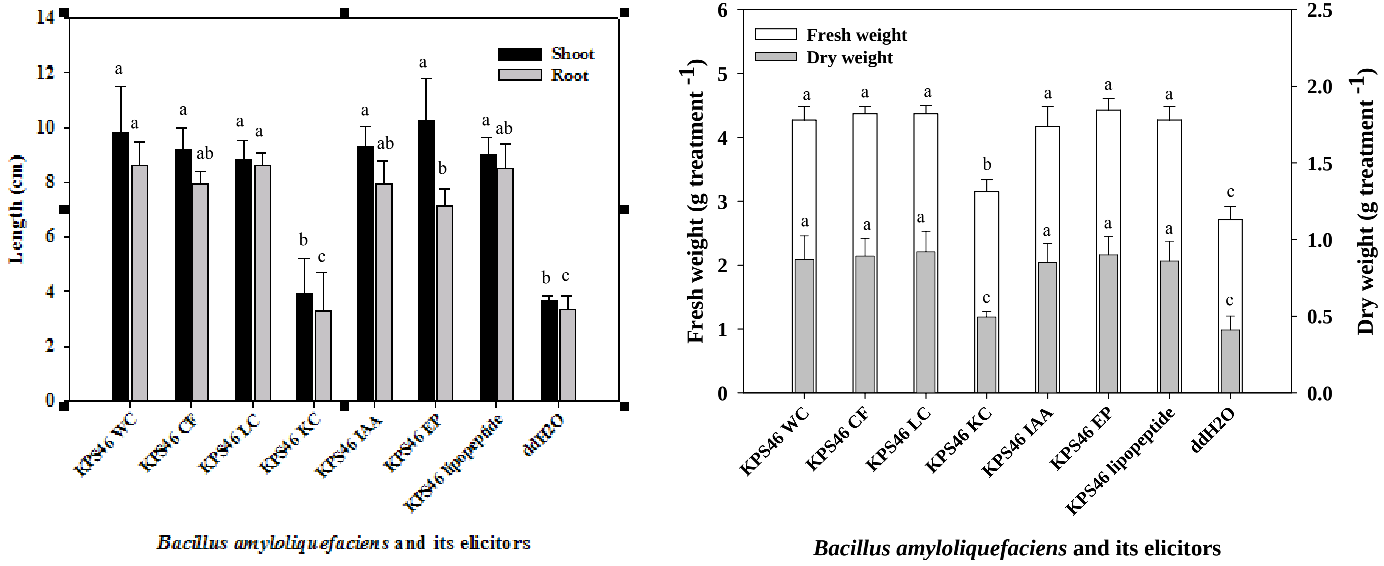
RESULTS AND DISCUSSION

Results

1. Effects of KPS46 extracellular factors on plant growth

Extracellular lipopeptides, proteins, and IAA extracted from cultures of strain KPS46 were effective in promoting the growth of soybean seedlings under gnotobiotic conditions. These culture fractions, when applied to soybean seed, increased root and shoot lengths, by more than 40 and 20%, respectively (Fig. 8A), and increased fresh and dry weights by more than 30% (Fig. 8B) compared to the distilled water control. Seed treatment with the extracted factors of KPS46 also increased the number of lateral root more than 20% compared to the control (Fig. 9). The lipopeptide, protein and IAA treatments had similar effects on soybean growth as seed treatments with a whole culture of KPS46, the cell-free fluid fraction from a KPS46 culture, and a suspension of live cells from the culture (Fig. 8; 9). There was no significant effect of seed treatment with a suspension of heat-killed cells of KPS46 compared with distilled water for any soybean plant growth parameter (Fig. 8; 9).

Seed treatments with extracted lipopeptides, proteins, IAA, and other fractions from a culture of KPS46 yielded similar results in greenhouse pot experiments (Fig. 10, 11, 12). All of the treatments involving KPS46 culture fractions, except the heat-killed cells, increased shoot and root lengths (Fig. 10A, 12) and plant biomass (Fig. 10B, 11, 12) compared to the distilled water control. However, the stimulating effect of these KPS46 extractes on plant growth depended on an appropriate concentration test (Buensanteai *et al.,* 2007b; Buensanteai *et al.,* 2008) was conducted. Two week after the beginning of seed treatment in greenhouse trails, soybean seedlings treated with active elicitors of KPS46 were average in each treatment more than 33.0 and 15.0 gram whereas non-treated seedlings were less than 24.0 and 11.5 gram fresh and dry weight respectively. According to DMRT ($p \leq 0.01$), the stimulation of seedling growth with KPS46's active elicitor was significant differences from KPS46 killed cell and ddH_2O negative control.



(A) **(B)**

Figure 8 Effects of cellular and extracelluar fractions from cultures of *Bacillus amyloliquefaciens* KPS46 on the growth of soybean under gnotobiotic conditions, as measured at 7 days after inoculation in: A) shoot and root length; B) fresh and dry weight. WC = whole culture, CF = culture fluid, LC = live cell suspension in distilled water, KC = heat killed cell suspension, IAA = indoles, EP = extracellular proteins. The data are the average of four replications (three plants per replication) for each treatment. The experiment was repeated three times. Error bars represent the standard deviation. Individual bars denoted with the same letter are insignificantly different ($p \leq 0.01$) from each other

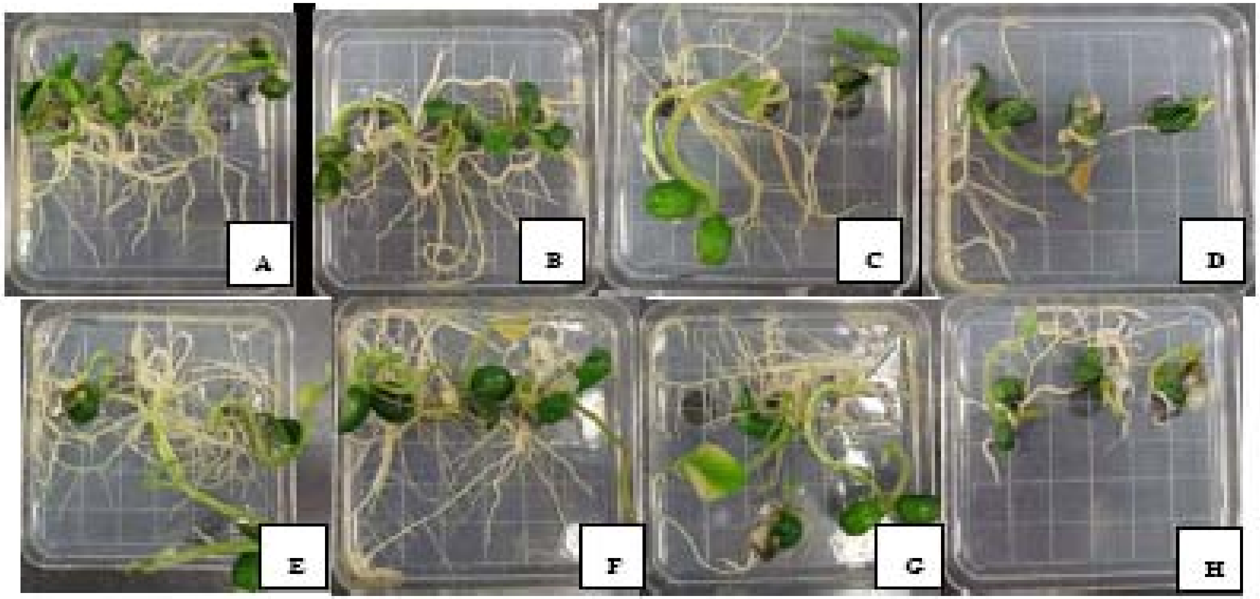
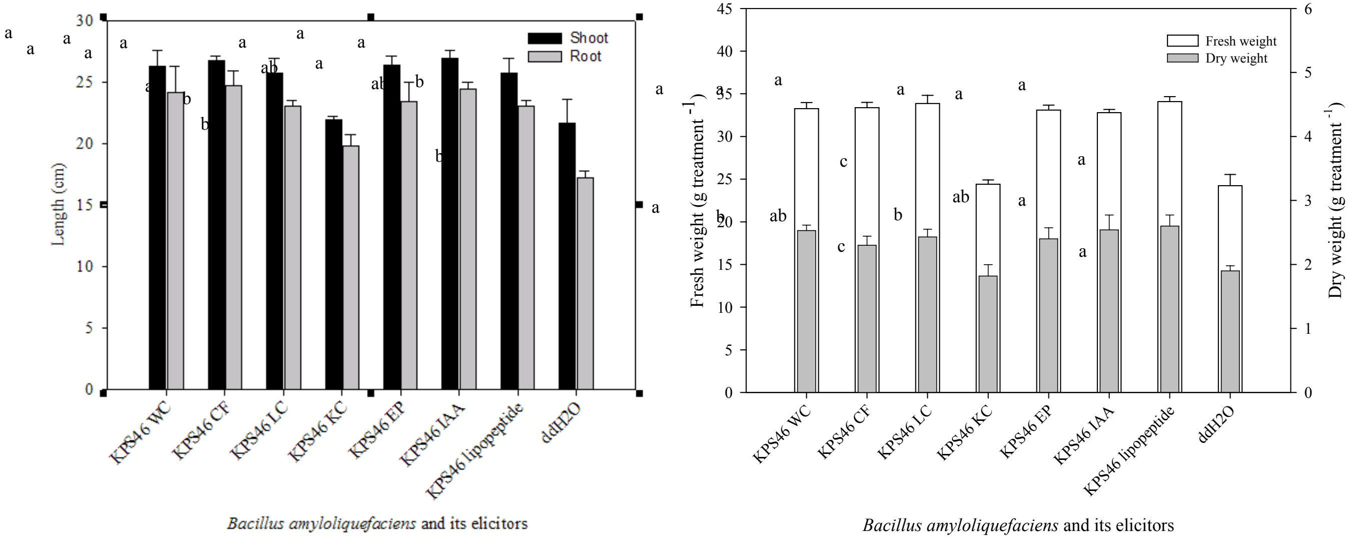


Figure 9 Effects of cellular and extracelluar fractions from cultures of *Bacillus amyloliquefaciens* KPS46 on the number of lateral root of soybean under gnotobiotic conditions, as measured at 7 days after inoculation. Soybean cv Spencer seed treated by KPS46 WC (A), KPS46 CF (B), KPS46 LC (C), KPS46 KC (D), KPS46 IAA (E), KPS46 EP (F), KPS46 lipopeptides (G) and ddH$_2$O (H). WC = whole culture, CF = culture fluid, LC = live cell suspension in distilled water, KC = heat killed cell suspension, IAA = indoles, EP = extracellular protein. The experiment was repeated three times



(A) (B)

Figure 10 Effects of cellular and extracelluar fractions from cultures of *Bacillus amyloliquefaciens* KPS46 on the growth of soybean under greenhouse conditions, as measured at 14 days after inoculation in: A) shoot and root length; B) fresh and dry weight. WC = whole culture, CF = culture fluid, LC = live cell suspension in distilled water, KC = heat killed cell suspension, IAA = indoles, EP = extracellular proteins. The data are the average of four replications (three plants per replication) for each treatment. The experiment was repeated three times. Error bars represent the standard deviation. Individual bars denoted with the same letter are insignificantly different ($p \leq 0.01$) from each other

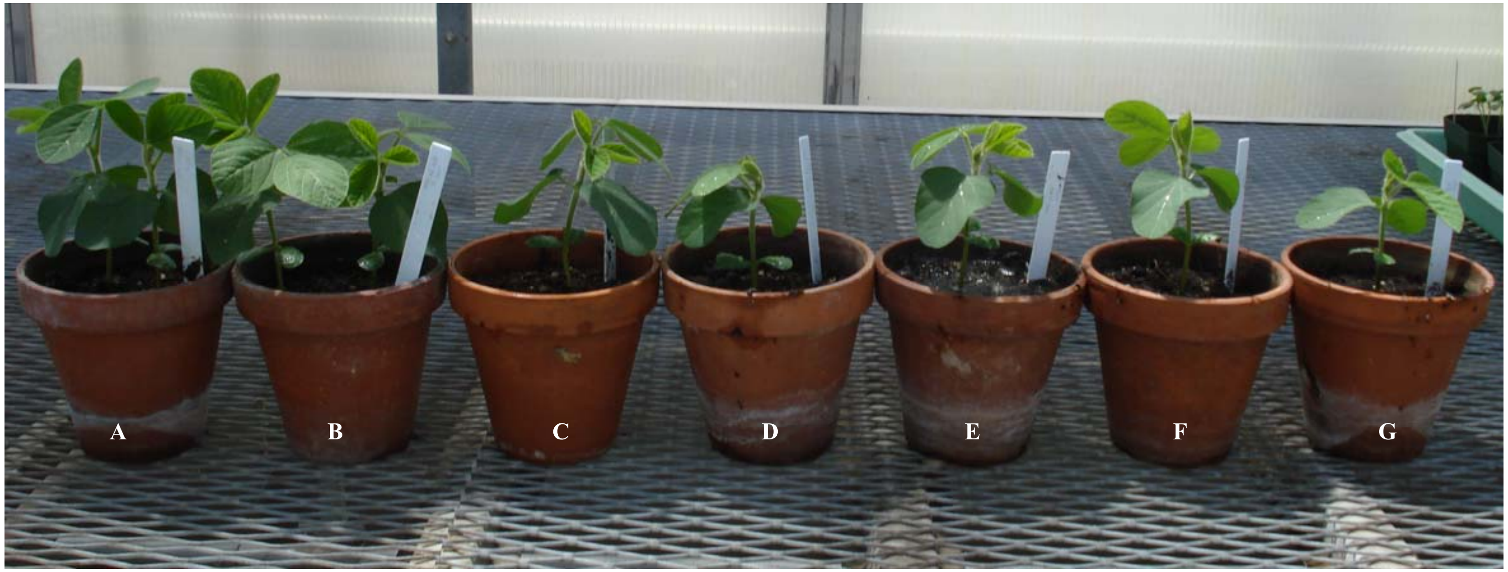


Figure 11 Effect of PGPR difference strains on growth promotion in greenhouse experiment. Soybean cv Spencer seed treated by *Bacillus amyloliquefaciences* KPS46 : WC (A), CF (B), LC (C), KC (D), KPS46 IAA (E), Lipopetides (F), and ddH_2O (G)

Figure 12 Effect of PGPR difference strains on shoot and root lenght in greenhouse experiment. Soybean cv Spencer seed treated by *Bacillus amyloliquefaciences* KPS46 : WC (A) compared with ddH$_2$O

2. Extraction and analysis of KPS46 metabolites

2.1 Indole-3-acetic acid analysis

Strain KPS46 secreted IAA, as determined by HPLC, when cultured in nutrient glucose broth medium (Fig. 13), IAA was the major auxin in the culture filtrate, with the highest concentration (31.0 ug ml- ^{1}) detected at stationary phase. The data obtained suggested that the extracts from KPS46 cultures exhibited IAA-like physiological activity in the host plant resembling the effect of authentic IAA on enhanced plant growth (data not show). In mineral medium amended with various concentrations of tryptophan, strain KPS46 secreted higher amounts of IAA with increasing tryptophan concentration (Fig. 14). Bacterial cultures amended with 125, 250, 500 2,500 and 5,000 mM of tryptophan in DF salt minimal medium, harvested significantly different ($p \leq 0.05$) when values of 10.6, 20.3, 25.1, 26.9, 32.7 ug ml^{-1} of IAA respectively. The result revealed that IAA production in KPS46 is tryptophan dependent.

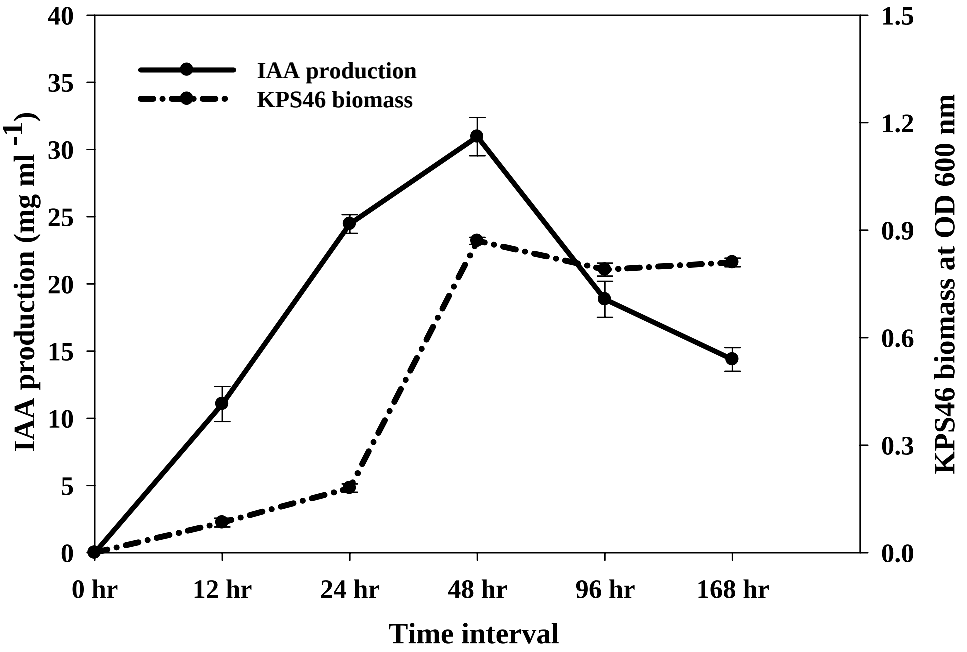


Figure 13 Concentration of indole-3-acetic acid (IAA) secreted by *Bacillus amyloliquefaciences* KPS46 into NGB medium, measured by RP-HPLC, and concurrent relative population levels of the bacterium as measured by absorbance. Data are the means and standard deviations of three replicates. Error bars represent the standard deviation

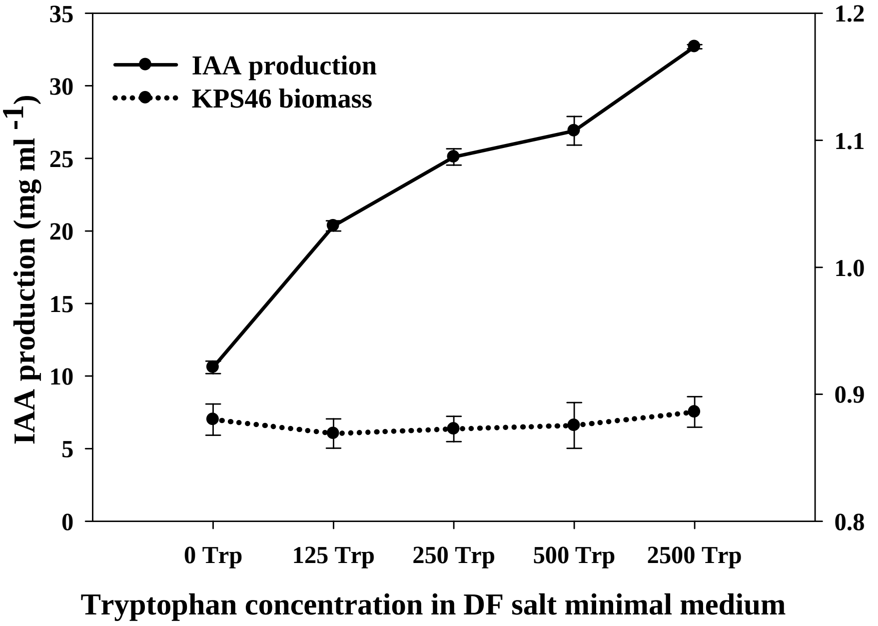


Figure 14 Production of indole-3-acetic acid by *Bacillus amyloliquefaciences* KPS46 in DF salt minimal medium amended with increasing concentrations of L-tryptophan. Data are the means and standard deviations of three replicates. Error bars represent the standard deviation

2.2 Lipopeptides production

When the purified lipopeptide fraction from the culture filtrate of KPS46 in NGB medium was analyzed by RP-HPLC analysis, high concentrations of bioactive non-polar antibiotics were detected (data not shown). Surfactin-type lipopeptides were identified by the basis of their retention times compared with those of purified surfactin standards. C18 homologues represented together more than 50% of the total amount of surfactin present in the culture supernatant. Based on RP-HPLC peak areas of the samples compared with values obtained for standards, the total amount of surfactins produced by strain KPS46 was 550 mg l^{-1} ± 20.267 (mean and standard deviation calculated from three independent cultures). One of lipopeptides produced by KPS46 is highly similar to the family of surfactins (Fig. 15).

