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Full Length Research Paper

Isolation and identification of N-acylhomoserin lactone degrading bacteria from potato rhizosphere

Esmaeil Mahmoudi[1]*, Nader Hasanzadeh[1], Badraldin Ebrahim Sayed Tabatabaei[2] and Vittorio Venturi[3]

[1]Department of Plant Pathology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Biotechnology, Faculty of Agriculture, Isfahan University of Technology, Isfahan, Iran.
[3]Bacteriology Group, International Center for Genetic Engineering and Biotechnology, Trieste, Italy.

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In many Gram-negative bacteria, including a number of pathogens such as *Pectobacterium carotovorum*, virulence factor production and many functions are linked to the quorum sensing (QS) systems that use diffusible *N*-acyl homoserine lactones (AHLs) as intercellular messenger molecules. A number of organisms also contain genes that hydrolyze AHLs into inactive products, thereby blocking the quorum-sensing systems. In this study, the diversity of bacteria that inactivate N -AHL signal in soil and potato rhizoshere was investigated. Among 139 isolated strains, eighteen N-AHL degrading isolates were finally identified as genera *Bacillus*, *Arthrobacter*, *Mesorhizobium*, *Pseudomonas* and *Streptomyces* using polyphasic approaches. All these isolates were capable to degraded both synthetic and natural N-AHL produced by *Pectobacterium atrosepticum* strain SM1. In quenching experiments selected isolates, especially *Bacillus* sp. EM84, were markedly reduced the pathogenicity of PaSM1 in potato tubers and totally suppressed tissue maceration on potato tubers. These results reveal the diversity of the QS interfering bacteria in the rhizosphere and demonstrate the validity of targeting QS signal molecules to control pathogens with natural bacterial isolates.

Key words: Quorum sensing, acyl-homoserin lactone, quorum quenching, biocontrol.

INTRODUCTION

Bacteria have evolved sophisticated mechanisms to coordinate gene expression at population and community Levels. For instance, gene expression may depend upon the perception of diffusible molecules that is synthesized by bacterial populations and communities. Because the concentration of the emitted signal in a confined environment reflects the bacterial cell number and density, such a regulatory pathway was termed 'quorum sensing' (QS) (Fuqua et al., 1994). In general, each individual bacterial cell produces a basal level of QS signals, which accumulate to a concentration threshold as the cells proliferating and interact with their cognate transcription factors to activate gene expression. Several groups of QS signals have already been identified among *N*- acyl homoserine lactones (AHLs) which are a family of QS signals has been identified in many Gram- negative bacteria such as Proteobacteria. AHL mediated quorum sensing regulates the expression of many genes responsible for biofilm formation, bioluminescence, production of pigments, pathogenicity, siderophore production, plasmid conjugal transfer, production of antibiotics and antifungal compounds and swarming (Whitehead et al., 2001).

Many authors proposed that this finding has allowed the discovery of alternative methods to control bacterial infections without using growth inhibitors such as antibiotics that enable the appearance of resistance (Dong et al., 2007; Sperandio, 2007). AHL signaling system could be regarded as a promising target for developing novel approaches to control bacterial infections, that is, to paralyze quorum- sensing of bacterial pathogens through inactivation of QS systems (Cirou et al., 2010). The term

*Corresponding author. E-mail: e.mahmoudi@khuisf.ac.ir. Tel: 098-03115354002-8. Fax: 098-03115354016.

quorum quenching (QQ) encompasses various natural phenomena or engineered procedures that lead to the perturbation and eventually the attenuation of the expression of QS-regulated functions (Dong and Zhang, 2005; Rasmussen and Givskov, 2006; Williams, 2007). Several anti-QS mechanisms have been identified in recent years (Cirou et al., 2010). AHL antagonists have been found to interfere with bacterial QS signaling, by inducing accelerated degradation of the AHL-dependent transcription factor. Different types of AHL-degrading enzymes have been described which can affect these signal molecules (Dong and Zhang, 2005; Turovskiy et al., 2007). Furthermore, several rhizobacteria belonging to different genera from gram-positive bacteria (e.g. *Arthrobacter*, *Bacillus*, *Rhodococcus, Streptomyces,…*) and gram negative bacteria (e.g. *Agrobacterium*, *Comamonas*, *Klebsiella*, *Pseudomonas, Ralstonia,…*) have been identified that they can produced N- AHLs degrading enzymes and interfering QS- regulated functions (Angelo-Picard et al., 2005; Faure and Dessaux, 2007).

Pectobacterium atrosepticum and *Pectobacterium carotovorum* are plant pathogenic bacteria responsible for diseases characterized by a maceration of the tissues, such as the black leg disease of potato, or the soft rot disease of carrot or melon. The pathogens are of major commercial importance as they are responsible for millions dollars loss of the potato crop in the world (Toth and Birch, 2005). The maceration occurs as the result of a set of bacterial enzymes such as cellulase, pectate lyases and pectine methyl esterase, the activities of which disrupt the pecto-cellulose wall of the plant cells (Toth and Birch, 2005; Grant et al., 2006) . Production of virulence factors in *Pectobacterium* (maceration enzymes, harpin and carbapenem antibiotic) are controlled by N-AHSL-dependent QS system that relies upon 3-oxo hexanoyl- N-homoserine lactone (3-oxo C6-HSL) or octanoyl homoserine lactone (C8-HSL) as the main signals (Whitehead et al., 2001; Von Bodman et al., 2003; Barnard and Salmond, 2007). Targeting the QS regulatory elements to develop biocontrol strategies for *Pectobacterium* species is therefore a pertinent option (Dong et al., 2000; Smadja et al., 2004; Liu et al., 2008). Two research strategies have already been developed: one aimed at producing transgenic plants interfering with QS, the other at isolating plant-associated bacteria naturally interfering with QS in *Pectobacterium*. Genetically modified *P. carotovorum* expressing AHL-lactonase or AHL- acylase showed decreased production of virulence factors and attenuated virulence of *P. carotovorum* (Reimmann et al., 2002; Lin et al., 2003; Dong et al., 2000). Plants expressing AHL-lactonase quenched pathogen QS signaling and showed significantly enhanced resistance to *P. carotovorum* infection (Dong et al., 2002). The aims of this work were: (i) to isolate and identify bacteria inhabiting the potato rhizosphere that are capable of degrading N-AHL molecules; (ii) to evaluate their N-AHL degradation pattern; (iii) to develop effective biocontrol strategies against soft rot disease of potato as the ultimate goal.