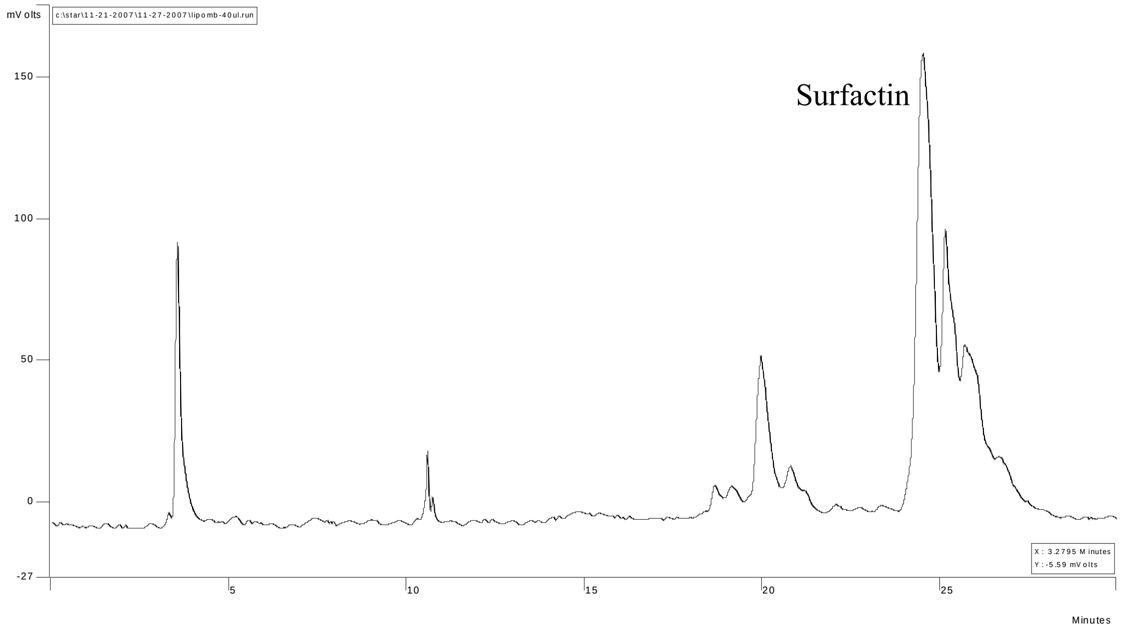


Figure 15 PR-HPLC profile of lipopeptides produced by *Bacillus amyloliquefaciences* KPS46 in NGB medium

3. UV mutant strain N19G1 phenotype

Strain N19G1 exhibited significantly ($p \leq 0.05$) reduced excretion of IAA compared to wildtype KPS46 (Fig. 16). The mutant strain also exhibited decreased more than one fold swarming motility, resulting in altered colony morphology on all test media compared with wildtype KPS46 (Fig. 17), the clearly diameter decreased for two fold on 0.7% LBA (Table 1). When extracellular proteins extracted from KPS46 and N19G1 were applied as a seed treatment in gnotobiotic (Fig. 18) and greenhouse experiments (Fig. 19), proteins from strain N19G1 had little or no affect on soybean growth parameters compared to the distilled water control. In contrast, proteins produced by KPS46 elevated all growth parameters under both experimental conditions.

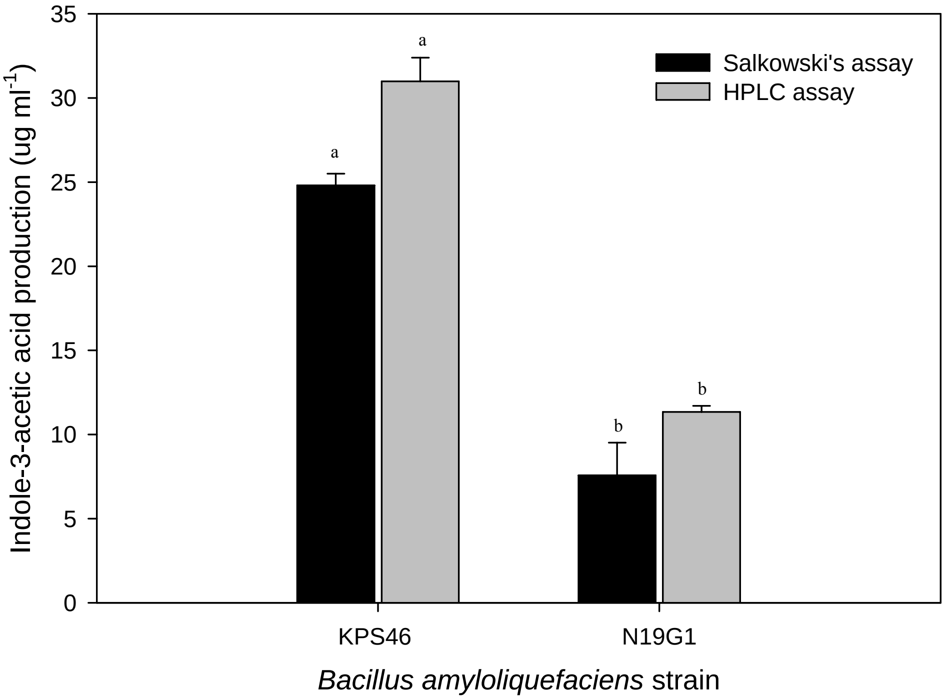


Figure 16 Concentrations of indole-3-acetic acid secreted by wildtype strain *Bacillus amyloliquefaciens* KPS46 and UV mutant strain N19G1 cultures grown in NGB medium. The concentration values were standardized to 10^8 CFU per ml of cells in the medium. The data are the means and standard deviations of three replicates. Error bars represent the standard deviation. Individual bars denoted with the same letter are insignificantly different ($p \leq 0.01$) from each other

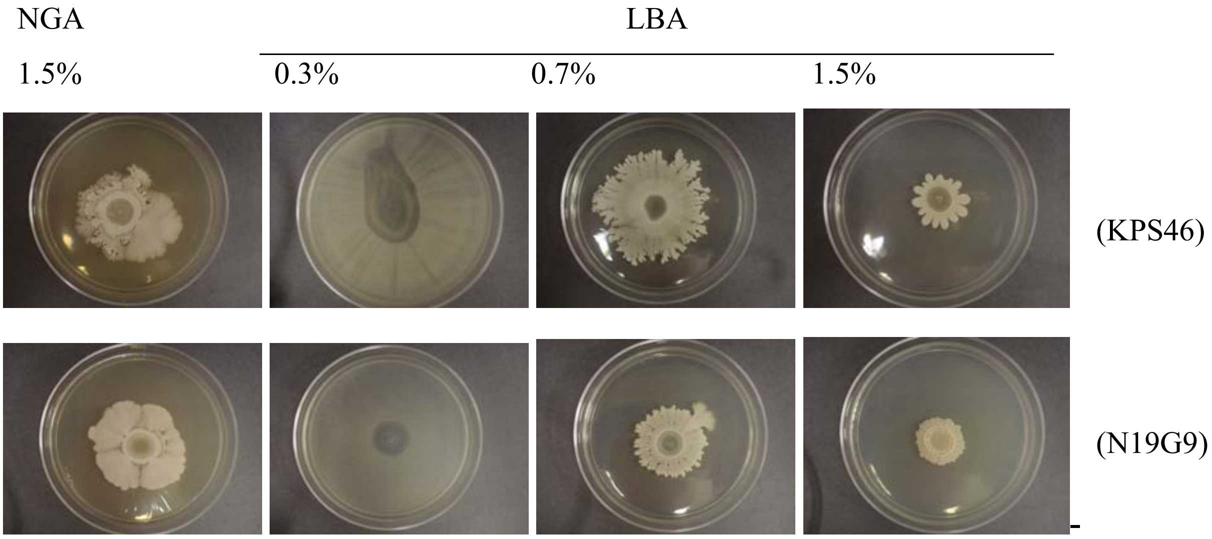
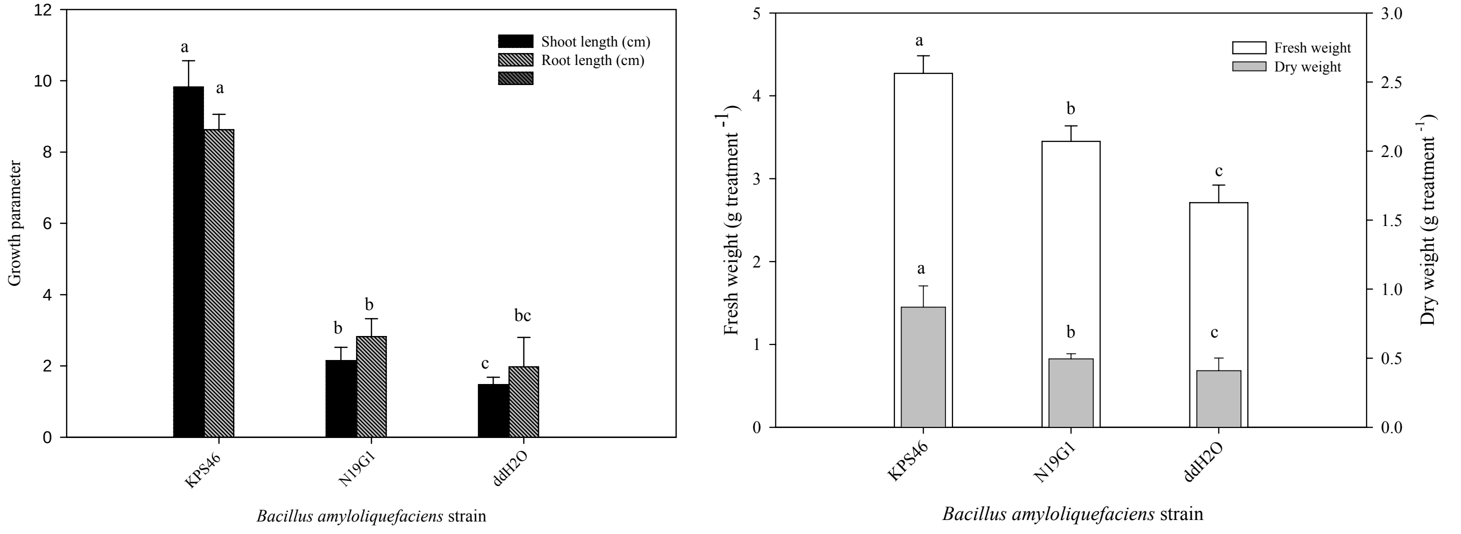


Figure 17 Swarming motility of *Bacillus amyloliquefaciens* wild type KPS46 and UV mutant strain N19G1 on NGB with 1.5% agar and LBA with 0.3, 0.7, and 1.5% agar

Table 1 Phenotypes of PGPR/PGPB, *Bacillus amyloliquefaciens* wild-type strain KPS46 and UV mutant strains

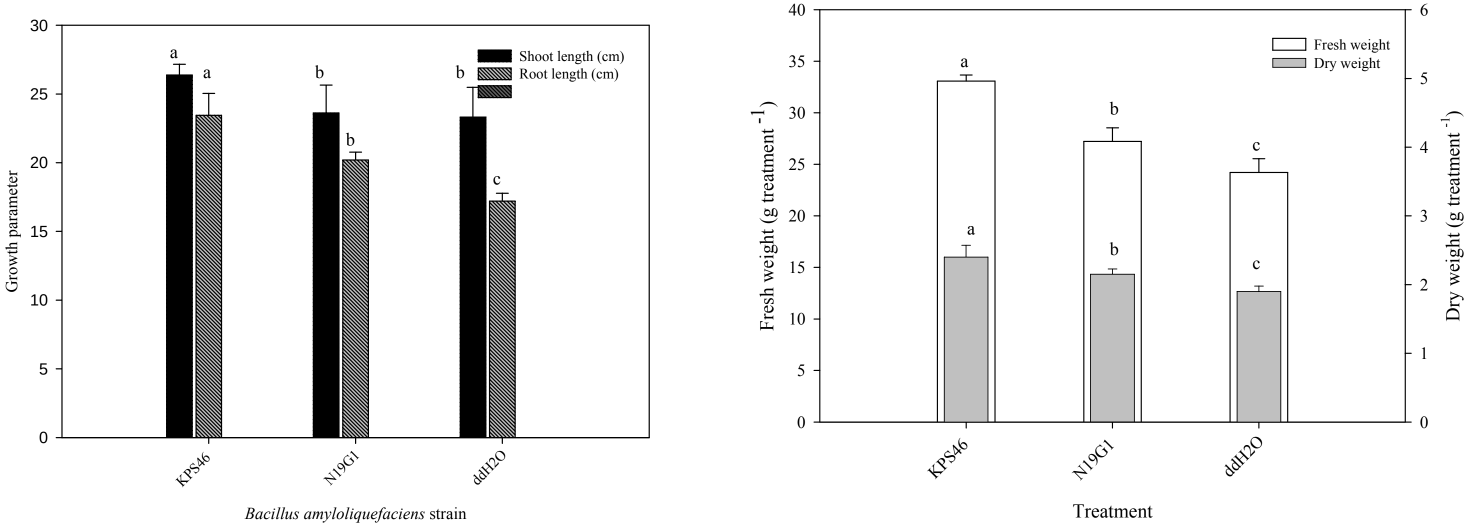
Phenotypes [a]	Strain						
	KPS46	N19G1	N13D5	N3B8	P12A2	P1C12	P22F8
IAA production via Salkowski and HPLC assay	+	-	-	-	+	+	+
Phosphate solubilization	+	-	+	-	+/-	-	+
Chitosanase activity	+	-	-	+/-	+	+	-
Swarming (Surfactance)							
LB 0.3% agar	+	+/-	+	+/-	+/-	+/-	+
LB 0.7% agar	+	-	-	-	-	-	-
LB 1.5% agar	+	-	-	-	-	-	-
NGB 1.5% agar	+	-	-	-	-	-	-
Antimicrobial activities							
Xanthomonas axonopodis pv. *glycines*	+	-	-	+	+/-	+	+

[a] In vitro activities as described in Materials and Methods. + positive activity, +/- modurate activity, - no detectable activity



(A) **(B)**

Figure 18 Effects of extracellular proteins from *Bacillus amyloliquefaciens* wild type strain KPS46 and UV mutant strain N19G1 in gnotobiotic experiment on soybean shoot and root length (A) and fresh and dry weight (B), as measured at 7 days after inoculation. The data are the average of four replications (three plants per replication) for each treatment. Error bars represent the standard deviation. Individual bars denoted with the same letter are insignificantly different ($p \leq 0.01$) from each other



(A)

(B)

Figure 19 Effects of extracellular proteins from *Bacillus amyloliquefaciens* wild type strain KPS46 and UV mutant strain N19G1 in greenhouse experiment on soybean shoot and root length (A) and fresh and dry weight (B). The data are the averages of four replications (three plants per replication) for each treatment, as measured at 7 days after inoculation. Error bars represent the standard deviation. Individual bars denoted with the same letter are insignificantly different ($p \leq 0.01$) from each other

4. KPS46 proteomic analysis

Two dimensional polyacrylamide gel electrophoresis of extracellular proteins excreted by wildtype KPS46 revealed around 190 detectable spots (Fig. 20). Among these, twenty corresponding to upregulated proteins, as compared to proteins from mutant strain N19G1, were assigned numbers as shown in Fig. 20.

The large majority of the identified proteins were homologous to proteins produced by *B. subtilis,* the remainder being homologous to proteins reported from other *Bacillus* spp., or other strains of *B. amyloliquefaciens* (Table2). The twenty proteins were placed in six categories based on predicted function (Table 2). Four spot were involved in detoxification and adaptation of cellular processing including catalase, two forms of superoxide dismutase, and general stress protein. Six proteins were in the transporter binding protein and lipoprotein category, three of them (spot numbers 2, 3 and 4) were found to be homologous to the same oligopeptide permease of *B. subtilis.* The other proteins in this category were homologs of three different ABC transporter proteins. Among the five proteins in the protein and amino acid biosynthesis group, two were homologous to forms of subtilisin, a serine endopeptidase, while two other were homologous to neutral proteases and the fifth to a protease that is toxic to phytopathogenic microorganisms. The remaining proteins fell into three categories: amino acid metabolism, energy metabolism, and nucleotide and nucleic acid metabolism. Notable in the energy metabolism category was a chitosanase homologue.

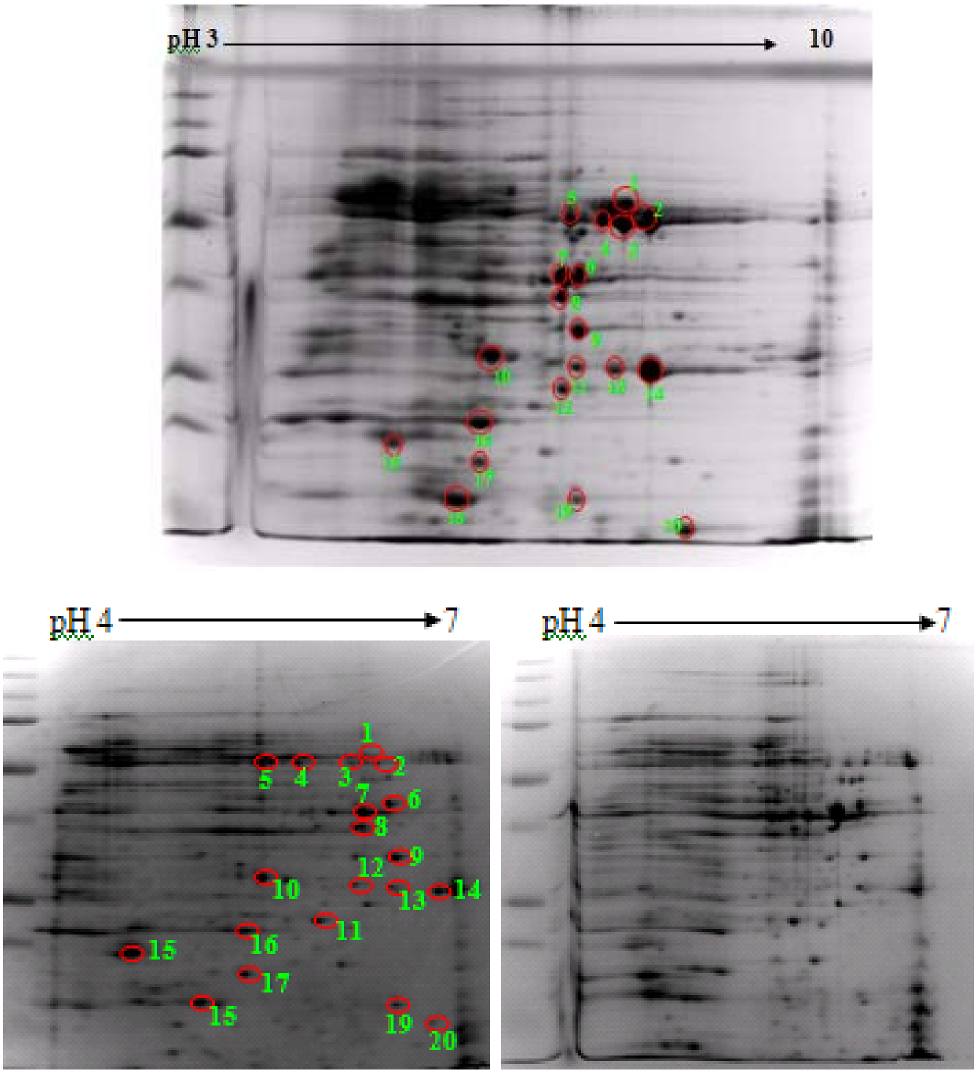


Figure 20 Two-dimensional gels of extracellular proteins extracted from *Bacillus amyloliquefaciens* KPS46: A) gel run on 11 cm of 3–10 IPG strips in the first dimension and 14% SDS-PAGE in the second dimension; B) gels of proteins from wildtype strain KPS46 (left) and mutant strain N19G1 (right) run on 11 cm of 4–7 IPG strips in the first dimension and 14% SDS-PAGE in the second dimension. Circled spots correspond to up-regulated proteins, identified by numbers, that were selected for analysis of amino acid sequence. *kD* = KiloDal.

Table 2 Identified proteins from the extracellular secretome of *Bacillus amyloliquefaciens* KPS46

Spot number	Protein name		Species	Accession number	Molecular mass (Da)	pI	Score	Sequence coverage (%)
Detoxification and adaptation of cellular process								
1	KatA	Catalase	*B. subtilis*	Q3HNK4_BACSU	54503	6.45	2021	75
13	SodA	Manesium superoxide dismutase (MnSOD)	*B.vallismortis*	Q6XZA2_BACVA	16176	5.27	540	44
14	SodF/ SodC	Copper/ Zinc superoxide dismutase (FeSOD, Cu/ZnSOD)	*B. cereus*	BCE33L4639	16176	8.24	235	29
18	YhdN	General stress protein 69	*B. subtilis*	G16U_BASU	20607	4.49	121	6
Transport/binding protein and lipoprotein								
2	OppA-F	Oligopeptide permease	*B.s subtilis*	CAA39787	61543	5.61	933	35
3	OppA-F	Oligopeptide permease	*B. subtilis*	CAA39787	61543	5.61	1095	33
4	OppA-F	Oligopeptide permease	*B. subtilis*	CAA39787	61543	5.61	809	33
5	AppA	Oligopeptide ABC transporter oligopeptide-binding protein	*B. subtilis*	I40545	61965	5.84	1379	34

Table 2 (Continued)

Spot number		Protein name	Species	Accession number	Molecular mass (Da)	pI	Score	Sequence coverage (%)
8	YufN	ABC transporter (lipoprotein) homolog yufN	*B. subtilis*	C70009	37383	5.03	355	20
12	PstB	Phosphate ABC transporter (binding protein) homolog yqgG	*B.subtilis*	A69956	31721	5.04	326	19
Protein and amino acid biosynthesis								
6	NprE	Neutral protease or extracellular neutral protease (Fragment)	*B. subtilis*	BSU1110	59317	7.89	779	45
7	NprB	Neutral protease B, Bacillolysin	*B. subtilis*	Q1L026_BACSU	56785	8.65	755	31
11	AprE	Serine alkaline protease (Pro-subtilisin)	*Bacillus sp. DJ-4.*	Q6IT79_9BACI	39071	9.23	538	33
16	AprE	Subtilisin	*B. subtilis*	Q4ZIL5_BACSU	27367	6.64	671	50
19	YraA	Probable intracellular protease	*B.licheniformis*	Q65FN6_BACLD	19604	5.30	160	30

Table 2 (Continued)

Spot number		Protein name	Species	Accession number	Molecular mass (Da)	pI	Score	Sequence coverage (%)
Amino acid metabolism								
9	GlnA/ GlnR	Glutamine synthetase	*B. subtilis*	BAA00730	50590	5.02	726	28
Energy metabolism								
10	LplD	Lytic enzyme or hydrolytic enzyme	*B. subtilis*	Q9R7J4_BACSU	27479	6.65	927	60
15	FbaA	Fructose-bisphosphate aldolase	*B. subtilis*	D32354	30552	5.19	595	38
17	Csn	Chitosanase precursor	*B.amyloliquefaciens.*	Q9ET84_BACAM	31442	8.83	706	42
Nucleotide and nucleic metabolism								
20	GuaB	Inosine-5-monophosphate dehydrogenase	*B. subtilis*	DEBSMP	53129	6.18	865	36

Source of reference: NCBI and SwissProt database

5. Soybean response to inoculation with KPS46 and *Xag*

In all experiments using soybean cultivars SJ4 and Spencer inoculated with *Xag* strains 2-2 and 20036a, respectively, seed treatment with *B. amyloliquefaciens* KPS46 reduced the severity of bacterial pustule in the foliage, confirming that induction of systemic resistance had occurred. The ability of KPS46 showed the most effective in reducing bacterial pustule disease severity more than 50 % (Table 3).

Seed treatment with KPS46 alone did not trigger the expression of biochemical markers associated with induced resistance, but it caused soybean plants to exhibit a higher level response to pathogen infection than non-treated plants (Fig. 21, 22, 23, 24, 25). In plants of cultivars SJ4 and Spencer treated treated with KPS46, peroxidase activity levels increased immediately after inoculatuon with strains of *Xag*, reaching peak levels within 3 days (Fig. 21A; 21B). In contrast, peroxidase activity in pathogen-inoculated plants having no KPS46 treatment was considerably lower. Non-KPS46 treated plants of cultivar SJ4 exhibited a similar temporal change in peroxidase activity as KPS46 treated plants, but there was no change in peroxidase levels over time in plants of cultivar Spencer that did not receive KPS46. In both cultivars, seed treatment with KPS46 without pathogen inoculation caused only a slight elevation in peroxidase activity levels compared to the respective distilled water control. Similar results were obtained as to β-1,3-glucanase activity (Fig. 22) and phenolic content (Fig. 23) measured in cultivar SJ4. Both markers were consistently higher in KPS46-treated plant than in non-treated plants, but the greatest response occurred in plants inoculated with *Xag*.

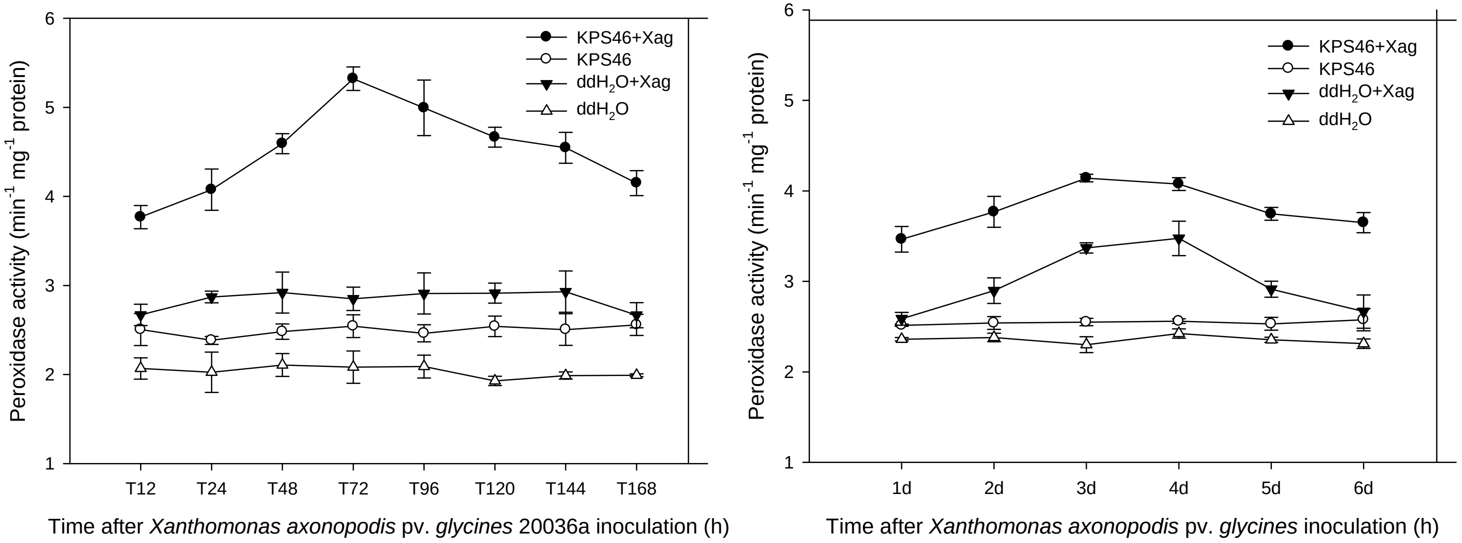
Plants of ‘Spencer’ treated as seed with KPS46 and mock inoculated with distilled water exhibited higher levels of SA and JA in the leaves than in plant with no KPS46 treatment and no pathogen inoculation (Fig. 24; 25). The elevated SA and JA concentrations remained unchanged during the 15-day assay period. KPS46-treated plants, when inoculated with *Xag*, exhibited spikes in SA and JA concentrations, detected within 15 days after pathogen inoculation. SA and JA levels then declined back down within 4 days to the steady state concentration found in the KPS46-treated/mock inoculated plants. Although plants with no KPS46 treatment also exhibited peaks in SA and JA content following pathogen inoculation, the rises in concentration were delayed and peak levels were considerably lower than in KPS46-treated plants.

Table 3 Effect of plant growth promoting rhizobacteria/ bacteria, *Bacillus amyloliquefaciens* KPS46and its elicitors on reduction disease severity of soybean bacterial pustule caused by *Xanthomonas axonopodis* pv. *glycines* (*Xag*)

Treatment[a]	**Disease severity**[b] **(%)**	**Disease reduction (%)**
1. KPS46 whole-cell (WC)	35.1a	55.2
2. KPS46 culture filtrate contain extracellular protein (CF)	35.7a	54.4
3. KPS46 Live cell (LC)	36.9a	55.5
4. KPS46 Killed cell (KC)	77.4c	4.9
5. KPS46 IAA	57.9ab	32.3
6. KPS46 lipopeptide	34.4 a	59.9
7. KPS46 extracellular proteins	33.9a	60.8
8. Positive Control (H_2O + *Xag*)	81.4c	0
9. Negative Control (H_2O alone)	0	-

[a] Seed treatment with inducer and challenged inoculation with pathogen (*Xag*) at 14 after planting

[b] Diseases severity was expressed as percent leaf area infection with *Xanthomonas axopodis* pv. *glycines* evaluated by the method of Prathuangwong *et al.* (1993) at 7 days after inoculation. Means in the column followed by the same letter are not significantly different (P< 0.05) according to Duncan's New Multiple Rang Test.



(A) **(B)**

Figure 21 Time courses of the accumulation of peroxidase activity of soybean cv. Spencer plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 20036a pathogen (A). Time courses of the accumulation of peroxidase activity of soybean cv. SJ4 plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 12-2 pathogen (B)

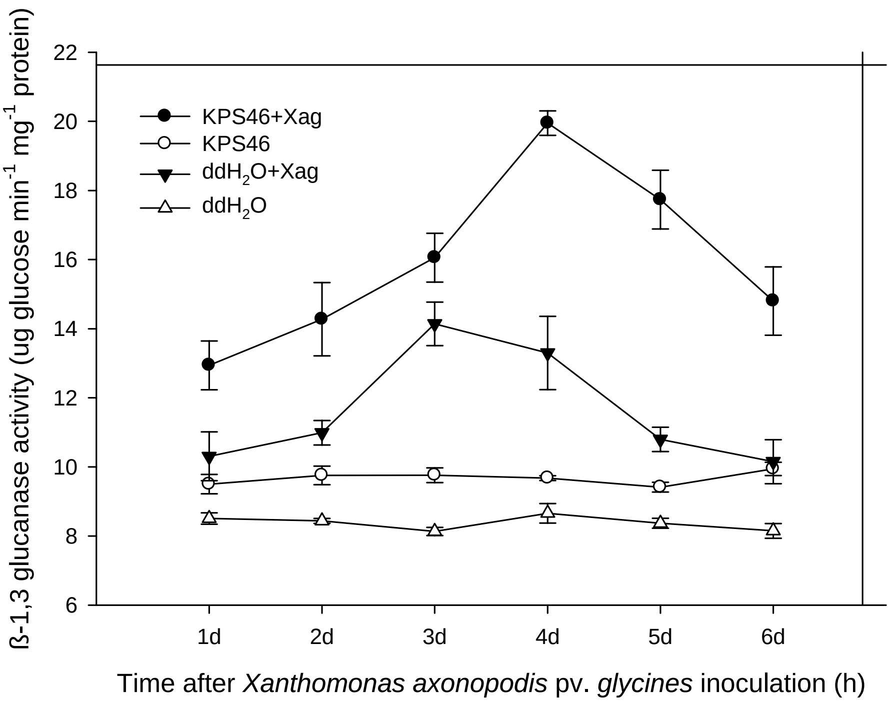


Figure 22 Time courses of the accumulation of *β*-1,3 glucanase activity of soybean cv. SJ4 plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 12-2 pathogen

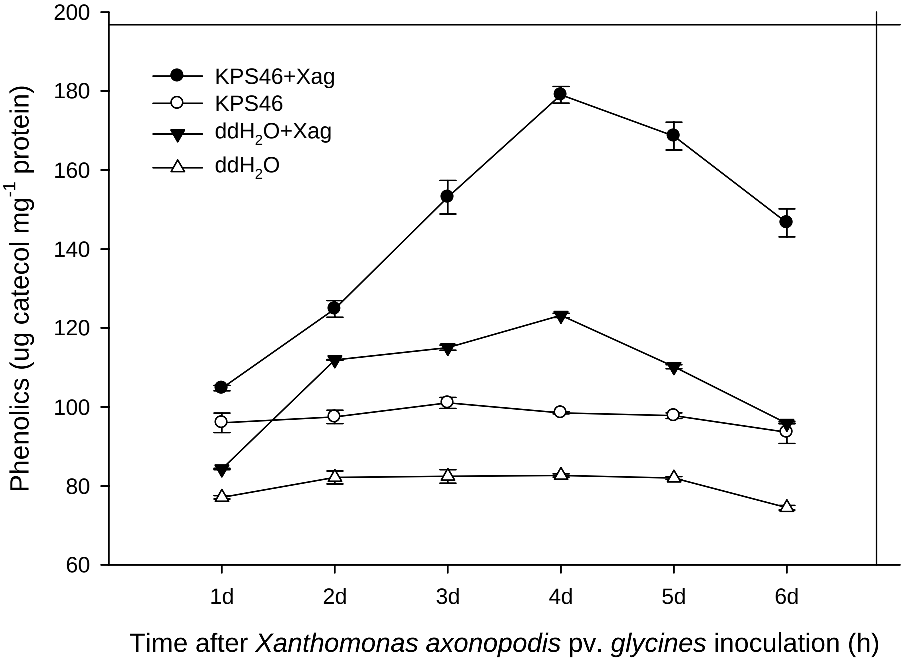


Figure 23 Time courses of the accumulation of phenolic compounds level of soybean cv. SJ4 plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 12-2 pathogen

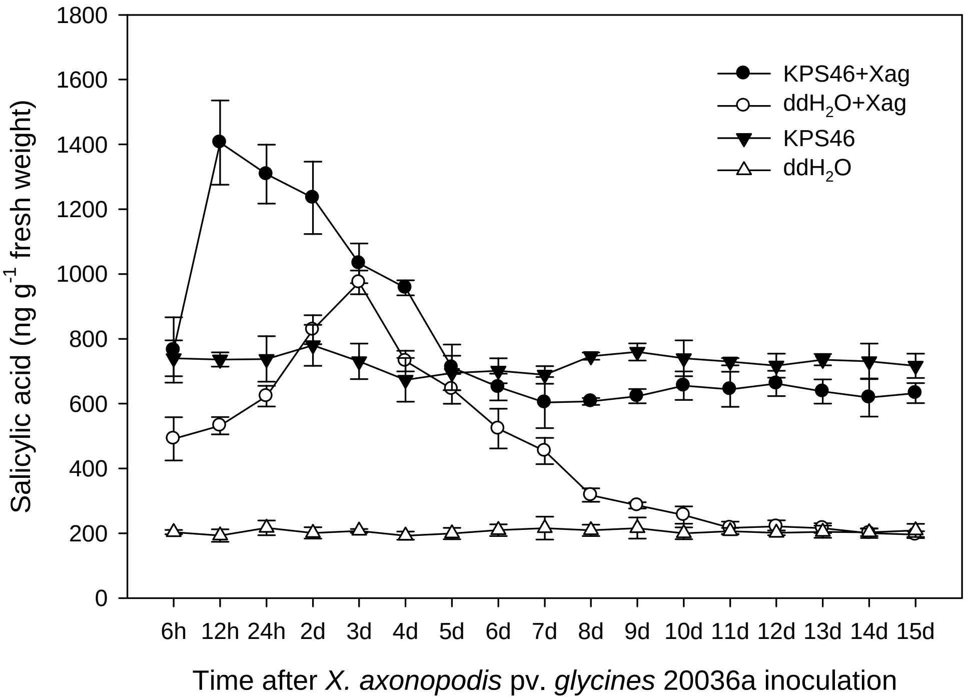


Figure 24 Time courses of the accumulation of salicylic acid (SA) content of soybean cv. Spencer plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 20036a pathogen

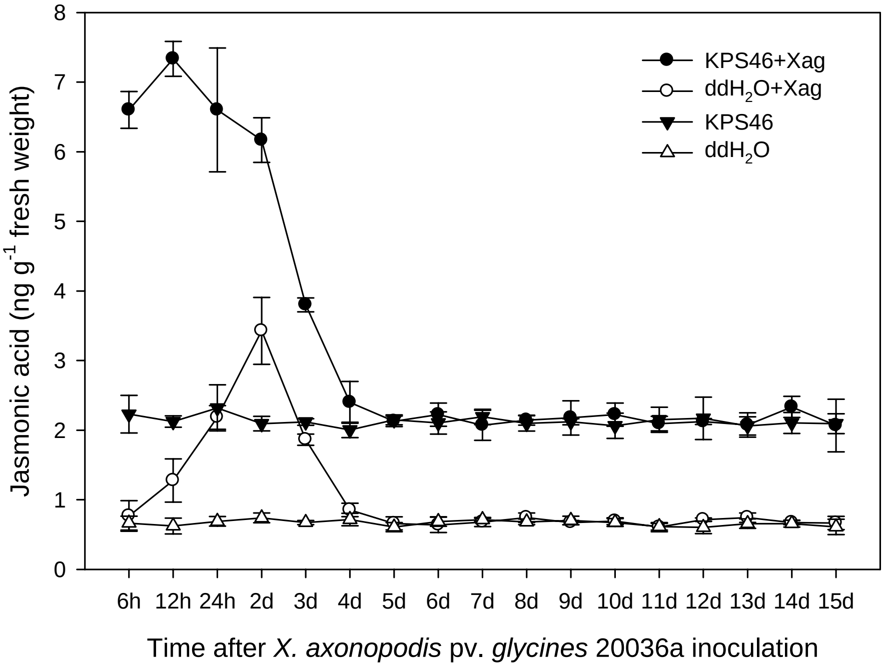


Figure 25 Time courses of the accumulation of jasmonic acid (JA) content of soybean cv. Spencer plants with or without *Bacillus amyloliquefaciens* KPS46 seed treatment and challenged with or without the *Xanthomonas axonopodis* pv. *glycines* 20036a pathogen

6. Changes in soybean proteome in response to KPS46 treatment and pathogen infection

In this study, we using proteomics approach, mass spectrometry and database searching to analyzed the changes in the proteomes of soybean plants when enhanced plant growth by sensitization mechanism with KPS46 and induced disease resistance by this strain plus challenged inoculation with *Xag*. The analysis of the 2D-PAGE images from all scanned gels allowed us to discriminate several different spots. Of these 20 identified proteins were found to be upregulated when sensitization by KPS46 and 20 identified proteins were indicated to be upregulated in KPS46 plus challenge inoculation with *Xag*. The number of peptide matches and the percentage of sequence coverage for each identified gene product are shown in Table 4 and 5. The identities of proteins were deduced by similarity to available plant sequences. Most of the proteins identified showed spots focusing at different p*I* or different apparent molecular weight, which probably correspond to differences in sequence.

When the proteome of KPS46-treated/non-pathogen inoculated plants was compared with the water-treated counterpart, the bacterial treatment was found to stimulate production of proteins primarily involved in growth and development (Table 4). The twenty proteins upregulated by KPS46 fell into five functional categories, four of which are related to growth and development. These four categories, attached to 18 proteins, included protein destination and storage; energy/ pentose phosphate; energy/glycolysis/glycoxylase cycle/ gluconeogenesis; and metabolism. Only two proteins, catalase and lectin, were associated with disease and defense response (Fig. 26).

In comparing the proteomes of KPS46-treated and non-treat plants that were inoculated with *Xag*, some of the same growth and development-related proteins upregulated in the KPS46 treated plants as found in non-inoculated plants, but there was also a greater representation by proteins associated with disease and defense response (Table 5). These defenses related proteins included catalase, lectin, heat shock protein, ascorbate peroxidase, stress inducible protein SAM22, peroxiredoxin, P21 protein and PR1A precursor. The proteins differentially expressed in this group, peroxidase is the most highly induced (30-fold) by inoculation with KPS46 compared to controls. There also were four additional functional categories found, each represented by a single protein: protein synthesis (elongation factor G); secondary metabolite (1-Deoxy-D-xylulose-5-phosphate reductoisomerase); energy and phothosynthesis (Ferredoxin-NADP$^+$ reductase); and energy and electron transport (ATPase beta subunit) (Fig. 27).

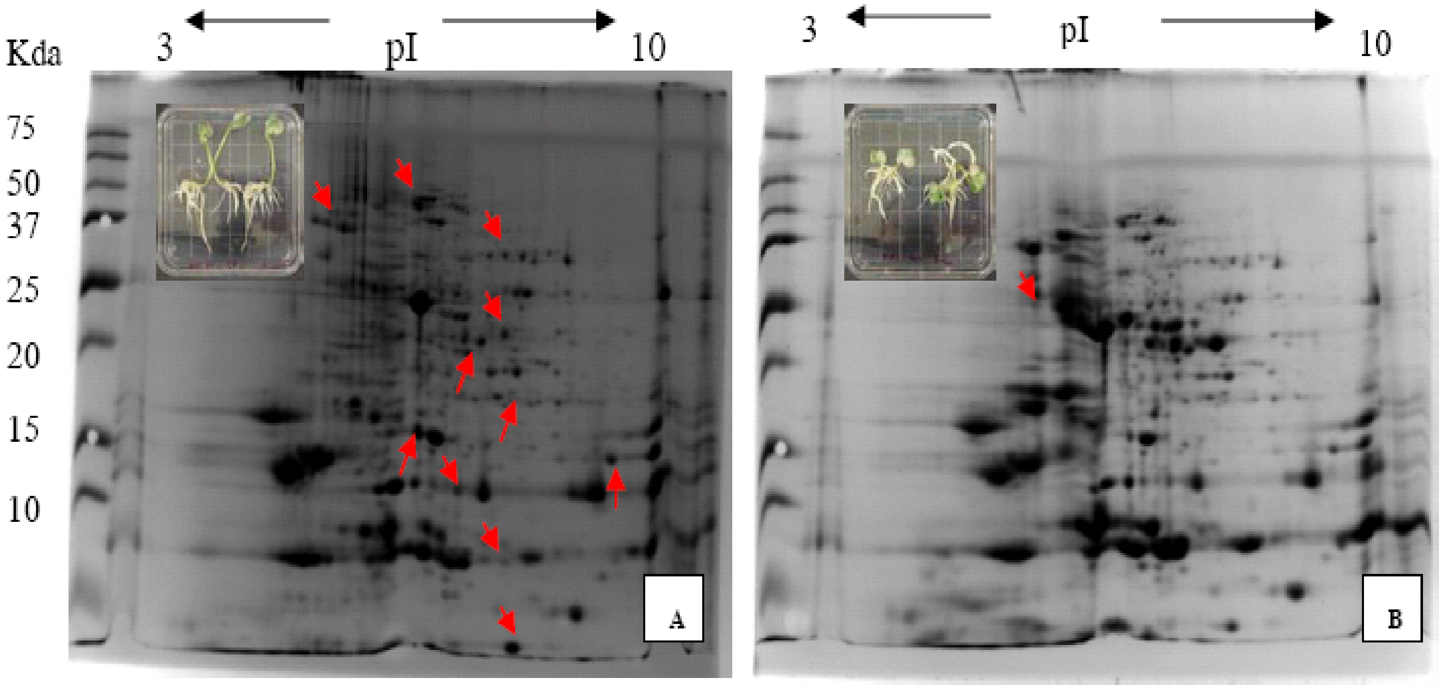


Figure 26 Representative image of 2 DE gel (11 cm) of soybean after enhanced growth promotion by *Bacillus amyloliquefaciences* KPS46 whole cell (A) ddH_2O (B) in greenhous experiment. Differentially-expressed protein spots are indicated by arrows on gel

Table 4 Identified difference proteins from soybean after induced by *Bacillus amyloliquefaciens* KPS46 compared with negative control at 7 day after planting

Spot number	**Protein name**	**Source**	**Accession number**	**Molecular mass (Da)**	***pI***	**No. of matched peptides**	**Sequence coverage (%)**
Functional category 1 : Protein destination and storage							
1	Glycinin G1 precursor	*Glycine max*	S10851	56299	4.47	4	8
2	Beta-conglycinin beta subunit	*Glycine max*	Q50JD8_SOYBN	48358	5.57	10	23
3	Vegetative storage protein, 27K, precursor	*Glycine max*	UESY27	29433		14	55
4	Alpha subunit of beta conglycinin	*Glycine max*	O22120_SOYBN	63184	4.25	9	19
5	Vegetative storage protein, 27K, precursor	*Glycine max*	UESY27	29433	-	1	5
7	Vegetative storage protein, 28K	*Glycine max*	S08511	29218	-	13	63
8	Vegetative storage protein, 25K, precursor	*Glycine max*	UESY25	29433	-	12	56
9	Beta-conglycinin alpha chain precursor	*Glycine max*	FWSYBA	70535	6.61	7	17
10	Beta-conglycinin alpha prime subunit	*Glycine max*	Q4LER6_SOYBN	72469	5.50	8	20

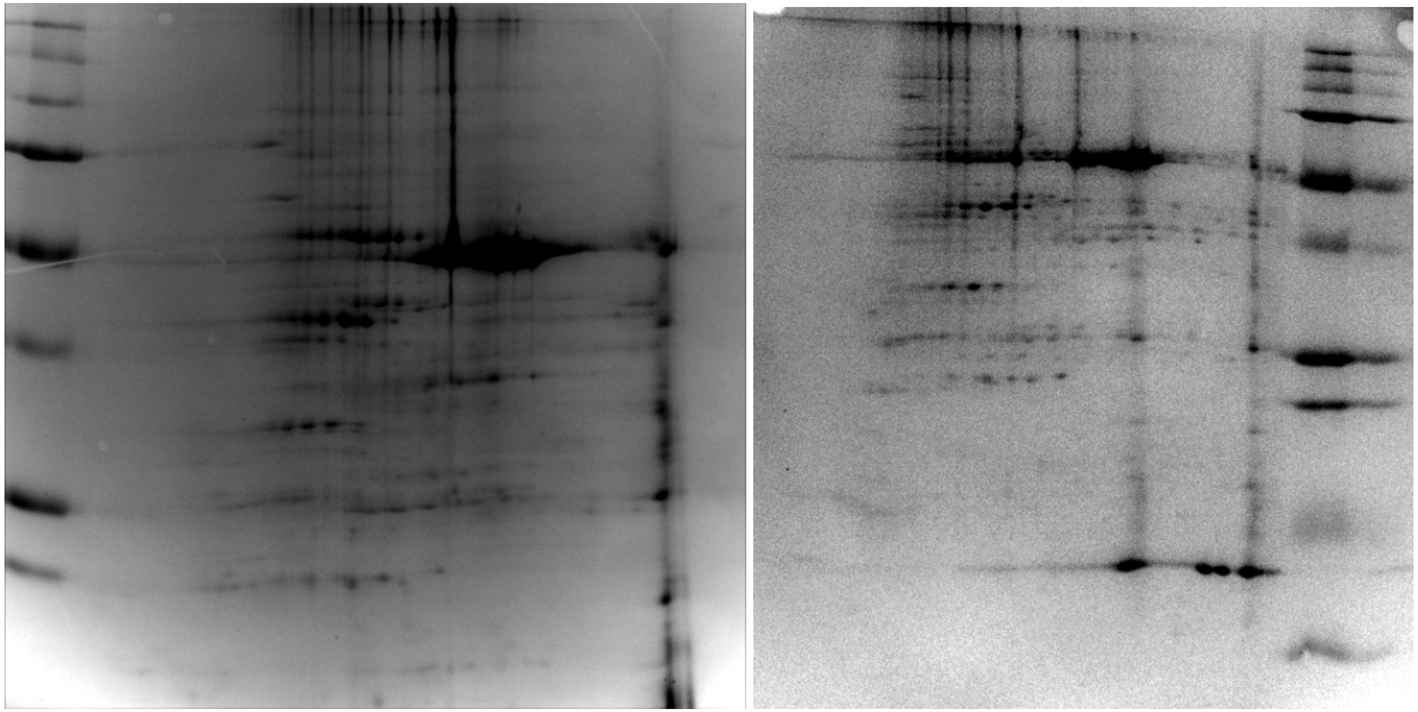
Table 4 (Continued)

Spot number	Protein name	Source	Accession number	Molecular mass (Da)	*pI*	No. of matched peptides	Sequence coverage (%)
17	Alpha subunit of beta conglycinin (Fragment)	*Glycine max*	O22120_SOYBN	63184	4.92	11	32
Functional category 2 : Energy/ pentose phosphate							
6	Ribulose-bisphosphate carboxylase	*Glycine max*	RKSYS	20288	6.49	15	48
Functional category 3 : Energy metabolism							
12	Soybean agglutinin	*Glycine max*	1SBF	25532	5.49	6	37
Functional category 4 : Energy/ glycolysis / glyoxylate cycle/ gluconeogenesis							
13	Glyceraldehyde-3-phosphate dehydrogenase	*Glycine max*	Q2I0H4_SOYBN	36855	6.72	11	45
18	Glyceraldehyde-3-phosphate dehydrogenase	*Glycine max*	Q2I0H4_SOYBN	36855	6.72	18	48
15	Malate synthase (EC 4.1.3.2) -	*Glycine max*	T07690	64325	7.02	17	37
Functional category 4 : Disease and defense response							
14	*Glycine max* catalase (cat4)	*Glycine max*	AAB88172	57043	6.80	12	29

Table 4 (Continued)

Spot number	Protein name	Source	Accession number	Molecular mass (Da)	*pI*	No. of matched peptides	Sequence coverage (%)
20	Lectin	*Glycine max*	Q2XV15_SOYBN Q2XV15_SOYBN	22568	9.59	7	39
Functional category 6 : Metabolism							
16	Alcohol dehydrogenase 1 (Fragment	*Glycine max*	Q8LJR2_SOYBN	40722	6.19	4	10
19	Probable gamma-glutamyl hydrolase	*Glycine max*	T08837	37824	6.08	8	23

Source of reference: NCBI and SwissProt database



(A) **(B)**

Figure 27 Representative image of 2 DE gel of soybean after induced systemic resistance by *Bacillus amyloliquefaciences* KPS46 with extracellular proteins (A) ddH$_2$O (B) and challenge inoculation with *Xanthomonas axonopodis* pv. *glycines* 20036a at 14 days after planting in greenhouse experiment. Differentially-expressed protein spots are indicated by arrows on gel

Table 5 Identified difference proteins from soybean after induced by *Bacillus amyloliquefaciens* KPS46 compared with negative control, and challenge inoculation with *Xanthomonas axonopodis* pv. *glycines* 20036a at 14 day after planting

Spot number	Protein name (Source)	Accession number	Molecular mass (Da)	*pI*	No. of matched peptides	Sequence coverage (%)
Functional category 1 : Protein destination and storage						
1	Glycinin G1 precursor	S10851	56299	4.47	14	28
2	Beta-conglycinin beta subunit	Q50JD8_SOYBN	48358	5.50	10	23
Functional category 2 : Protein synthesis						
4	Elongation factor G	Gi402753	77866	5.04	9	19
Functional category 3 : Energy/ pentose phosphate						
6	Ribulose-bisphosphate carboxylase	RKSYS	20288	6.49	16	48
Functional category 4 : Energy/ pentose phosphate						
12	Soybean agglutinin	1SBF	25532	5.49	6	37
Functional category 5 : Energy/ glycolysis / glyoxylate cycle/ gluconeogenesis						
13	Glyceraldehyde-3-phosphate dehydrogenase	Q2I0H4_SOY	36855	6.72	11	45
18	Phosphoglycerate kinase	Gi1161600	50317	8.48	11	56
15	Malate synthase	T07690	64325	7.02	17	37

Table 5 (Continued)

Spot number	Protein name (Source)	Accession number	Molecular mass (Da)	*pI*	No. of matched peptides	Sequence coverage (%)
Functional category 6 : Disease and defense response						
14	Catalase (cat4)	AAB88172	57043	6.80	12	29
20	Lectin	Q2XV15_SOYBNQ2XV15	22568	9.59	7	39
3	Heat shock protein	Gi1143427	75480	5.15	14	55
9	Ascorbate peroxidase	Gi1336082	27180	5.65	7	58
5	Stress inducible protein SAM22	P26987	16762	4.69	13	67
7	Peroxiredoxin	S08511	14120	5.43	13	63
8	P21 protein	P25096	22365	4.84	12	56
10	PR1A precusor	Gi13479525	18108	8.20	18	45
Functional category 7 : Energy/ photosynthesis						
11	Ferredoxin-NADP$^+$ reductase	Gi20465661	40643	8.32	6	32
Functional category 8 : Energy/ photosynthesis						
17	ATPase beta subunit	Gi1693118	55595	6.15	16	47

Table 5 (Continued)

Spot number	Protein name (Source)	Accession number	Molecular mass (Da)	*pI*	No. of matched peptides	Sequence coverage (%)
Functional category 9 : Metabolism						
16	Alcohol dehydrogenase 1	Q8LJR2_SOYBN	40722	6.19	4	10
19	Probable gamma-glutamyl hydrolase	T08837	37824	6.08	23	43

Source of reference: NCBI and SwissProt database

7. Induction of system resistance in Arabidopsis lines by KPS46

7.1 KPS46 induced resistance *Arabidopsis* against phytopathogenic bacterial infection

Induction of resistance against Pst in Arabidopsis signalling mutants by KPS46 varied among the mutants (Table 6). Induced resistance by KPS46 occurred on wildtype Arabidopsis (Col-1) and transgenic lines NahG affected in SA signalling, with KPS46 root treatment reducing disease severity in the leaves compared to the water control. KPS46 did not induce resistance in mutant lines affected in JA signalling (*jar1-1 and coi1-35*), ethylene signalling (*etr1-1*) or in auxin sensitivity (*aux 1-1*), are shown in Fig. 26.

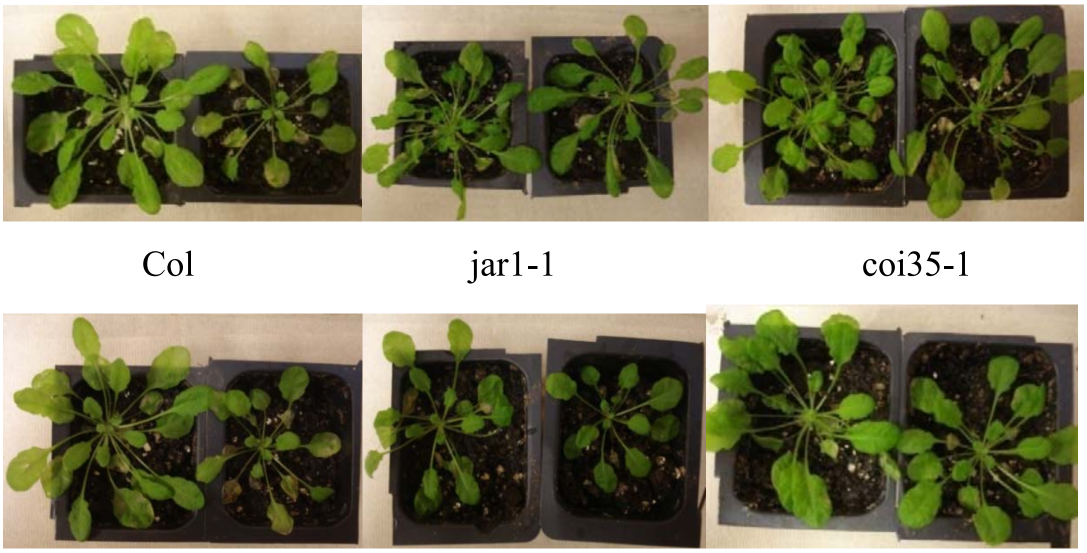
The bioprotection capacity of KPS46 was assessed in *A. thaliana* ecotype Col-0, which is susceptible to Pst. Yellowing and water-soaking symptoms were observed on the leaves of sterile distilled water treated plants, whereas leaves of KPS46-treated plants did not exhibit typical disease progression. Minute brown spots were observed 36 h after inoculation. No further symptom of the disease was observed. In addition, KPS46 treatments protected *Arabidopsis* approximately more than 70% disease severity reduction (Fig. 28). Pathogen proliferation also was inhibited significantly by KPS46 treatment. On the other hand, striled distill water did not affect disease progression (data not shown).

To analyze the mode of action of KPS46 on the resistance of *Arabidopsis* against Pst, the ecotype Col-0 and the five mutant lines were treated with KPS46 at 1 week prior to pathogen inoculation. KPS46 treatment protected NahG (a line expressing bacterial NahG) plants from DC3000 invasion. However, it did not confer, *etr1* (an altered perception of ethylene mutant), and *jar1* (a mutant that displays reduced sensitivity to methyl jasmonate) with disease resistance (Table 6). Therefore, the disease-protecting effect induced by KPS46 is dependent on ethylene, and jasmonic acid. The

KPS46 was recovered from the rhizosphere of all plant lines without significant differences (data not show); therefore, the loss of disease protecting capacity in *etr1*, and *jar1* is not due to poor colonization. These results indicate that sensitization by KPS46 is translocated systemically (data not shown).

7.2 Sensitization by KPS46 via produce hydrogen peroxide

The effects of KPS46 and Pst challenge on hydrogen peroxide accumulation in Arabidopsis wild type and mutant lines at 12 h after pathogen inoculation was determined. The responses provide reliable evidence of whether or not plants are sensitizated. Hydrogen peroxide was absent in sterile distilled water and was slightly increase in KPS46 treated *Arabidopsis* (Fig. 29). In contrast, these defense-related responses were highly regulated within the leaf tissue or trichome of plants treated with KPS46 and recovered 12 h after Pst challenge. Phytopathogenic bacteria inoculation was performed 1 week after KPS46 treatment. Similarly, higher peroxidase activity was observed in soybean treated and inoculated with KPS46 and *Xag* pathogens (Prathuangwong and Buensanteai, 2007). Hence, induced resistance in KPS46 is plant species specific. Taken together, KPS46 should be a good candidate for biological control of multiple potent diseases in soybean and Arabidopsis plant.



Col jar1-1 coi35-1

NahG aux1-1 etr1-1

Figure 28 Disease severity of priming by *Bacillus amyloliquefaciens* KPS46 and pathogen challenge on the cellular defense responses in Arabidopsis. Arabidopsis ecotype Col-0 and its mutant lines were treated by *Bacillus amyloliquefaciens* KPS46 and ddH_2O. One week after KPS46 treatment, Arabidopsis was inoculated with virulent *Pseudomonas syringae* pv. *tomato* DC3000

Table 6 Effect of treatment with KPS46 on severity of bacterial spot (casued by *Pseudomonas syringae* pv. *tomato* DC3000) on *Arabidopsis thaliana* lines

Arabidopsis line	Phenotype	Relative disease severity	
		Control	KPS46 treated
Col1-1	wildtype	+++	+
NahG	degrades SA	+++	++
etr1-1	ethylene insensitive	+++	+++
aux1-1	auxin insensitive	+++	+++
jar1-1	JA-insensitive	+++	+++
coi1-35	JA-insensitive	+++	+++

+,++,+++ denote low, moderate, and high disease severity, respectively

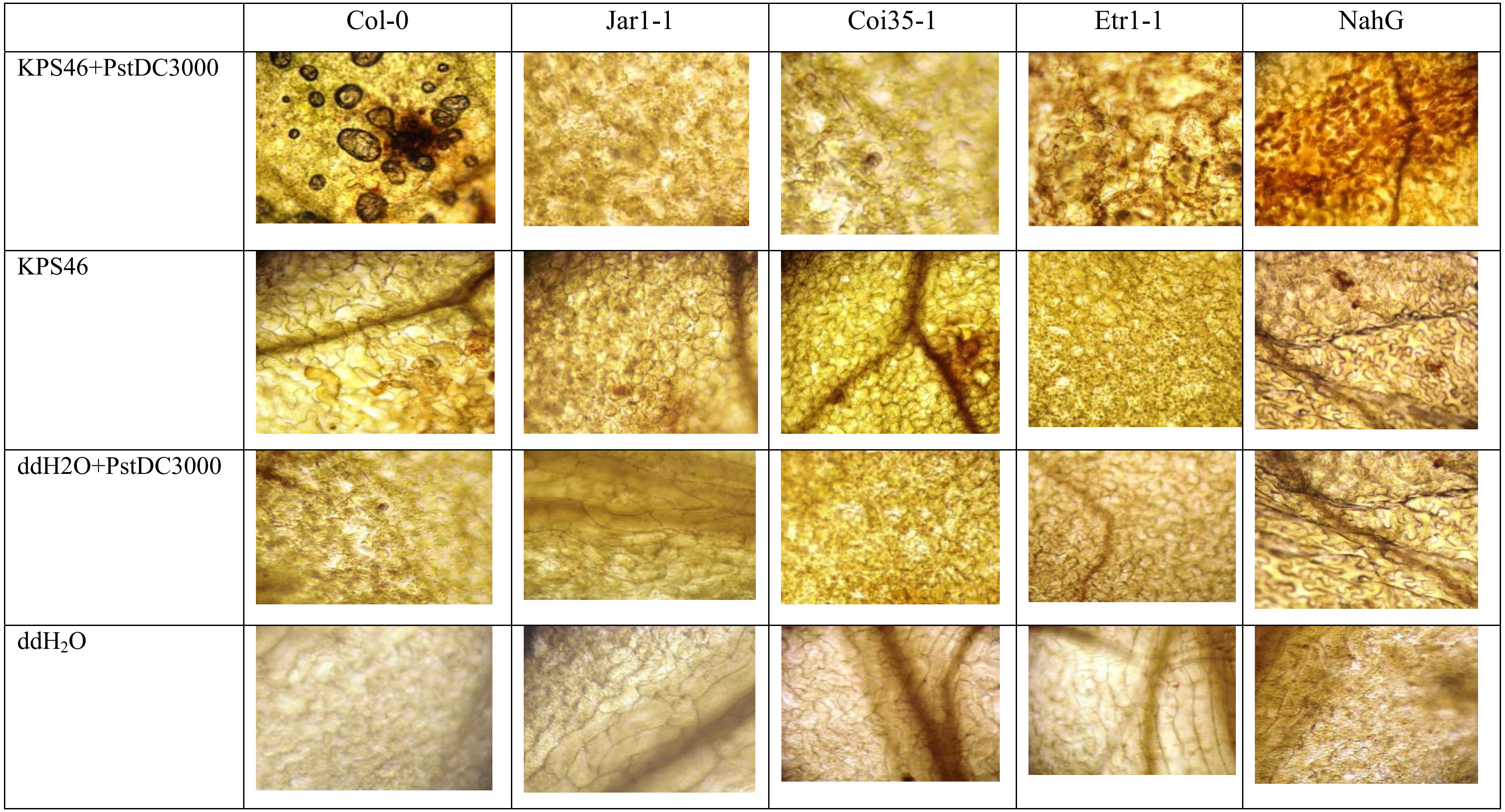


Figure 29 Effects of priming by KPS46 and pathogen challenge on the cellular defense responses in Arabidopsis. Arabidopsis ecotype Col-0 and its mutant lines were treated by KPS46 and ddH_2O. One week after KPS46 treatment, Arabidopsis was inoculated with virulent DC3000. Microscopic observation and quantification of hydrogen peroxide was performed on leaves recovered at 12 hpi, deep-brown color indicates H_2O_2 production

Discussion

The results of the present study are consistent with the hypothesis that strain KPS46 promotes the growth of soybean by production of several elicitors to enhance direct and indirect mechanisms of plant growth promotion. The authors found that KPS46 secreted high levels of IAA, surfactin as lipopeptides, and extracellular proteins in pure culture at stationary phase, whereas the reduced production of these compounds was observed with KPS46-UV mutagenesis, the strain N19G1. The presence and absence or reduced secretion of these compounds as represented by KPS46 wildtype and N19G1 mutant inoculation have been interpleted as elicitors that subsequent affecting soybean growth variation. Plant growth experiments have shown that the adapting concentrations of these exogeneous IAA, surfactin, and extracellular proteins secreted by KPS46 could increase growth of soybean seedlings particularly root and shoot lengths, number of lateral root, and fresh and dry weights under gnotobiotic and greenhouse conditions that were equivalent to the synthetic IAA tested plant. The decrease in soybean growth promotion achieved by these compounds from N19G1 mutant demonstrates that they are capable of interacting with plant and involved in elicitation of soybean growth.

The first announcement of elicitors in this study revealed that the PGPR's IAA is an essential component responsible for direct and indirect mechanisms that activated plant growth promotion (Araujo *et al.,* 2005, Buensanteai *et al.,* 2007b; Spaepen *et al.,* 2007), by triggering plant growth mediated by auxin synthesis and transport in the plant. In another word, the phytohormone-like acting compounds, IAA, have been suggested to be involved in the phytostimulation, bioremediation and biofertilization exerted by several plant-beneficial rhizobacteria (Vessey, 2003; Khan, 2005; Idriss *et al.,* 2007). Under gnotobiotic and greenhouse conditions have demonstrated that the involvement of the plant growth regulators as IAA could enhance the growth of soybean seedlings (Buensanteai *et al.,* 2007b). Results of our studies implied that the extracted compounds from KPS46 cultures exhibited IAA-like physiological activity in the host plant resembling the effect of authentic IAA on enhanced plant growth. The significant increase in shoot weight of KPS46-treated seeds, explained that the

KPS46'IAA might be increase amount of auxin into plant cell since IAA obtained from seedlings was originally present in the bacterial media. In the initial extraction of KPS46 medium, the higher amount of IAA was harvested which was the highest reported for any PGPRs (Araujo *et al.,* 2005, Leveau and Lindow, 2005; Kang *et al.,* 2006; Buensanteai *et al.,* 2007b). It is also interesting that the trigger of IAA formation in KPS46 appeared to be tryptophan depletion in the DF salt minimal medium similar to other efficient IAA-producing microorganisms. For example, *B. amyloliquefaciens* FZB42 also increased IAA production with high tryptophan ratio increased (Idriss *et al.,* 2007).

We quantified the variable doses of exogenous IAA secreted into KPS46 culture media and applied to the seeds that showed effective increase in enhanced growth of soybean seedlings. The strong eliciting ability of soybean growth promotion achieved by the IAA dose concentrated equal 50 ug ml^{-1}. The response of seedlings at 15 ug ml^{-1} concentrated treat seeds was not apparent, whereas at 75 and 100 ug ml^{-1} showed toxicity with inhibited seed germination and caused seed rot. Similarity, Kutschera and Briggs (1987) and Lambrecht et al. (2000) reported that suitable auxin concentrations could be enhanced cell elongation, cell enlargement and cell differentiation in tobacco which is one reason why high concentration of KPS46's IAA showed inhibited effect on soybean seed germination (data not show). In general, higher IAA levels in seeds or seedlings should be better associated with increase in plant biomasses (Araujo *et al.,* 2005, Buensanteai *et al.,* 2007b), but in this study demonstrated only one positive effect dose of enhanced plant growth. PGPR might promote plant growth in several pathways in that its IAA synthesis known as associated to auxin responsive element of plant, AuxRe, involved in pathways that the particular levels of IAA molecule degrade amino acids to pyruvate or succinate which may be a possible mechanism of triggered signaling transduction pathway (Wang *et al.,* 2001; Vande Broek *et al.,* 2005; Chant and Summers, 2007). These results led to speculate that signaling transduction by bacterial IAA may have other roles in enhanced growth promotion where amino acid catabolism is an important energy source (Wang *et al.,* 2001). According to these previous reports, the IAA biosynthesis in plants could be triggering by PGPR's IAA as secondary signaling transduction molecules that can occur via different pathways and produce intermediate group including: indole-3-acetamide (IAM), indole-3-pyruvate (IPyA), tryptamine, and indole-3-acetonitrile (Vande Broek *et al.,* 1999; Folke *et al.,* 2003). Therefore, the growth promotion of soybean by strain KPS46 may be attributed to any one of these mechanisms or corroboration and synergism effect.

Our experiments also found that strain KPS46 have increased plant growth by surfactin lipopeptide. Surfactin extracted from KPS46 could significantly ($p \leq 0.05$) promote plant growth by increasing shoot and root elongation, and plant biomass of soybean seedlings. Surfactin lipopeptide could be enhanced the potential of exerting biochemical effects upon growth and metabolic processes of living organisms that involved complex growth phenomena, such as differentiation, induction, or auxin-mediated events (Parr and Norman, 1965; Ernst *et al.,* 1971; Pfahler *et al.,* 1981). From these supporting documents, we can conclude that KPS46 surfactin have a potential on soybean growth enhancement that the action like phytohormone function, particulary at the seedling stage of soybean plant. However, most reports of extracellular biosurfactin produced by PGPR identified its characteristic on bacterial adhesion at plant surface to modify their hydrophobicities and potential antibiotic activities against plant pathogens (Ahimou *et al.,* 2000; Kinsinger *et al.,* 2003; Ongena *et al.,* 2007). These mechanisms might be expressed and affected by various factors including environment, plant, and bacterial species themselves (Kloepper, 1996). Little is known why and when those bacterial strains regulate one or specific or more functions. Their biosurfactins may have evolved from a lipopeptide precursor but have lost some binding functions while retaining phytohormone activity (D'Souza *et al.,* 1994; Hamvas *et al.,* 2007).

The deduced results of extracellular proteins secreted by KPS46 revealed for the first time that the 20-out of approximately 190-visible protein spots participating in soybean growth enhancement. Base on 2D-gel analysis, phenotypic seedling growth assays, and prediction of value structures of exogenous proteins obtained from KPS46 culture filtrate, these 20-extracellular proteins were capable of interacting with biosynthesis pathways that reflecting plant growth promotion. The existence of three distinct groups of secreted proteins were found to be homologous to the oligopeptide permease (Opp) operon of *B. subtilis*, which has been reported as a plant growth regulator protein likely related to peptide plant hormones (Ryan and Pearce, 2004). It is also reported to be an essential component of enzyme systems for the biosynthesis of membrane components and related to a large family of membrane transport signaling systems, antifungal lipopeptide production (Rudner *et al.,* 1991; Kuwana *et al.,* 2002; Lai *et al.,* 2003; Monnet, 2003; Gohar *et al.,* 2005; Rodionov *et al.,* 2006), and is important for thermotolerance, as membrane-components are in contact with high temperature condition to increase its PGPR competition (Ryan and Pearce, 2004). One more protein spot obtained in this study is an ABC transporter (lipoprotein) homology yufN protein, which is not only one of the key enzymes of cell membrance metabolism, but is also important for binding proteins of tryptophan amino acids. It is known that tryptophan as a precursor of plant growth regulator, IAA and thus is considered an important enhanced growth promoter of several planta (Araujo *et al.,* 2005; Leveau and Lindow, 2005; Buensanteai *et al.,* 2007b). Another ABC transporter phosphate binding protein is pstS. The *pst* operon is a member of the PhoP-PhoR two-component signal transduction system, which controls the phosphate response in *B. subtilis*. The phosphate ABC transporter binding protein could bind with phytases (myo-inositol hexakisphosphate phosphohydrolase) (Idriss *et al.,* 2002; Konietzny and Greiner, 2002). For these reasons, phosphate ABC transporter binding protein is related to phosphate solubilizing abilities of several PGPR/PGPB such as *Bacillus* and *Trichoderma* might be associated with phosphorus uptake of several crops (De Freitas *et al.,* 1997; Rodriguez and Fraga, 1999).

The one among twenty protein spots known to be regulated by nucleotide and nucleic metabolism, inosine monophosphate dehydrogenase (IMPDH), a key enzyme is involved in the biosynthesis of nucleotides, nucleosides, purines and pyrimidines in the biological process of ribonucleotide biosynthesis process. The IMPDH protein required by *Rhizobium tropici* strain CIAT8999-10.T could be activated dinitrogenase reductase protein (NifH) (Christiansen-Weneger, 1992; O'gara *et al.,* 1997; Collavino *et al.,* 2005). This protein could also be corroborated with the iron of the nitrogenase complex and nitrogen metabolisms, thus might be important of nitrogen and nitrate uptake as biofertilizer and bioremediation to enhanced plant growth and development (Wang *et al.,* 1988; Fisher, 1999; Mantelin and Touraine, 2003; Vessey, 2003).

The left of twenty members of KPS46 extracellular proteins have been known as a fundamental principle underlying all groups of these molecules to adapt the PGPR survival with highest competition. They could contribute to lipopeptide production, nitrogen fixation, production of siderophores, phosphorus solubilization, release of volatile compounds, increase in nitrate uptake, survival, colonization, competition, biofertilization, biodegradation and bioremediation (Vessey, 2003). From these particular cases, we found, the four protein spots extracted from KPS46 involved in detoxification and adaptation of cellular processing that differentially regulated the three proteins including: catalase, superoxide dismutase, and general stress protein. The catalase, same was described for *B. licheniformis* and *B. subtilis* is essential to ensure complete protection of cells against oxidative stress (Naclerio *et al.,* 1995; Voigt *et al.,* 2006) that associated to colonization and competition mechanisms of PGPR. Because, one of the generally accepted concepts is that beneficial PGPR are effective when they successfully colonize and persist in the plant rhizosphere (Yan *et al.,* 2003). The extracellular superoxide dismutase (SOD), is a free radical metabolizing enzyme that protects the cell membrane from damage by the highly reactive superoxide free radicals (Bowler *et al.,* 1990). Superoxide dismutases are metalloenzymes, which catalyse the dismutation of the superoxide anion radical into molecular oxygen and hydrogen peroxide (H_2O_2). There are four types of enzymes with Mn, Fe, Cu/Zn or Ni, as the prosthetic group that trigger photosystemI, photosystemII reaction. Moreover, Beyer and Fridovich (1987) have suggested that the reaction of H_2O_2 with FeSOD also attacked tryptophan amino acid residues, a precursor of phytohormone, IAA, in the *E. coli* enzyme.

There was also some amino acid metabolism protein present in the KPS46 extracellular medium. This protein spot is a regulator of nitrogen metabolism proteins in bacteria and is closely connected with the intracellular levels of glutamine and glutamate, the main nitrogen donors in the cell. Glutamine is formed from glutamate and ammonium by glutamine synthetase (GlnA), which is a major way for the cell to assimilate ammonium (Fisher, 1999; Larsen *et al.,* 2006). In the gram-positive model organism *B. subtilis*, the two transcriptional regulators as the nitrogen transcription factor (TnrA) and glutamine synthetase (GlnA/ GlnR) are important for the regulation of nitrogen metabolism and biofertilization (Schreier and Sonenshein, 1986; Vessey, 2003).

These data from our works demonstrate that the positive effect of KPS46 on soybean plant growth promotion was associated with IAA, surfactin and extracellular protein production, since the strain produced high suitable concentrations of these elicitors both in bacterial cultures and in the leaves of soybean plants (Prathuangwong and Buensanteai, publishing). However, the possibility that strain KPS46 may possess other direct and indirect mechanisms of plant growth promotion can not be ruled out in this study. Other non-auxin, lipopeptide and proteins related phytohormone and volatile compounds might also be plant-growth enhancement association, like proteins involved in cytokinin biosynthesis that it has been found to be involved in signal transduction trigger plant cell development (Hass *et al.,* 2004; Michael *et al.,* 2005). Another compound is the VOCs such as 2,3-butanediol that it is correlated with growth promotion with cell enlargement. VOCs also regulates plant auxin homeostasis tissue-specifically to promote leaf expansion as well as lateral roots appear to successfully provide such a balance (Ryu *et al.,* 2003a; Farag *et al.,* 2006), is thereby suggested to be important components of hormonal cross-talk in plant growth enhancement mechanism.

In this study, we have also examined that the response of soybean plant to KPS46 associated with growth promotion and induced resistance against bacterial pustule disease. Treatment of soybean seed with KPS46 resulted in systemic elevation of all biochemical markers for resistance, which in line with previous reports on the response of soybean. Peroxidase active was noted to increase following insect herbivory (Felton *et al.,* 1994; Bi and Fleton, 1995; Graham and Graham, 1996; Endo *et al.,* 2007). Thus, no definitive conclusions can be drawn from analyzing the biochemical data as to whether resistance induced by the JA-dependent pathway or the SA-dependent pathway. Plant samples analyzed in this study were collected 14 days after seed treatment. It is possible that a similar set of analyses conducted on seedling earlier following treatment might reveal differential expression of markers for the two pathways. Expression of both sets of markers may reflect cross-talk between the two pathways, a phenomenon reported more in tomato than in other model plant systems (Anfoka *et al*., 1998; Kumkel and Brucks, 2002; Silva *et al.,* 2004; Thaler *et al.,* 2004). The more rapid and higher level expression of markers after inoculation with *Xag* in KPS46 treated plants as compared to nonbacterized plants is indicative of sensitization reported by other PGPR strains in other plant species (Thaler *et al.,* 2004).

This study was designed to identify transduction signaling pathway of induced resistance mechanism including defense enzyme, phenolic compound, salicylic acid and jasmonic acid changes, we found, in the first day of three way interaction between soybean plant, KPS46 and *Xag* pathogen that salicylic and jasmonic acid, phenolic compounds levels, 1,3-*β*-glucanase activity and typical antipathogenic peroxidase activity increase in the soybean leaves compared with the sterile distilled water treated controls. The observation demonstrates that pathogen challenge is essential for the expression of phenolic accumulation response in sensitization soybean seeds. The results presented here reveal that the strain KPS46 previously shown to elicit induced resistance mechanism via increase defense enzyme and other biochemical marketrs activity before challenge inoculation with phytopathogens. Similarly, many publications have reported that PGPR/ PGPB can accumulate various plant defense-related enzymes, pathogenicity-related proteins, phytoalexin, lignin and phenolic compounds (Hammerschmidt and Kuc, 1982; Hammerschmidt *et al.,* 1984; Zdor and Anderson, 1992; Alvarez *et al*., 1998; Yedidia *et al.,* 1999; Chen *et al.,* 2000; Buensanteai *et al.,* 2007, Prathuangwong and Buensanteai, 2007).

Taken together these results suggest that KPS46 seed treatment and challenge inoculation with the *Xag* bacterial pathogen stimulated more resistance inducting defense enzymes and transduction signaling molecules accumulated in soybean leaf tissues. These observations confirm that KPS46 influences in soybean plant defense metabolism in such the way that increased resistance is conferred upon bacterial pathogen attack. In our study, we observed that when KPS46 is applied at high concentrations a SAR-like response is produced with the typical concentration-dependent increase in SA, 1,3-*β*-glucanase, peroxidase activity and early plant defense response biochemical compounds (Borg-Olivier *et al.,* 1993; Brisson *et al.,* 1994; Bolwell *et al.,* 1995; Wojtaszek *et al.,* 1997; Bernards *et al.,* 1999; Graham *et al.,* 2000). This SAR-like reaction may be sustained (data not shown) without affecting plant health or development. Although SAR has traditionally been associated with a direct effect of the inducer on the plant, it is now considered that SAR inducers may play a dual role in the activation of plant defense responses: low doses of SA sensitization for potentiated induction of certain defense genes after pathogen attack, whereas higher doses directly induce another set of defense genes (Conrath *et al.,* 2001, 2002). In addition, it is well known that JA levels increase rapidly and transiently in response to elicitor treatment (Rakwal *et al.,* 2002) and in a slower and sustained manner after soybean seed colonization by KPS46. These results suggest that KPS46 induces plant resistance against diseases by pathways that requires either SA or JA/ET signaling molecules depending on pathogen and plant species.

In this study, we used 2D-PAGE, MS and internet database searching to analyze the changes in the proteomes of soybean plants when enhanced plant growth with KPS46 and induced disease resistance by this strain plus challengd inoculation with *Xag*. The analysis of the images from all scanned gels allowed us to discriminate several different spots. We analyzed the normalized volumes of the spots using the data from each of the three biological replicates from control and KPS46 treated plants. Of these 20 identified proteins were found to be upregulated when sensitization by KPS46 and 20 identified proteins were indicated to be upregulated in KPS46 plus challenge inoculation with *Xag*. The number of peptide matches and the percentage of sequence coverage for each identified gene product are shown in Table 2 and 3. The identities of proteins were deduced by similarity to available plant sequences. Most of the proteins identified showed spots focusing at different p*I* or different apparent molecular weight, which probably corresponded to differences in sequence. Results of proteomic analysis support to the conclusion that KPS46 sensitizes soybean plants. Whereas only two pathway-neutral proteins out of 20 were identified as being defense related in KPS46-treated plants lacking pathogen inoculation, a substantially higher number of defense related proteins were induced upon inoculation of KPS46-treated plants with the pathogen. This correlates with the high increase in peroxidase activity observed in soybean plant of KPS46-treated plants compared to controls. Catalase, lectin, heat shock protein, stress inducible protein SAM22, peroxiredoxin, P21 protein, PR1A precursor were also up-regulated in KPS46-treated soybean plant and challenge inoculation with *Xag,* to inhibit bacterial pustule disease development. This is the first study to examine the interaction among soybean, KPS46, and *Xag* using biochemical and proteomic level assays that the 20- protein spots secreted by KPS46 appear to function by triggering in the plant pathway responsible for PGPR and ISR. In normal physiological conditions, all of these antioxidant enzymes act as scavenging enzymes that remove ROS, thus protecting plant cell from oxidative damage. Other than the defense related protein, protein related to metabolism and growth were induced by KPS46. This most likely reflects the growth promotion effect that KPS46 has on soybean (Buensanteai *et al.,* 2007b). The results of proteome of soybean presented here similar to many publications have reported that PGPR/ PGPB can accumulate various plant defense and stress response proteome complex in rice (Kim *et al.,* 2004), Arabidopsis (Rajjou *et al.,* 2006), bean (Marra *et al.,* 2006), maize (Chen *et al.,* 2004), cucumber (Verhagen *et al.,* 2004; Segarra *et al.,* 20007).

Results from experiment using mutant lines of Arabidopsis plant demonstrates that induced resistance against Pst by KPS46 is SA independent and JA dependent. Induced resistance by KPS46 also reguired sensitivity to jasmonic acid, ethylene and auxin, which supports the importance of JA signalling (Pieterse *et al.,* 2000). Unlike KPS46, another strain of *B. amyloliquefacians*, IN937A, did not induce resistance against Pst (Ryu *et al.,* 2003), although it was shown to induce resistance against other pathogens on Arabidopsis via induced resistance system (Desikan *et al.*, 1998a, 1998b, 2001a, 2001b; Kilic-Ekici and Yuen, 2004). Yet another strain of *B. amyloliqufaciens*, EXTN-1, applied to activated *PR-1* and *Pdf1.2* in wildtype Arabidopsis but not in transgenic NahG and mutant line *jar1*, respectively, suggesting involvement of both JA- and SA-dependent pathways (Ahn *et al.,* 2002; van Loon, 2000). Thus, there is great diversity among strains of *B. amyloliquefaciens* as to signaling pathways on the same plant species.

These study findings suggest that plant disease protection is proportional to the amount of enhanced enzyme activity, pathogenicity-related protein, phenolic compounds, SA, JA and soybean proteome profiles. The higher level of all biochemical and protein markers elicited by the train KPS46 found in the present study might be play an important role in improving damage to plant organelles and cells caused by bacterial pathogens, which would partially account for the observed delay in disease symptom development with KPS46 treatment. In nonbacterized pathogen control plants, peroxidase, β-1,3-glucanase activity, phenolic compound, JA and SA increased at a slower rate, while plant disease symptoms developed more faster. While the response of soybean to pathogenesis has been reported, this is the first study to examine soybean responses in connection with induced resistance using analysis of biochemical markers for induced resistance and proteomic analysis. We found direct evidence for cross talk in soybean and for sensitization by KPS46. Based on the response of Arabidosis to KPS46, we surmise that KPS46 activates the JA dependent pathway in soybean, but this requires confirmation by examining early responses in soybean to KPS46 induction.

These findings from this research support the hypothesis that induced resistance are involved in the biological control of bacterial pustule disease in soybean by KPS46, with induced resistance being manifested later than growth promotion mechanism. There is a consensus that involvement of multiple mechanisms in a biological control agent KPS46 could lead to more effective biological control (McDorell and Dangl, 2000; van Wees *et al.,* 2000; Martin *et al.,* 2003). While the relative contribution of each mechanism possessed by KPS46 to its biocontrol efficacy in the field has yet to be determined, the action of dual mechanisms could explain the effectiveness of KPS46 in controlling a range of bacterial pathogens over a wide range of environmental conditions. Together of these results strongly indicated that KPS46 seed sensitization treatment and challenge inoculation with the *Xag* bacterial pathogen stimulated more resistance inducting defense enzymes and transduction signaling molecules accumulated in soybean leaf tissues. These observations confirm that KPS46 influences in soybean plant defense metabolism in such the way that increased resistance is conferred upon bacterial pathogen infection. In this investigation, the strain KPS46 was clearly found to reduce the incidence of bacterial pustule disease in soybean plants.

In summary, this research presented here provides a beginning extracellular protein map for the strain KPS46 and will encourage future proteomic studies of other PGPR/ PGPB. The expression level of some extracellular proteins exhibited decreased expression via UV mutagenesis strain N19G1, suggests that these proteins may be involved in the mode of action on plant growth promotion and plant development. We can also infer from these studies that PGPR co-existing in the rhizosphere that have single modes of action may act synergistically to stimulate the growth of the soybean plant, such as that indicated in Vessey *et al.* (2003). Furthermore, this research focusing on the metabolite mechanisms of the stain KPS46 is of great importance for development and improvement of agricultural production as a way to reduce the chemical pesticide and fertilizer usage. In addition, this study could be comfirmed that the defense response of a resistant of soybean plant can be directly and indirectly linked to signaling events leading to the specific defense responses. The timely response to pathogens also plays a critical role in inducing resistance (Maleck *et al.,* 2000). Susceptible plants often take longer to activate their defense response after infection by a pathogen (Buensanteai *et al.,* 2007a). In some instances they do not respond at all (Moerschbacher *et al.,* 1999). The latter might be due to the fact that the signal transduction leading to the response is in some way blocked by the attacking pathogen (Moerschbacher *et al.,* 1999; Park *et al.,* 2000). However, KPS46 was highly effective for sensitization and induces defense of resistance mechanisms in soybean and Arabidopsis against the bacterial pustule *Xag* pathogen and leaf spot pathogen *Pst* bacterization with KPS46 sensitization the plant into a highly competent state for a long time in the absence of detectable physiological and biochemical variations. Sensitization has physiological and biochemical genetic importance, and it is one of the most economical and effective modes of resistance because it prevents dispensable metabolic consumption in plants. Plants divert this metabolic investment for growth and other fitness-related processes (Delledonne *et al*., 1998, 2001; Purrington 2000;

Jalali *et al*., 2001; Nunkel and Brooks, 2002; Bostock 2004; Jeuh *et al*., 2004; Pare *et al.,* 2005). In this soybean plant, both the SA-dependent and JA-dependent signaling transduction pathways while Arabidopsis plant depending on JA pathway are elicited with an additive effect by this strain. Recently, costs and benefits of sensitization were analyzed. Finally, the obtained data increase understanding of the modes of action at molecular and biochemistry levels of *B. amyloliquefaciens* KPS46 that enhances soybean growth promotion and induced resistance. From all of these information, we could potentially enhance the value of these species as effective elicitors or stimulators such as enhanced production of phytohormones, lipopeptide, extracellular proteins and other secondary metabolite compounds for developing new bioproduct formulations in the future. Application of KPS46 appears as a alternative to agricultural crop production technology as they are directed and in-directed at lowering chemical pesticide and fertilizer inputs in commercial farmer scale. KPS46 could be a novel biological disease management strategy that is eco natural-friendly.

CONCLUSION AND RECOMMENDATION

Conclusion

From these experimental results and discussion of this study, the conclusion can be drawn as follow:

1. The KPS46 strains produced high amount of elicitors including IAA, surfaction and extracellular proteome at stationary phase.

2. The KPS46 strains produced indole compounds when the precursor L-tryptophan was added to the culture medium. The concentration of indole increased with increasing concentrations of L-tryptophan.

3. The extracellular proteome of the strain KPS46 includes proteins from different functional classes, such as enzymes for detoxification and adaptation of cellular process, transport/ binding protein and lipoprotein, protein and amino acid biosynthesis, amino acid metabolism, energy metabolism, and nucleotide and nucleic metabolism, and some proteins of yet unknown and fully understood function.

4. The strain KPS46 and its elicitors as IAA, surfactin and extracellular protein are able to corroboratively elicit with growth development of soybean by increasing the shoot length, root length, root dry weight, shoot dry weight, number of lateral root and root surface area under gnotobiotic and greenhouse assay.

5. The strain KPS46 could be induced resistance on soybean and Arabidopsis plant against bacterial disease by pattern of induced systemic resistance (ISR) associated with the accumulation of phenolic content and defense related enzymes of increased β-1,3-glucanase and peroxidase activity levels in plants.

6. The strain KPS46 was able to activate both rapid salicylic acid (SA) and delayed jasmonic acid/ ethylene (JA/ET) dependent pathways of induced systemic resistance in soybean plants cv. Spencer with high and low production levels respectively.

7. The strain KPS46 and Pst challenge on was able to accumulayte to hydrogen peroxide production in Arabidopsis wild type and this strain induces Arabidopsis resistance against bacterial diseases by pathways that requires JA/ET signaling pathway

8. The strain KPS46 induces plant resistance against diseases by pathways that requires either SA or JA/ET signaling molecules depending on pathogen and plant species.

9. Our studies and data did not show any proof on negative cross-talk between SA and JA signaling in soybean plants as has been shown in *Arabidopsis*.

10. The proteome of KPS46-treated cv. Spencer seeds and primed with *Xag* inoculation secreted various defense related proteins including PR1A precursor, catalase, lectin, heat shock protein, ascorbate peroxidase, stress inducible protein SAM22, peroxiredoxin and P21 protein to inhibit disease development. This work showed that the strain KPS46 actively induces systemic changes in plant physiology and disease resistance through systemic modulation mechanism complex of the expression of defense response and metabolism proteins complex.

11. The soybean proteome changes found in this work help to give an understanding of how Bacillus -treated plants become more resistant to pathogen attacks through the changes in expression of a complex of defence-oriented proteins which can directly protect the plant or switch the metabolism to a defensive protection state.

12. These results show the potential of using the strain KPS46 to enhance growth of soybean, as well a biocontrol agent for crop productions management.

13. To our knowledge, this is the first two-dimensional extracellular protein reference map of a PGPR, *B. amyloliquefaciens* associated with soybean-growth enhancement.

14. This research is the first to employ the proteomics approach to study soybean responses to PGPR/PGPB. The proteome changes found in this study help to give a better understanding of how bacteria can directly influence plant growth and metabolism.

15. The use of the strain KPS46 may minimize the cost of control strategies and reduce the risk associated with the high use of chemical pesticides and chemical fertilizer in commercial soybean production in the future.

The above results support the hypothesis that strain KPS46 utilizes combination of the growth promoting and induced resistance mechanisms of actions to increase the growth enhancement and disease resistance reduction of soybean against *Xag* pathogen in both gnotobiotic and greenhouse assay.

Recommendation

More experiment will be necessary to prove the mechanism of plant growth enhancement and induced resistance. Although in this study we can ruled out the possibility that strain KPS46 can promote plant growth under gnotobiotic and greenhouse condition through control the plant pathogens, we found that the bacterium can enhance the growth of soybean by directly and indirectly stimulation of several functional category of plant protein expression. While the examination of plant proteins in this study was not exhaustive, it appears that most of the proteins upregulated by KPS46 are related to growth development and disease resistance of soybean. Althought, these three elicitors in soybean growth promotion under biological conditions, UV mutant strains of N19G1 genetically blocked in the production of IAA, surfactin and extracellular proteins was compared with its wild type strain KPS46 to examine the effect on plant-growth promotion. In this comparison, UV knockout mutant of IAA biosynthesis, surfactin production and extracellular proteins secretion, strain N19G1, could not enhance plant growth whereas KPS46 wild type significantly did. However, the knock-out mutant with destroyed function of the genes encode these elicitors might be useful to prove the mechanism of alternative pathway of plant growth promotion by PGPR KPS46 strain. To our knowledge, the extracellular proteome of *B. amyloliquefaciens* KPS46 associated with soybean-growth enhancement has been studied for the first time. The complete *B. amyloliquefaciens* proteome is not sequenced and no public protein sequencing effort is underway (Gohar *et al.,* 2005; Idriss *et al.,* 2007). Thus, having solved the sample preparation issues, the biggest problem with identifying proteins from *B. amyloliquefaciens* is the limited information available in protein databases. The number of proteins that have been characterised in the Swiss-Prot and EMBL protein databases is almost from the *B. subtilis*. However, the proteome changes found in this study help to give a better understanding of how bacteria can directly and indirectly influence plant growth and plant defense metabolism but not clearly complex mechanism yet. Then, further research is need also to determine correlation of soybean proteins mechanism and developing mutants of strain KPS46 with decreased each mode of action of IAA production, surfactin production and specific extracellular protein would help in elucidating the major direct mechanism of action of strain KPS46 in promoting the growth and induced resistance of soybean under gnotobiotic, greenhouse and field conditions. This would also help in developing a potential inoculant for use in agriculture in the future.

LITTERATURE CITED

Achuo, E.A., K. Audenaert, H. Meziane and M. Hofte. 2004. The salicylic acid-dependent defence pathway is effective against different pathogens in tomato and tobacco. **Plant Pathol.** 53: 65–72.

Agrios, G. N. 1988. **Plant Pathology,** 3rd Edition. Academic Press Inc., San Diego USA.

Ahimou, F., P. Jacques and M. Deleu. 2000. Surfactin and iturinA effect on *Bacillus subtilis* surface hydrophobicity. **Enz. and Microbial. Tech.** 27(10): 749-754.

Ahn, I. P., K. Park and C. H. Kim. 2002. Rhizobacteria-induced resistance perturbs viral disease progress and triggers defense-related gene expression. **Mol. Cells** 13: 302-308.

Alstrom, S., 1991. Induction of disease resistance in common bean susceptible to halo blight bacterial pathogen after seed bacterization with rhizoshpere pseudomonads. **J. Gen.Appl. Microbiol.** 37: 495-501.

Alvarez, M. E., R. I. Pennell, P. Meijer, A. Ishikawa, R. A. Dixon and C. J. Lamb. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. **Cell** 92: 773-784.

Anfoka, G. and H. Buchenauer. 1997. Induction of systemic resistance in tomato and tobacco plants against cucumber mosaic virus. **J. of Plant Dis. and Protect.** 104: 506-516.

Antelmann, H., E. Darmon, D. Noone, J. W. Veening, H. Wester, S. Bron, O. P. Kuipers, K. M. Devine, M. Hecker and J. M. van Diji. 2003. The extracellular proteome of *Bacillus subtilis* under secretion stress conditions. **Molecul. Microbiol.** 49(1):143-156.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. **Annu. Rev. of Plant Biol.** 55: 373 – 399.

Araujo, F. F., A. A. Henning and M. Hungria. 2005. Phytohormones and antibiotics produced by *Bacillus subtilis* and their effects on seed pathogenic fungi and on soybean root development. **World J. of Microbiol. and Biotech.** 21: 1639-1645.

Bakker, P. A. H. M., L. X. Ran, C. M. J. Pieterse and L. C. van Loon. 2003. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. **Can. J. Plant Pathol**. 25: 5-9.

Bakker, P.A.H.M., C.M.J. Pieterse and L.C. van Loon. 2007. Induced systemic resistance by fluorescent Pseudomonas spp. **Phytopathol.** 97: 239-243.

Bagyan, I. and P. Setlow. 2002. Localization of the cortex lytic enzyme CwlJ in spores of *Bacillus subtilis*. **J. Bacteriol.** 184(4): 1219-1224

Barbieri, P., T. Zanelli, E. Galli and G. Zanetti. 1986. Wheat inoculation with *Azospirillum brasilense* Sp6 and some mutants altered in nitrogen fixation and indole-3-acetic acid production. **FEMS Microbiol. Lett.** 36: 87-90.

Barea, J.M. and M. E. Brown. 1974. Effects on plant growth produced by *Azotobacter paspali* related to synthesis of plant growth regulating substances. **J. of Appl. Bacteriol.** 37: 583-593.

Barthlott, W. and C. Neinhuis. 1997. Purity of the sacred lotus or escape from contamination in biological surfaces. **Planta** 202: 1 – 8.

Bashan, Y. and G. Holguin. 1997. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPG (plant growth-promoting bacteria) and PGPB. **Soil Biol. Biochem.** 30, 1225–1228

Bayles, C. J., M. S. Ghemawat and J. R. Aist. 1990. Inhibition by 2-deoxy-D-glucose of callose formation, papillae deposition and resistance to powdery mildew in a *mlo* barley mutant. *Physiological Molecular* **Plant Pathol.** 36: 63 - 72.

Beffa, R. S., R. M. Hofer, M. Thomas and F. Meins, 1996. Decreased susceptibility to viral diseases of β-1,3-glucanase-deficient plants generated by antisense transformation. **Plant Cell** 8: 1001 - 1011.

Bell, E., R. A. Creelman and J. E. Mullet. 1995. A chloroplast lipoxygenase is required for wound induced accumulation of jasmonic acid in *Arabidopsis.* **PNAS** 92: 8675 – 8679.

Benizri, E., A. Courtade, C. Picard and A. Guckert. 1998. Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens* M.3.1. **Soil Biol. and Biochem.** 30: 1481-1484.

Bernards, M.A., W.D. Fleming, D.B. Llewellyn, R. Priefer, X. Yang, A. Sabatino and G.L. Plourde. 1999. Biochemical characterization of the suberization-associated anodic peroxidase of potato. **Plant Physiol.** 121: 135–145.

Bertini, L., L. Leonardi, C. Caporale, M. Tucci, N. Cascone, I. D. Berardino, V. Buonocore and C. Caruso. 2003. Pathogen-responsive wheat PR4 genes are induced by activators of systemic acquired resistance and wounding. **Plant Science** 164: 1067 – 1078.

Beyer, W. F. J. and I. Fridovich. 1987. Effect of hydrogen peroxide on the iron containing superoxide dismutase of *Escherichia coli*. **Biochem.** 26:1251–1257.

Bi, J.L., G.W. Felton and A. Mueller. 1994. Induced resistance in soybean to *Helicoverpa zea*: Role of plant protein quality. **J. of Chem. Ecol.** 20: 183–198.

Bi, J.L. and G.W. Felton. 1995. Foliar oxidative stress and herbivory: primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. **J. of Chem. Ecol.** 21: 1511–1530.

Bisgrove, S. R., M. T. Simonich, N. M. Smith, A. Sattler and R. W. Innes. 1994. A disease resistance gene in Arabidopsis with specificity for two different pathogen avirulence genes. **Plant Cell** 6: 927-933.

Block, A., E. Schmelz, P. J. O'Donnell, J. B. Jones and H. J. Klee. 2005. Systemic acquired tolerance to virulent bacterial pathogens in tomato. **Plant Physiol.** 138: 1481-1490.

Bloemberg, G.V. and B. J. J. Lugtenberg. 2001. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. **Curr. Opin. Plant Biol.** 4: 343–350

Boiero, L., D. Perrig, O. Masciarelli, C. Penna, F. Cassán and V. Luna. 2007. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. **Appl. Microbiol. Biotechnol**. 74(4): 874-80

Bolwell, G.P., V.S. Butt, D.R. Davies and A. Zimmerlin. 1995. The origin of the oxidative burst in plants. **Free Rad. Research** 23: 517–532.

Borg-Olivier, O. and B. Monties, 1993. Lignin, suberin, phenolic acids and tyramine in the suberized, wound-induced potato periderm. **Phytochem.** 32: 601–606.

Bork, P. 1993. Hundreds of ankyrin-like repeats in functional diverse proteins: Mobile modules that cross phyla horizontally. **Proteins** 17: 363 – 374.

Bostock, R. M. 2005. Signal crosstalk and induced resistance: straddling the line between cost and benefit. **Annu. Rev. Phytopathol.** 43: 545-580.

Bowler, C., L. V. Kaer, W. V. Camp, M. V. Montagu, D. Inze and P. Dhaese. 1990. Characterization of the *Bacillus stearothermophilus* manganese superoxide dismutase gene and its ability to complement copper/zinc superoxide dismutase deficiency in *Saccharomyces cerevisiae*. **J. Bacteriol.** 172(3): 1539-1546.

Bradford, M. M. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Anal. Biochem.** 72: 248–254.

Bradley, C.A. 2008. Effect of fungicide seed treatments on stand establishment, seedling disease, and yield of soybean in North Dakota. **Plant Dis.** 92(1): 120-125.

Brisson, L.F., R. Tenhaken and C.J. Lamb. 1994. Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. **The Plant Cell** 6: 1703–1712.

Buensanteai, N., D. Athinuwat and S. Prathuangwong. 2007a. *Bacillus amyloliquefaciens* induced systemic resistance against *Xanthomonas axonopodis* pv. *glycines* caused agent soybean bacterial pustule with increased phenolic compounds and phenylalanine ammonia lyase. **Proc. of the 45th Kasetsart University Annu. Con.** Thailand. 115-123.

Buensanteai, N., G. Y. Yuen and S. Prathuangwong. 2007b. Indole-3-acetic acid produced by *Bacillus amyloliquefaciens* KPS46 and effects on growth promotion of soybean. **Proc. of the 8th Nat. Plant Protec. Con**, Phitsanulok, Thailand. 185-205.

Buensanteai, N., D. Athinuwat, T. Chatnaparat, G. Y. Yuen and S. Prathuangwong. 2008. The extracellular proteome of plant growth promoting-bacteria, *Bacillus amyloliquefaciens* KPS46 and its effect on enhanced growth promotion and induced systemic resistance on soybean. **Proc. of the 46th Kasetsart University Annu. Con,** Bangkok, Thailand. 342-252.

Cao, H., X. Li and X. Dong. 1998. Generation of broad-spectrum resistance by overexpression of an essential regulatory gene in systemic acquired resistance. **PNAS** 95: 6531 – 6536.

Cartieaux, F.P., L. Nussaume and C. Robaglia. 2003. Tales from the underground: molecular plant-rhizobacteria interactions. **Plant Cell and Environ.** 26: 189-199.

Cartieaux, F., M.-C. Thibaud, L. Zimmerli, P. Lessard, C. Sarrobert, P. David, A. Gerbaud, C. Robaglia, S. Somerville and L. Nussaume. 2003. Transcriptome analysis of *Arabidopsis* colonized by a plant growth promoting rhizobacterium reveals a general effect on disease resistance. **Plant J.** 36: 177-188.

Cattelan, A.J., P.G. Hartel, J. J. Fuhrmann. 1999. Screening for plant growth–promoting rhizobacteria to promote early soybean growth. **Soil Sci. Soc. of Amer. J.** 63: 1670-1680.

Chant, E.L. and D. K. Summers. 2007. Indole signalling contributes to the stable maintenance of *Escherichia coli* multicopy plasmids. **Mol. Microbiol.** 63: 35–43.

Chen, Z., H. Silva and D. F. Klessig. 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. **Science** 262: 1883 – 1886.

Chen, C., R. R. Belanger, N. Benhamouand and T. C. Paulitz. 2000. Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. **Physiol . Mol. Plant Pathol.** 56: 13-23.

Chen J., G. G. Harman, A. Comis, G.W. Cheng and H.N. Liu. 2004. The change of maize plant proteome affected by *Trichoderma harzianum* and *Pythium ultimum.* **Acta Phytopathol. Sinica** 34(4): 319-328.

Chen, X. H., A. Koumoutsi, R. Scholz, A. Eisenreich, K. Schneider, I. Heinemeyer, B. Morgenstern, B. Voss, W. R. Hess, O. Reva, H. Junge, B. Voigt, P. R. Jungblu, J. Vater, R. Süssmuth, H. Liesegang, A. Strittmatter, G. Gottschalk and R. Borriss. 2007. Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. **Nat. Biotechnol**. 25(9): 1007-1014.

Christiansen-Weneger, C. 1992. N_2-fixation by ammonium-excreting *Azospirillum brasilense* in auxin-induced tumours of wheat (*Triticum aestivum* L.). **Biol. Fertil. Soils.** 12: 85–100.

Collavono, M., P. M. Riccillo, D. H. Grasso, M. Crespi and O. M. Aguilar. 2005. GuaB activity is required in *Rhizobium tropici* during the early stages of nodulation of determinate nodules but is dispensable for the *Sinorhizobium meliloti*-alfalfa symbiotic interaction. **Mol. Plant-Microbe Interact.** 18(7): 742-750.

Conrath, U., O. Thulke, V. Katz, S. Schwindling and A. Kohler. 2001. Priming as a mechanism in induced systemic resistance of plants. **Eur. J. Plant Pathol.** 107: 113-119.

Conrath, U., C. M. J. Pieterse and B. Mauch-Mani. 2002. Priming in plant-pathogen interactions. **Trends Plant Sci.** 7: 210-216.

Crozier, A., P. Arruda, J. M. Jasmim, A. M. Monteiro and G. Sandberg. 1988. Analysis of indole-3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense*. **Appl. Environ. Microbiol.** 54(11): 2833-2837.

Dashti, N., B. Prithivirai, R.K. Hynes and D.L. Smith. 2000. Root and rhizosphere colonization of soybean [*Glycine max* (L.) Merr.] by plant-growth-promoting rhizobacteria at low root zone temperatures and under short-season conditions. **J. of Agr. Crop Sci.** 8: 15-22.

De Freitas, J. R., M. R. Banerjee and J. J. Germida. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but no phosphorus uptake of canola (*Brassica napus* L.). **Biol. Fertl. Soils.** 24: 358–364.

Delaney, T. P., S. Uknes, B. Vernooij, L. Friedrich, K. Weymann, D. Negroto, T. Gaffney, M. Gut-Rella, H. Kessmann, E .Ward and J. Ryals. 1994. A central role of salicylic acid in plant disease resistance. **Science** 226: 1247 – 1250.

Del Gallo, M. and I. Fendrik. 1994. **The rhizosphere and *Azospirillum*. *In Azospirillum*/plant associations**. Edited by Y. Okon. CRC Press, Boca Raton. pp. 57-74.

Delledonne, M., Y. Xia, R. A. Dixon and C. Lamb. 1998. Nitric oxide functions as a signal in plant disease resistance. **Nature** 394: 585 – 588.

Delledonne, M., J. Zeier, A. Marocco and C. Lamb. 2001. Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response. **PNAS** 98: 13454 – 13459.

de Meyer, G., K. Capieau, K. Audenaert, A. Buchala, J. P. Metraux and M. Hofte. 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *P.*

aeruginosa 7NSK2 activate the systemic acquired resistance pathway in bean. **Mol. Plant-Microbe Interact.** 12:450-458.

Desikan, R., A. Reynolds, J. T. Hancock and S. J. Neill. 1998a. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on gene expression in *Arabidopsis* suspension cultures. **Biochemical J.**330: 115 – 120.

Desikan, R., E. C. Burnett, J. T. Hancock and S. J. Neill. 1998b. Harpin and Hydrogen peroxide induce the expression of a homologue of gp91-phox in *Arabidopsis thaliana* suspension cultures. **J. of Exp. Bot.** 49: 1767 – 1771.

Desikan, R., S. A.-H. Mackerness, J. T. Hancock and S. J. Neill. 2001a. Regulation of the *Arabidopsis* transcriptome by oxidative stress. **Plant Physiol.** 127: 159 – 172.

Desikan, R., J. T. Hancock, K. Ichimura, K. Shinozaki and S. J. Neill. 2001b. Harpin induces activation of the *Arabidopsis* MAP kinase AtMPK4 and AtMPK6. **Plant Physiol.** 126: 1579 – 1587.

Doke, N., Y. Miura, L. M .Sanchez, H. J. Park, T. Noritake, H. Yoshioka and K. Kawakita. 1996. The oxidative burst protects plants against pathogen attack: mechanism and role as an emergency signal for plant bio-defence. **Gene** 179: 45 – 51.

Domon, B. and R. Aebersold. 2006. Mass spectrometry and protein analysis. **Science** 312: 212-217.

Dorey, S., M. Kopp, P. Geoffroy, B. Fritig and S. Kauffmann. 1999. Hydrogen peroxide from the oxidative burst is neither necessary nor sufficient for hypersensitive cell death induction, phenylalanine ammonia lyase stimulation, salicylic acid accumulation or scopoletin consumption in cultured tobacco cells treated with elicitin. **Plant Physiol.** 121: 163 -171.

D' Souza, C., M. M. Nakano and P. Zuber. 1994. Identification of comS, a gene of the srfA operon that regulates the establishment of genetic competence in *Bacillus subtilis*. **PNAS** 91(20): 9397–9401.

Durner, J. and D. F. Klessig. 1995. Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. **PNAS** 92: 11312 – 11316.

Duy, N. V., U. Mader and N. P. Tran. 2007. The proteome and transcriptome analysis of *Bacillus subtilis* in response to salicylic acid. **Proteomics** 7:698–710.

Duzan, H.M., F. Mabood, X. Zhou, A. Souleimanov and D.L. Smit. 2005. Nod factor induces soybean resistance to powdery mildew. **Plant Physiol. and Biochem.** 43: 1022-1030.

El-Gendy, W., M. D. Brownleader, H. Ismail, P. J. Clarke, J. Gilbert, F. El-Bordiny, M. Trevan, J. Hopkins, M. Naldrett and P. Jackson. 2001. Rapid deposition of wheat cell wall structural proteins in response to *Fusarium*-derived elicitors. **J. of Exp. Bot.** 54: 85 – 90.

Elstner, E. F. and A. Heupel. 1976. Formation of hydrogen peroxide by isolated cell walls from horseradish (*Armoracia lapathifolia*). **Planta** 130: 175 – 180.

Endo, N., I. Hirakawa, T.,Wada and S. Tojo, 2007. Induced resistance to the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) in three soybean cultivars. **Appl. Entomol. and Zool.** 42: 199-204.

Engelberth, J., E.A. Schmelz, H.T. Alborn, Y.J. Cardoza, J. Huang, J.H. Tumlinson. 2003. Simultaneous quantification of jasmonic acid and salicylic acid in plants by vapor-phase extraction and gas chromatography-chemical ionization-mass spectrometry. **Anal. Bichem.** 312 (2): 242-250.

Ernst, R., J. Arditti and P. L. Healey. 1971. Biological effects of surfactants. I. Influence on the growth of orchid seedlings. **New Phytologist.** 70(3):457-475.

Fallik, E., and Y. Okon. 1989. Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. **Soil. Biol .and Biochem.** 21: 147-153.

Farag, M. A., C. M. Ryu, L. W. Sumner and P. W. Pare. 2006. GC–MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. **Phytochem.** 67(20):2262-2268.

Felton, G.W., C.B. Summers and A.J. Mueller. 1994. Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-cornered alfalfa hopper. **J. of Chem.l Ecol.** 20 (3): 639-650.

Fisher, S. H. 1999. Regulation of nitrogen metabolism in *Bacillus subtilis*: vive la difference. **Mol. Microbiol.** 32**:**223-232.

Folke, S., A. Crister, E. Agneta, C. Alan and S. Goran. 2000. The relative importance of tryptophan-dependent and tryptophan-independent biosynthesis of indole-3-acetic acid in tobacco during vegetative growth. **Planta** 211:715-721.

Gaffney, T., L. Friedrich, E. Vernooij, D. Negrotto, G. Nye, S. Uknes, E. Ward, H. Kessmann and J. Ryals. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. **Science** 261 : 754-756.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. **Can. J. of Microbiol.** 41: 109-117.

Glick, B.R., C. Liu, S. Ghosh and E. B. Dumbroff. 1997. Early development of canola seedlings in the presence of the plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2. **Soil. Biol. and Biochem.** 29: 1233-1239.

Goeschl, J., L. Rappaport and H. Pratt. 1966. Ethylene as a factor regulating the growth of pea epicotyls subjected to physical stress. **Plant Physiol.** 41: 877 – 884.

Gohar, M., N. Gilois, C. Garreau, V. Sanchis and D. Lereclus. 2005. A comparative study of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus anthracis* extracellular proteomes. **Proteomics** 5(14): 3696-711.

Gorg, A., C. Obermaier, G. Boguth, A. Harder, B. Scheibe, R. Wildgruber and W. Weiss. 2000. The current state of two-dimensional electrophoresis with immobilized pH gradients. **Electrophoresis** 21: 1037–1053.

Graham, T.L. and M.Y. Graham. 1996. Signaling in soybean phenylpropanoid responses: dissection of primary, secondary, and conditioning effects of light, wounding, and elicitor treatments. **Plant Physiol.** 110: 1123–1133.

Graham, T.L., M.Y. Graham, A.R. Rose, R.S. Poling and M.A. Omer. 2000. Identification and distribution of a specific peroxidase isoform functioning as a genistein-activated NADH oxidase central to defense competency in soybean. **Perox. News.** 14: 103–109.

Grant, J. .J and G. J. Loake. 2000. Role of reactive oxygen intermediates and cognate redox signaling in disease resistance. **Plant Physiol.** 124: 21 – 29.

Grbic, V. and A. B. Bleecker. 1995. Ethylene regulates the timing of leaf senescence in *Arabidopsis.* **Plant J.** 8: 595 – 602.

Gundlach, H., M. J. Muller, T. M. Kutchan and M. H. Zenk. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. **PNAS** 89: 2389 - 2393.

Hain, R., H.J. Reif, E. Krause, R. Langebartels, H. Kindl, B. Vornam, W. Wiese, E. Schmeizer, P. H. Schreier, R. H. Stocker and K. Stenzel. 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. **Nature** 361: 153 –156.

Hammerschmidt, R., E. M. Nuckles and J. Kuc. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium.* **Physiol. Plant Pathol.** 20: 73–82.

Hammerschmidt, R., J. Kuc. 1995. **Induced Resistance to Disease in Plants**. Dordrecht, Netherlands : Kluwer Academic Publishers. pp. 86-110.

Hammerschmidt, R. and J. C. Schultz. 1996**. Multiple defenses and signals in plant defense against pathogens and herbivores.** In: Romeo *et al*. Phytochemical Diversity and Redundancy in Ecological Interactions, Plenum Press, New York.

Hammerschmidt, R. 1999. Induced disease resistance : How do induced plants stop pathogens? **Physiol. and Mol. Plant Pathol.** 55 : 77-84.

Hammond-Kosack, K. E. and J. D. G. Jones. 1996. Resistance gene-dependent plant defense responses. **Plant Cell** 8: 1773 – 1791.

Hamvas, A., D. J. Wegner, C. S. Carlson, K. R. Bergmann, M. A. Trusgnich, L. Fulton, Y. Kasai, P. An, E. R. Mardis, R. K. Wilson and F. S. Cole. 2007. Comprehensive genetic variant discovery in the surfactant protein B gene. **Pediatr. Res.** 62(2):170-175.

Handelsman, J. and E. V. Stabb. 1996. Biocontrol of soilborne plant pathogens. **The Plant Cell** 8: 1855-1869.

Hartman, G. L., J. B. Sinclair and J. C. Rupe. 1999. **Compendium of Soybean Diseases,** 4 th ed. American Phytopathological Society, St. Paul, MMinnesota.

Herbert, H. 1999. Advances in protein solubilization for two-dimensional electrophoresis. **Electrophoresis 20**:660–663.

Hoffman, T., J. S. Schmidt, X. Zheng and A. F. Bent. 1999. Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. **Plant Physiol.**119: 935 – 950.

Huang, X., K. Stettmaier, C. Michel, P. Hutzler, M. J. Mueller and J. Durner. 2004. Nitric oxide is induced by wounding and influences jasmonic acid signalling in *Arabidopsis thaliana.* **Planta** 218: 938 – 946.

Hurkman, W. J. and C. K. Tanaka. 1996a. Effect of salt stress on germin gene expression in barley roots. **Plant Physiol.** 110: 971 – 977.

Hurkman, W. J. and C. K. Tanaka. 1996b. Germin gene expression is induced in wheat leaves by powdery mildew infection. **Plant Physiol.** 111: 735 – 739.

Idriss, E. E., O. Makarewicz, A. Farouk, K. Rosner, R. Greiner, H. Bochow, T. Richter and R. Borriss. 2002. Extracellular phytase activity of *Bacillus amyloliquefaciens* FZB45 contributes to its plant-growth-promoting effect. **Microbiol.** 148: 2097-2109.

Idriss, E. E., D. J. Iglesias, M. Talon and R. Borriss. 2007. Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by *Bacillus amyloliquefaciens* FZB42. **Mol. Plant-Microbe Interact.** 20(6): 619-626.

Jacques, P., C. Hbid, J. Destain, H. Razafindralambo, M. Paquot, E. De Pauw and P. Thonart. 1999. Optimisation of biosurfactant lipopeptide production from *Bacillus subtilis* S499 by Plackett-Burman design. **Appl. Biochem. and Biotech.** 77: 223–233.

Jalali B, S. Bhargava and A. Kamble. 2006. Signal transduction and transcriptional regulation of plant defence responses. **J. of Phytopathol.** 154 : 65–74.

Jeun, Y. C., K. S. Park, C. H. Kim, W. D. Fowler and J. W. Kloepper. 2004. Cytological observations of cucumber plants during induced resistance elicited by rhizobacteria. **Biol. Control**. 29: 34-42.

Jock, S., B. Volksch, L. Mansvelt and K. Geider. 2002. Characterization of *Bacillus* strain from apple and pear trees in South Africa antagonistic to *Erwinia amylovara*. **FEMS microbial. Lett.** 211(2): 247-252.

Kachroo, P., K. Yoshioka, J. Shah, H. Dooner and D. F. Klessig. 2000. Resistance to turnip crinkle virus in *Arabidopsis* requires two host genes and is salicylic acid dependent but *NPR1*, ethylene and jasmonate independent. **Plant Cell** 12: 677 – 690.

Kang, B. R., K. Y. Yang, B. H. Cho, T. H. Han, I. S. Kim, M. C. Lee, A. J. Anderson and Y. C. Kim. 2006. Production of indole-3-acetic acid in the plant-beneficial strain *Pseudomonas chlororaphis* O6 is negatively regulated by the global sensor kinase GacS. **Curr. Microbiol.** 52 (60): 473-476.

Kasem, S., C. Wataradee, J. Thaotamphitak and S. Prathuangwong, 2005. Efficacy of mixed beneficial bacteria and bio-products for induced resistance to bacterial pustule of soybean. **Proc. of the 7th Nat. Plant Protect. Con.** Thailand, 113-114.

Kearns, D. B. and Losick, R. 2003. Swarming motility in undomesticated *Bacillus subtilis*. **Mol. Microbiol.** 49(3): 581-590.

Khan, A. G. 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. **J. Trace Elem. Med. Biol.** 18(4): 355-364.

Kilic-Ekici, O. and G. Y. Yuen. 2003. Induced resistance as a mechanism of biological control by *Lysobacter enzymogenes* strain C3. **Phytopathol.** 93: 1103-1110.

Kilic-Ekici, O. and G. Y. Yuen. 2004. Comparison of strains of *Lysobacter enzymogenes* and PGPR for induction of resistance against Bipolaris sorokiniana in tall fescue. **Biol. Cont.** 30: 446-455.

Kim, S. T., S. G. Kim, D. H. Hwang, S. Y. Kang, H. J. Kim, B. H. Lee, J. J. Lee and K. Y. Kang. 2004. Proteomic analysis of pathogen-responsive proteins from rice leaves induced by rice blast fungus, *Magnaporthegrisea*. **Proteomics** 4: 3569-3578.

Kinsinger, R. F., M. C. Shirk and R. Fall. 2003. Rapid surface motility in *Bacillus subtilis* is dependent on extracellular surfactin and potassium ion. **J. Bacteriol**. 185(18): 5627-5631.

Kloepper, J.W. and Schroth, M.N. 1978. Plant growth-promoting rhizobacteria on radishes. In Proc. of the 4th Int. **Conf. on Plant Pathogenic Bacter** (Vol. 2), pp. 879–882, Station de Pathologie Ve´ge´tale et Phytobacteriologie, INRA, Angers, France

Kloepper, J.W., J. Leong, M. Teintze and M. N. Schroth. 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. **Nature** 286: 885-886.

Kloepper, J.W. and M. W. Schroth. 1981. Plant growth-promoting rhizobacteria under gnotobiotic conditions. **Phytopathol.**71: 642-644.

Kloepper, J.W., D. J. Hume, F. M. Scher, C. Singleton, B. Tipping, M. Laliberté, K. Frauley, T. Kutchaw, C. Simonson, R. Lifshitz, I. Zaleska and L. Lee. 1988. Plant growth-promoting rhizobacteria on canola (rapeseed). **Plant Dis.**72: 42-45.

Kloepper, J.W., R. Lifshitz and R. M. Zablotowicz. 1989. Free living bacterial inocula for enhancing crop productivity. **Trends in Biotechnol.** 7: 39-44

Kloepper J. W.1996. Host specificity in microbe-microbe interactions. **Bioscience** 46(6):406-409.

Kloepper, J. W., C. M. Ryu and S. A. Zhang. 2004a. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. **Phytopathol.** 94:1259-1266.

Kloepper, J. W., C. M. Ryu and S. A. Zhang. 2004b. The nature and application of biocontrol microbes: *Bacillus* spp.-induced systemic resistance and promotion of plant growth by *Bacillus* spp. **Phytopathol.** 94:1259-1266.

Kloepper, J.W. and C.M. Ryu. 2006. Bacterial endophytes as elicitors of induced systemic resistance. **Soil Biol.** 9: 33-52.

Konietzny, U. and R. Greiner. 2002. Molecular and catalytic properties of phytase degrading enzymes (phytases). **Int. J. Food Sci. Technol.** 37:791–812.

Kunst, F., N. Ogasawara, I. Moszer, A. M. Albertini, G. Alloni, V. Azevedo, M. G. Bertero, P. Bessières, A. Bolotin, S. Borchert, R. Borriss, L. Boursier, A. Brans, M. Braun, S. C. Brignell, S. Bron, S. Brouillet, C. V. Bruschi, B. Caldwell, V. Capuano, N. M. Carter, S. K. Choi, J. J. Codani, I. F. Connerton and A. Danchin. 1997. The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. **Nature** 390(6657):249-256.

Kumar, D. and D. F. Klessig. 2003. High-affinity salicylic acid binding protein 2 is required for plant innate immunity and has salicylic acid-stimulated lipase activity. **PNAS** 100: 16101 – 16106.

Kunkel, B.N. and D.M. Brooks. 2002. Cross talk between signaling pathways in pathogen defense. **Curr. Opin. in Plant Biol.** 5(4): 325-331.

Kutschera, U. and W. R. Briggs. 1987. Rapid auxin-induced stimulation of cell wall synthesis in pea internodes. **PNAS.** 84(9):2747-2751.

Kuwana, R., Y. Kasahara and M. Fujibayashi. 2002. Proteomics characterization of novel spore proteins of *Bacillus subtilis*. **Microbiol.** 148(12): 3971-3982.

Lai, E. M., N. D. Phadke, M. T. Kachman, R. Giomo, S. Vazquez, J. A. Vazquez, J. R. Maddock and A. Driks. 2003. Proteomic analysis of the spore coats of *Bacillus subtilis* and *Bacillus anthracis.* **J. Bacteiol.** 185 (4): 1443–1454.

Lam, E., N. Kato and M. Lawton. 2001. Programmed cell death, mitochondria and the plant hypersensitive response. **Nature** 411: 848 - 853.

Lambrecht, M., Y. Okon, A. V. Broek and J. Vanderleyden. 2000. Indole-3-aceric acid; a reciprocal signaling molecule in bacteria-plant interactions. **Sciences** 8(7):298-300.

Larsen, R., T. G. Kloosterman, J. Kok and O. P. Kuipers. 2006. GlnR-mediated regulation of nitrogen metabolism in *Lactococcus lactis***. J. Bacteriol.** 188(13): 4978–4982.

Lawton, K. A., L. Friedrich, M. Hunt, K. Weymann, T. Delaney, H. Kessmann, T. Staub and J. Ryals. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. **The Plant J.** 10: 71 – 82.

Leveau, J. H. and S. E. Lindow. 2005. Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. **Appl. Environ. Microbiol**. 71(5): 2365-2371.

Levine, A., R. Tenhaken, R. Dixon and C. Lamb. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. **Cell** 79: 583 - 593.

Lifshitz, R., J. W. Kloepper, F. M. Scher, E. M. Tipping and M. Laliberté. 1986. Nitrogen-fixing pseudomonads isolated from the roots of plants grown in the Canadian high arctic. **Appl. and Environ. Microbiol.** 51: 251-255.

Lynch, J.M. and Whipps, J.M. 1991. Substrate flow in the rhizosphere. In The Rhizosphere and Plant Growth (Keister, D.L. and Cregan, B., eds), pp. 15–24, **Beltsville Sympos. in Agric Res 14**, Kluwer, Dordrecht, The Netherlands

Maleck, K., A. Levine, T. Eulgem, A .Morgan, J. Schmid, K. A. Lawton, J. L. Dangl and R. A. Dietrich. 2000. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. **Nature Genetics** 26: 403 – 410.

Mantelin, S. and B. Touraine. 2003. Plant growth promoting-bacteria and nitrate available : impact on root development and nitrate uptake. **J. of Exper. Bot.** 1-8.

Martin, G. B., A. J. Bogdanove and G. Sessa 2003. Understanding the functions of plant disease resistance proteins. **Annu. Rev. Plant Biol.** 54: 23-61.

Martinez-Morales, L. J., L. Soto-Urzua, B. E. Baca and J. A. Sanchez-Ahedo. 2003. Indole-3-butyric acid (IBA) production in culture medium by wild strain *Azospirillum brasilense.* **FEMS Microbiol. Lett.** 228(2):167–173.

Mauch-Mani, B. and A. J. Slusarenko. 1996. Production of salicylic acid precursors is a major function of the phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Pernospora parasitica*. **Plant Cell** 8: 203 - 212.

McDowell, J. M. and J. L. Dangl. 2000. Signal transduction in the plant immune response. **Trends Biochem Sci.** 25:79-82.

Meziane, H., I. Van der Sluis, L. C. van Loon, M. Hofte and P. A. H. M. Bakker. 2005. Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. **Mol. Plant Pathol**. 6 : 177-185.

Miyazaki, J. H. and S. F. Yang. 1987. The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. **Physiol. of Plants** 69: 366 – 370.

Moerschbacher, B. M., U Noll, L. Gorrichon and H. J. Reisener. 1990. Specific inhibition of lignification breaks hypersensitive resistance of wheat to stem rust. **Plant Physiol.** 93: 465 - 470.

Monnet, V. 2003. Bacterial oligopeptide-binding proteins. **Cell and Mol. Life Sci.** 60(10): 2100-2114.

Mulosevic, N. and A. J. Slusarenko. 1996. Active oxygen metabolism and lignification in the hypersensitive response in bean. *Physiological and Molecular* **Plant Pathol.** 49: 143 – 158.

Naclerio, G., G. L. Baccigalupi, C. Caruso, M. De Felice and E. Ricca. 1995. *Bacillus subtilis* vegetative catalase is an extracellular enzyme. **Appl. Environ. Microbiol**. 61(12): 4471–4473.

Nehl, D.B., S. J. Allen and J. F. Brown. 1996. Deleterious rhizosphere bacteria: an integrating perspective. **Appl. Soil Ecol.** 5: 1-20.

Neupane F.P. and D.M. Norris. 1991. α-Tocopherol alteration of soybean antiherbivory to *Trichoplusia ni* larvae. **J. of Chem.Ecol.** 17(10): 1941-1951.

Nunkel, B. N. and D. M. Brooks. 2002. Crosstalk between signaling pathways in pathogen defense. **Curr. Opin. Plant Biol.** 5: 325-331.

O'gara, M. J., C. H. Lee, G. A. Weiberg, J. M. Nott and S. F. Queener. 1997. IMP dehydrogenase from *Pneumocystis carinii* as a potential drug target. **Antimicrob. agents Chemother.** 1: 40-48.

Oldroyd, G. E., E. M. Engstrom and S. R. Long. 2001. Ethylene inhibits the Nod factor signal transduction pathway of *Medicago truncatula*. **Plant Cell** 13: 1835 – 1849.

Ongena, M., E. Jourdan, A. Adam, M. Paquot, A. Brans, B. Joris, J. L. Arpigny and P. Thonart. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtillis* as elicitors of induced systemic resistance in plants. **Environ. Microbiol.** 9(4): 1084-1090.

Oosthuizen, M. C., B. Steyn and J. Theron. 2002. Proteomic analysis reveals Differential protein expression by *Bacillus cereus* during biofilm formation. **Appl. and Environ. Microbiol.** 68(6): 2770-2780.

Neupane F.P. and D. M. Norris. 1991. α-Tocopherol alteration of soybean antiherbivory to *Trichoplusia ni* larvae. **J. of Chem. Ecol.** 17(10), 1941-1951.

Pan, S.Q., X.S. Ye and J. Kuc. 1991. Association of β-1,3-glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. **Physiol.y Mol. Plant Pathol.** 39: 25–39.

Pare, P. W., M. A. Farag, V. Krishnamachari, H. Zhang, C. M. Ryu and J. W. Kloepper. 2005. Elicitors and priming agents initiate plant defense responses. **Photosynthesis Res.** 85: 149-159.

Park, K. S. and J. W. Kloepper. 2000. Activation of *PR-1a* promoter by rhizobacteria that induce systemic resistance in tobacco against *Pseudomonas syringae* pv. *tabaci*. **Biol. Control** 18: 2-9.

Park, D.-S., S. Landini, M. Y. Graham and T. L. Graham. 2002. Induced distal defence potentiation against *Phytophthora sojae* in soybean. **Physiol. Mol. Plant Pathol.** 60: 293-310.

Parr, J. F. and A. G. Norman. 1965. Considerations in the use of surfactants in plant systems: A review. **Botanical Gazette.** 26(20): 86-96.

Patten, C. L. and B. R. Glick. 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. **Appl. and Environ.** 68(8): 3795-3801.

Pelletier, A. and J. Sygusch. 1990. Purification and characterization of three chitosanase activities from *Bacillus megaterium* P1. **Appl. Environ. Microbiol**. 56(4): 844-848.

Pereira, M. C. B. and A. Mounteerd. 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. **Biol .Cont.** 29: 288-295.

Pfahler, P. L., H. F. Linskens, H. W. Schoot and M. Wilcox. 1981. Surfactant effects on petunia pollen germination *in vitro.* **Bulletin of Environ. Contamin and Toxicol.** 26(1): 567-570.

Pieterse, C. M. J., S. C. M. van Wees, E. Hoffland, J. A. van Pelt and L. C. van Loon. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. **Plant Cell** 8:1 225-1237.

Pieterse, C. M. J., S. C. M. van Wees, J. A. van Pelt, M. Knoester, R. Laan, N. Gerrits, P. J. Weisbeek and L. C. van Loon. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. **Plant Cell** 10: 1571-1580.

Pieterse, C., M. J., J. A. van Pelt, J. Ton, S. Parchmann, M. J. Mueller, A. J. Buchala, J.-P. Metraux and L. C. van Loon. 2000. Rhizobacteriamediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. **Physiol. Mol. Plant Pathol.** 57: 123-134.

Pieterse, C. M. J., J. A. van Pelt, S. C. M. van Wees, J. Ton, K. M. Leon-Kloosterziel, J. J. B. Keurentjes, B. W. M. Verhagen, M. Knoester, I. van der Sluis, P. A. H. M. Bakker and L. C. van Loon. 2001. Rhizobacteria-mediated induced systemic resistance: triggering, signaling and expression. **Eur. J. Plant Pathol.** 107: 51-61.

Pieterse, C. M. J., S. C. M. van Wees, J. Ton, J. A. van Pelt and L. C. van Loon. 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. **Plant Biol.** 4: 535-544.

Ping L. and B. Wilhelm. 2004. Signals from the underground: bacterial volatiles promote growth in *Arabidopsis.* **Trends in Plant Sci**. 9(6): 263-266.

Pozo, M. J., L. C. van Loon and C. M. J. Pieterse. 2004. Jasmonates-signals in plant–microbe interactions. **J. Plant Growth Regul.** 23: 211-222.

Prathuangwong, S. 1983. Soybean bacterial pustule research in Thailand, pp.40- 41. In G.J.Persly(ed). Tropical legume improvement, **ACIAR Proceeding Series No.8**. Oct.10 -12,1983. Bangkok

Prathuangwong, S. 1984. A simple technique for inoculation of *Xanthomonas campestris* pv. *glycines.* **Thai J. of Agr. Sci.** 17(3): 183-189.

Prathuangwong, S. and K. Amnuaykit. 1989. Studies on tolerance and rate reducing bacterial pustule of soybean cultivars/lines. **Kasetsart J(Nat Sci)** 21: 408-420.

Prathuangwong, S., C. Preecha and N. Thaveechai. 1993. Development standard method and format measuring severity of soybean bacterial pustule. **Proc. of the 6th Internat. of Plant Pathol Con.** Canada, 6.4.1.

Prathungwong, S., S. Saisangthong, A. Fareaendee and P. Chutuwantana. 1996. The incidence and new occurrence of soybean diseases at central area of Thailand during 1994-1996. **Proc. of the 6th Nat. Soybean Res. Con**., Sept. 3-6, 1996. Chiang Mai, Thailand. 242-258.

Prathuangwong, S. and S. Kasem. 2001. New bacterial antagonistic isolates as effectively as chemical control of soybean diseases. **Proc. of the 5th Nati. Plant protect. Con.** Thailand, 237-248.

Prathuangwong, S., S. Tawatphum and S. Kasem. 2002. Endophytic bacterial response to plant growth promoting rhizobactria and root rot suppression of soybean. **Proc. of the 1st Internat. Con. on Trop. and Subtrop. Plant Dis.** Thailand, 177.

Prathuangwong, S. and S. Kasem. 2003. Potential of new antagonists for controlling soybean bacterial pustule and reducing bactericide application. **Proc. of the 7th Internat. Cong. of Plant Pathogy.** New Zealand, 2, 11.

Prathuangwong, S. and S. Kasem. 2004. Screening and evaluation of thermotolerant epiphytic bacteria from soybean leaves for controlling bacterial pustule disease. **Thai J. Agr. Sci.** 37: 1-8.

Prathuangwong, S., S. Kasem, J. Thowthampitak and D. Athinuwat. 2004a. Multiple plant response to bacterial mediated protection against various disease. **J. of ISSAAS.** 11(3): 79-87.

Prathuangwong, S., J. Thowthampitak, S. Kasem and S. Tsuyumu. 2004b. New application strategies of thermotolerant bacteria for managing soybean diseases under farming production. **Proc. of the 4th JSPS-NRCT Joint Seminar on Devel. Microb. Res.** Japan, 118.

Prathuangwong, S., R. Detmanee and S. Kasem. 2005a. Potential roles of *Bacillus firmus* KPS46 filtrates and its secondary metabolites in controlling soybean bacterial pustule. **Proc of the 2nd Asian Con on Plant Pathol,** National University of Singapore, Singapore. 2D-1.

Prathuangwong, S., S. Kasem, N. Techati and P. Jutawantana. 2005b. Suppression of important diseases and improvement of maize through ISR bacteria. **Proc. of the 32th Nat. Corn and Sorghum Ann. Con.** Thailand, 182-193.

Prathuangwong, S., C. Preecha, S. Vudhivanich, S. Kasem, N. Buensanteai and W. Chuaboon. 2005c. Occurrence and biological control of cauliflower at Suphanburi. **Proc. of the ISSAAS Internat. Cong.** Vietnam, S1-10.

Prathuangwong S. and N. Buensanteai. 2007. *Bacillus amyloliquefaciens* induced systemic resistance against bacterial pustule pathogen with increased phenols, peroxides, and 1,3-β-glucanase in soybean plant. **Acta Phytopathol. et Entomol. Hungarica** 42: 321-330.

Prathuangwong S. and N. Buensanteai. 2008. *Bacillus amyloliquefaciens* KPS46 produces indole-3-acetic acid in culture medium and its effect on growth promotion via increased proteome expression and indole-3-acetic acid content in soybean plant**. Proc. of the APS centennial meeting 2008,** Minneapolis MN, USA. (Accepted)

Purrington, C. B. 2000. Costs of resistance. **Curr. Opin. Plant Biol.** 3: 305-308.

Rakwal, R., S. Tamgami, G. K. Agrawal and H. Iwahashi. 2002. Octadecanoid signaling component “burst” in rice (*Oryza sativa* L.) seedling leaves upon wounding by cut and treatment with fungal elicitor chitosan. **Biochem. and Biophy. Res.Commu.** 295(5): 1041-1045.

Ramamoorthy, V., T. Raguchander and R. Samiyappan. 2002a. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. **Eur. J. of Plant Pathol.** 108: 429–441.

Ramamoorthy, V., T. Raguchander and R. Samiyappan. 2002b. Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici*. **Plant Soil.** 239: 55-68.

Rao, M. V., H. Lee, R. A. Creelman, J. E. Mullet and K. R. Davis. 2000. Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. **The Plant Cell** 12: 1633 – 1646.

Rasmussen, J. B., R. Hammerschmidt. and M. N. Zook. 1991. Systemic induction of salicylic-acid accumulation in cucumber after inoculation with *Pseudomonas syringae* pv. *syringae*. **Plant Physiol.** 97: 1342-1347.

Rivas, L. A., V. Parro, M. Moreno-Paz and R. P. Mellado. 2000. The *Bacillus subtilis* 168 *csn* gene encodes a chitosanase with similar properties to a *Streptomyces* enzyme. **Microbiol.** 146: 2929-2936.

Rodionov, D. A., P. Hebbeln, M. S. Gelfand and T. Eitinger. 2006. Comparative and functional genomics analysis of prokaryotic nickel and cobalt uptake transporters: evidence for a novel group of ATP-binding cassette transporters. **J. Bacteriol.** 188(1): 317–327.

Rodriguez, H. and R. Fraga. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. **Biotechnol. Adv**. 17: 319–339

Rudner, D. Z., J. R. LeDeaux, K. Ireton and A. D. Grossman. 1991. The spo0K locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. **J Bacteriol.** 173(4):1388–1398.

Rustérucci, C., V. Stallaert, M. L. Milat, A. Pugin, P. Ricci and J. P. Blein. 1996. Relationship between active oxygen species, lipid peroxidation, necrosis, and phytoalexin production induced by elicitins in *Nicotiana.* **Plant Physiol.**111: 885 – 891.

Ryals, J. A., U. H. Neuenschwander, M. G. Willits, A. Molina, H. Y. Steiner and M. D. Hunt 1996. Systemic acquired resistance. **Plant Cell** 8: 1809-1819.

Ryan, C. J. and G. Pearce. 2004. **Peptide hormones: Plant hormones biosynthesis, signal transduction, action.** Kluwer Academic, Dordrecht, Boston.

Ryu, C. M., C. H. Hu, M. S. Reddy and J. W. Kloepper. 2003a. Different signaling pathways of induced resistance by rhizobacteria in *Arabidopsis thaliana* against two pathovars of *Pseudomonas syringae*. **New Phytol.** 160: 413-420.

Ryu, C. M., M. A. Farag, C. H. Hu, M. S. Reddy, P. W. Pare and J. W. Kloepper. 2003b**.** Bacterial volatiles promote growth in Arabidopsis. **PNAS.** 100(8): 4927-4932.

Ryu, C. M., M. A. Farag, C. H. Hu, M. S. Reddy, J. W. Kloepper and P. W. Pare. 2004a**.** Bacterial volatiles induce systemic resistance in Arabidopsis. **Plant Physiol.** 134: 1017-1026.

Ryu, C. M., J. F. Murphy, K. S. Mysore and J. W. Kloepper. 2004b. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against Cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. **Plant J.** 39: 381-392.

Saxena, D., E. Ben-Dov, R. Manasherob, Z, Barak, S. Boussiba and A. Zaritsky. 2002. A UV tolerant mutant of *Bacillus thuringiensis* subsp. *kurstaki* producing melanin. **Curr. Microbiol.** 25-30.

Schreier, H. J. and A. L. Sonenshein. 1986. Altered regulation of the glnA gene in glutamine synthetase mutants of *Bacillus subtilis*. **J. Bacteriol.** 167(1): 35-43.

Schmelzer, E., P. H .Schreier, R. H. Stocker and K. Stenzel. 1993. Disease resistance results from foreign phytoalexin expression in a novel plant. **Nature** 361: 153 - 156.

Segarra, G., Casanova E., Bellido D., Odena M. A., Oliveira E. and Trillas I. 2007. Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. **Proteomics** *7:* 3943–3952.

Senesi, S., F. Celondroni, S. Salvetti,D. J. Beecher, A. C. L. Wong and E. Ghelardi. 2002. Swarming motility in Bacillus cereus and characterization of a *fil*Y mutant impaired in swarm cell differentiation. **Microbiol.** 148: 1785-1794.

Shah, J. 2003. The salicylic acid loop in plant defense. **Curr. Opin. Plant Biol** 6: 365-371

Shen, S., Y. Jing and T. Kuang. 2003 Proteomics approach to identify wound-response related proteins from rice leaf sheath. **Proteomics.** 3: 527-535

Sibylle, M., L.W. Ericsson and M.P. Gordon. 1998. Quantification of jasmonic acid, methyl jasmonate, and salicylic acid in plants by capillary liquid chromatography electrospray tandem mass spectrometry. **Anal. Biochem.** 257(2): 186-194.

Silva, H.S.A., R.S. Romeiro, D. Macagnan, B.A. Halfeld-Vieira, M.C.B. Pereira and A. Mounteer. 2004. Rhizobacterial induction of systemic resistance in tomato plants non-specific protection and increase in enzyme activities. **Biol. Cont.** 29: 288–295.

Sinclair, J.B. and O.D. Dhingra. 1975. **An annotated bibliography of soybean disease** 1882-1974. **International Soybean Program**. University of Illinois, Urbama, Illinois.

Sinclair, J.B. 1982. **Compendium of Soybean Disease.** American Phytopathl. Society, St. Paul, MN. 104 p.

Slaymaker, D. H., D. A. Navarre, D. Clark, O. del Pozo, G. B. Martin and D. F. Klessig. 2002. The tobacco salicylic acid-binding protein 3 (SABP3) is the chloroplast carbonic anhydrase, which exhibits antioxidant activity and plays a role in the hypersensitive defense response. **PNAS** 9: 11640 – 1645.

Spaepen, S., J. Vanderleyden and R. Remans. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. **FEMS Microbiol. Rev.** 31: 425-448.

Steenhoudt, O. and J. Vanderleyden. 2000. Azospirillum, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. **FEMS Microbiol. Rev.** 24: 487–506

Sticher, L., B. Mauch-Mani and J. P. Metraux. 1997. Systemic acquired resistance. **Annu. Rev. of Phytopathol.** 35: 235-270.

Tien, T.M., M. H Gaskins and D. H. Hubbell.1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). **Appl. and Environ. Microbiol.** 37: 1016-1024.

Thaler, J.S., R. Kanban, D.E. Ullman, K. Baege and R.M. Bostock. 2004. Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. **Oecologia** 131(2): 227-235.

Thordal-Christensen, H., Z. Zhang, Y. Wei and D. B. Collinge. 1997. Subcellular localization of H_2O_2 in plants. H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. **Plant Journal** 11: 1187 – 1194.

Tjalsma, H., H. Antelmann and J. D. H. Jongbloed. 2004. Proteomics of protein secretion by *Bacillus subtilis*: separating the “secrets” of the secretome. **Microbiol. and Molecul. Biol. Rev.** 68(2): 207-233.

Underwood, N.C. 1998. The timing of induced resistance and induced susceptibility in the soybean-Mexican bean beetle system. **Oecologia** 114: 376-381.

Underwood, N., W. Morris, K. Gross and J.R. Lockwood. 2000. Induced resistance to Mexican bean beetles in soybean: variation among genotypes and lack of correlation with constitutive resistance. **Oecologia** 122: 83–89.

Vande Broek, A., M. Lambrecht, K. Eggermont and J, Vanderleyden. 1999. Auxins upregulate expression of the indole-3-pyruvate decarboxylase gene in *Azospirillum brasilense*. **J. Bacteriol.** 181: 1338–1342.

Vande Broek, A., P. Gysegom, O. Ona, N. Hendrickx, E. Prinsen, J. Van Impe and J. Vanderleyden. 2005. Transcriptional analysis of the *Azospirillum brasilense* indole-3-pyruvate decarboxylase gene and identification of a *cis*-acting sequence involved in auxin responsive expression. **Mol. Plant-Microbe Interact.** 18: 311–323.

van Loon, L. C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. **Eur. J. Plant Pathol.** 103: 753–765.

van Loon, L. C, P. A. H. M. Bakker and C. M. J. Pieterse. 1998. Systemic resistance induced by Rhizosphere bacteria. **Annu. Rev. of Phytopathol.** 36: 453–483.

van Loon, L. C. and E. A. van Strien. 1999. The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. **Physiol. Mol. Plant Pathol.** 55: 85–97.

van Loon, L. C. 2000. **Systemic induced resistance**. In : Mechanisms of resistance to plant diseases. Kluwer, Dordrecht, pp 521-574.

van Wees, S.C.M., M. Luijendijk, I. Smoorenburg, L.C. van Loon, C.M.J. Pieterse. 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene Atvsp upon challenge. **Plant Mol. Biol.** 41: 537–549.

van Wees, S. C. M., E. A. M. DeSwart, J. A. van Pelt, L. C. van Loon and C. M. J. Pieterse. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. **PNAS** 97:8711-8716.

van Wees, S. C. M. and J. Glazebrook. 2003. Loss of non-host resistance of *Arabidopsis NahG* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. **Plant J.** 33: 733 – 742.

Vauterin, L., B. Hoste, K.Kerters and J. Swings. 1995. Reclassification of Xanthomonas. Int. **J. Syst. Bacteriol**. 45: 472-489.

Verhagen, B. W. M., J. Glazebrook, T. Zhu, H. S. Chang, L. C. Van Loon and C. M. J. Pieterse. 2004. The transcriptome of rhizobacteria-induced systemic resistance in Arabidopsis. **Mol. Plant-Microbe Interact.** 17**:** 895-908.

Vernooij, B., L. Friedrich, A. Morse, R. Reist and R. Kolditz-Jawhar. 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. **Plant Cell** 6: 959–965.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. **Plant and soil.** 255: 571-586.

Voigt, B., T. Schweder, M. J. J. B. Sibbald, D. Albrecht, A. Ehrenreich, J. Bernhard, J. Feesche, K. H. Maurer, G. Gottschalk, J. M. van Diji and M. Hecker. 2006. The extracellular proteome of *Bacillus licheniformis* grown in different media and under different nutrient starvation conditions. **Proteomics** 6: 1704–1705.

Waldmuller, T., E.G. Cosio, H. Grisebach and J. Ebel. 2004 Release of highly elicitor-active glucans by germinating zoospores of *Phytophthora megasperma* f. sp. *glycinea.* **Planta** 188 (4): 1431-2048.

Walling, L.L. 2001. Induced resistance: from the basic to the applied. **Trends Plant Sci.** 6, 445–447

Wang, S. Z., J. S. Chen and J. L. Johnson. 1988. The presence of five *nifH*-like sequences in *Clostridium pasteurianum*: sequence divergence and transcription properties. **Nucleic Acids Res.** 16(2): 439-454.

Wang, D., X. Ding and P. N. Rather. 2001. Indole can act as an extracellular signal in *Escherichia coli*. **J Bacteriol.** 183:4210–4216.

Wang, K. L. C., H. Li and J. R. Ecker. 2002. Ethylene biosynthesis and signaling networks. **The Plant Cell** S131 – S151.

Wei, G., J.W. Kloepper, S. Tuzun. 1991. Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. **Phytopathol.** 81: 1508-1512.

Whistler, C. A., V. O. Stockwell and J. E. Loper. 2000. Lon protease influences antibiotic production and UV tolerance of *Pseudomonas fluorescens* Pf-5. **Appl. Environ. Microbiol.** 66(7): 2718-2725.

Wildermuth, M. C., J. Dewdney, G. Wu and F. M. Ausubel. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. **Nature** 414: 562 – 565.

Wohlgemuth, H., K. Mittelstrass, S. Kschieschan, J. Bender, H. J. Weigel, K. Overmyer, J. Kangasjarvi, H. Sandermann and C. Langebartels. 2002. Activation of an oxidative burst is a general feature of sensitive plants exposed to the air pollutant ozone. **Plant Cell Environ.** 25: 717-726.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. **Biochem. J.** 322: 681–692.

Xu, Y., P. F. L Chang, D. Liu, M. L. Narasimhan and K. G. Raghothama. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. **Plant Cell** 6: 1077–1085.

Xue, L., P.M. Charest and S.H. Jabaji-Hare. 1998. Systemic induction of peroxidases, 1,3-β-glucanases, chitinases, and resistance in bean plants by binucleate *Rhizoctonia* species. **Phytopathol.** 88: 359-365.

Yan, Z., M. S. Reddy and J. W. Kloepper. 2003. Survival and colonization of rhizobacteria in a tomato transplant system. **Can. J. of Microbiol.** 49(6): 383-389.

Yang, Y., J. Shah and D. F. Klessig. 1997. Signal perception and transduction in plant defense responses. **Genes Dev.** 11: 1621-1639.

Yedidia, I., N. Benhamou and I. Chet. 1999. Induction of defense responses in cucumber plants (C*ucumis sativus* L.) by the biocontrol agent *Trichoderma harzianum.* **Appl. Environ. Microbiol.** 65: 1061-1070.

Yoshida, K., K. Kobayashi, Y. Miwa, C. M. Kang, M. Matsunaga, H. Yamaquchi, S. Tojo, M. Yamamoto, R. Nishi, N. Oqasawara, T. Nakayama and Y. Fujita. 2001. Combined transcriptome and proteome analysis as a powerful approach to study genes under glucose repression in *Bacillus subtilis*. **Nucleic Acids Res.** 29(3): 683-692.

Zavahir, J. S. and G. Seneviratne. 2007. Potential of developed microbial biofilms in generating bioactive compounds. **Res. J. Microbiol.** 2: 397–401.

Zdor, R.E. and A.J. Anderson. 1992. Influence of root colonizing bacteria on the defence responses in bean. **Plant and Soil** 140: 99–107.

Zehnder, G. W., J. F. Murphy, E. J. Sikora and J. W. Kloepper. 2001. Application to rhizobacteria for induced resistance. **Eur. J. Plant Pathol.** 107: 39-50.

Zhang, S., M. S. Reddy and J. W. Kloepper. 2004. Tobacco growth enhancement and blue mold disease protection by rhizobacteria: Relationship between plant growth promotion and systemic disease protection by PGPR strain 90-166. **Plant Soil** 262:277-288.

Zhou, T. and T. C. Paulitz. 1994. lnduced resistance in the biocontrol of *Pythium aphanidermatum* by *Pseudomonas spp.* on cucumber. **J. Phytopathol.** 142: 51-63.

Zieslin, N. and R. Ben–Zaken. 1993. Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. **Plant Physiol. Biochem.** 31: 333–339.

APPENDIX

1. Recipes of media in this study

1.1 Nutrient glucose agar (NGA) and nutrient broth (NGB)*

	per L
Beef extract	3.0 g
Bacto peptone	5.0 g
Glucose	2.5 g
Agar	15.0 g

Nutrient agar (NA) or nutrient broth may be purchased in dehydrated form from Difco, * Do not add agar if nutrient broth is desired.

1.2 Nutrient-broth yeast extract agar (NBY) and nutrient-broth yeast extract broth*

	per L
Nutrient broth	8.0 g
Yeast extract	2.0 g
K_2HPO_4	2.0 g
KH_2PO_4	0.5 g
Glucose	2.5 g
Agar	15.0 g

After autoclaving, add 1.0 ml of a sterile solution of 1M $MgSO_4$. $7H_2O_2$ * Do not add agar if nutrient broth is desired.

1.3 Luria bertani (LB) agar and Luria bertani broth *

	per L
Bacto typtone	10.0 g
Yeast extract	5.0 g
NaCl	10.0 g
Agar	15.0 g

Do not add agar if nutrient broth is desired.

1.4 N-free Hoagland's Nutrient Solution (Hoagland and Boyer, 1936)

Stock solutions were prepared as follows :

Macronutrients Stock solutions

	per L
1M KH_2PO_4	136.09 g
0.5M K_2SO_4	87.135 g
1M $MgSO_4.7H_2O$	246.48 g

Micronutrients

Boric acid	1.00 g
Manganous chloride	1.00 g
Zinc sulfate	0.58 g
Cupric sulfate	0.13 g
Sodium molybdate	0.10 g
Iron stock solution:	20 g

The final medium contained:

KH_2PO_4	2 ml of stock
K_2SO_4	4 ml of stock
$CaSO_4$	1 g of stock
$MgSO_4.7H_2O$	1 ml of stock
Microstock	1 ml of stock
IRON	1 ml of stock

The pH was adjusted to 7.0 using 0.5 M KOH and sterilized for 20 minutes at 121^0C for 15 minutes.

1.5 Salkowski's Reagent (Gordon and Weber – 1951)

150 mL concentrated Sulphuric acid

250 mL distilled water

7.5 mL (0.5M) $FeCl_3.6H_2O$

1.6 DF salts minimal medium utilized for indole production (Dworkin and Foster-1958)

per L

KH_2PO_4	4.0 g
Na_2HPO_4	6.0 g
$MgSO_4.7H_2O$	0.2 g
$FeSO_4.7H_2O$	0.001 (Stock solution of 100 mg/10 ml)
Glucose	2.0 g
Gluconic acid (K salt)	2.0 g
Citric acid (Tri-Na salt)	2.0 g
$(NH_4)2SO_4$	2.0 g

Dissolved in 1000 ml of distilled H2O

Micro-nutrients (Stock solution: 0.1ml l-1 was added to above DF salts minimal medium)

H_3BO_3	10 mg
$MnSO_4$	11.2 mg
$ZnSO_4$	124.6 mg
$CuSO_4$	78.2 mg
MoO_3	78.2 mg

Dissolved in 1000 ml of distilled H2O

1.7 Minimal medium + Glucose (Salamone 2000)

Component 1:

	per L
KH_2PO_4	1.36 g
K_2HPO_4	1.74 g

in 408 ml of deionized water.

Component 2:

$MgSO_4$	0.5 g
NH_4Cl	1.0g

in 572 ml of deionized water

The two components were autoclaved separately to avoid formation of a phosphate precipitate and mixed after autoclaving. Forty milliliters of glucose 25 g dissolved in 100 ml of distilled water and filter sterilized was added to the minimal medium.

1.8 YEDP (Phosphate solubilizing medium)

	per L
Yeast extract	5 g
Dextrose	10 g
Calcium phosphate	2 g
Agar	12 g