MATERIALS AND METHODS

Bacterial strains, media and culture conditions

Aside from bacterial strains isolated from potato rhizosphere in this study, *P. atrosepticum* strain SM1 (Kindly provided from Islamic Azad University- Science and Research Branch, Iran) was used as the source of naturally produced AHL molecules. *Chromobacterium violaceum* CV026 (McClean et al., 1997) (provided by Vittorio Venturi, ICEGB, Area Science park, Italy) and *Agrobacterium tumefaciens* NT1 (Shaw et al., 1997) (provided by Yves Dessaux, CNRS, Gif-sur-Yvette, France) were used as the indicator strains for AHLs detection.

The media used were Luria- Bertani (LB), King's-B (KB) (Schaad et al., 2001) and AB minimal medium, which was supplemented when necessary with 2% mannitol (Chilton et al., 1974) or with cycloheximide (50 $\mu g\ l^{-1}$). The bacteria were grown at 27 °C, except for biosensors and *P. atrosepticum* SM1, which were grown at 28 and 25°C respectively. All AHLs standard that used in this study were purchased from Sigma (Sigma-Aldrich, Inc., St. Louis, Mo., USA).

Isolation of bacterial strains

Soil samples and potato roots were collected from potato field cultivated for commercial purposes in Iran. One gram of soil sample or roots with adhering soil was used for isolating of culturable bacteria. Soil or rhizospheric samples were resuspended in 10 ml of sterile 0.8% NaCl by very vigorous shaking for 3 min, and the resulted suspension was serially diluted. Appropriate dilutions were spread on King-B medium Agar and LB medium (1% tryptone, 0.5% yeast extract and 0.5% NaCl) containing 50 $\mu g\ mL^{-1}$ cycloheximide for isolation of florescent *Pseudomonas* and total culturable bacteria respectively. Plates were incubated in the dark at 27ºC for 72 h. Thereafter, For AHL-degradation screening, the bacterial colonies were randomly picked from different media, grown to pure cultures, and kept as frozen stocks in glycerol medium at -80°C.

Screening of bacterial isolates for N-AHSL degradation activity

Because N-AHSL are sensitive to alkaline pH (Yates et al., 2002), all degradation assays were done in AB and LBm media that were buffered to pH 6.5 by addition of 100 mM KH_2PO_4/K_2HPO_4. Individual colonies (taken from frozen stocks spread onto LB plates) were inoculated in 5 ml LB medium supplemented with each one of A-HSL standard with following concentration: 5 $mg\ l^{-1}$ C6- HSL, 5.7 $mg\ l^{-1}$ C8-HSL, 6 $mg\ l^{-1}$ C12-HSL, and 6 $mg\ l^{-1}$ C14-HSL. Cultures were incubated at 27°C for 24 h with shaking. A control experiment involving non-inoculated degradation medium processed as for the inoculated media was performed at the same time as the degradation assays. After this time, bacterial cells were removed by centrifugation at 12000 rpm for 5 min. The culture supernatant was extracted twice with equal volumes of ethyl acetate. The organic phase was taken to dryness under an evaporator. Residues were redissolved in 50 μl volumes of ethyl acetate and store at -20°C. Component in the ethyl acetate extracts were separated by chromatography on C_{18}-reversed phase plate (Sigma Aldrich, Inc., St. Louis, Mo., USA, Cat.no. Z265446) with a solvent system of methanol-water (60:40, vol/vol) at room temperature. After development, the solvent was evaporated, and the dried plates were overlaid with a culture of the biosensors bacteria as

described previously by Shaw et al. (1997) and McClean et al. (1997).

AHL production by *Pectobacterium atrosepticum*

P. atrosepticum strain SM1 was streaked as homogeneous line on LB medium and biosensor strain, *C. violaceum* CV026, was spotted at a distance of 6 to 7mm from the PaSM1 line. After incubation at 28°C for 24 h, appearance of violet pigment in CV026 colony revealed the production of violacein by CV026 as well as production of N-AHL by PaSM1.

Degradation ability of natural N-AHL produced by *Pectobacterium atrosepticum*

P. atrosepticum strain SM1 was inoculated in 5 ml LB medium and was incubated at 25°C by shaking for 24 h. After this time, bacterial cells were removed by centrifuge at 12000 rpm for 5 min. Culture supernatant was extracted twice by equal volume ethyl acetate. Residue was added to fresh LB medium and selected bacteria were inoculated in this medium. Bacterial cultures were incubated at 27°C for 20 h and AHL residue was detected as earlier described.

Identification of the selected isolates

To identify the bacterial species, the DNA coding regions for the 16S rRNA of each isolate were amplified by PCR using the universal primers pA (5´-AGAGTTTGATCCTGGCTCAG) and pH (5´-AGGAGGTGATCCAGCCGCA), which allowed the amplification of almost the entire gene (Bruce et al., 1992). DNA extraction for strains was performed as described previously by Manzano et al. (2003). Polymerase chain reactions were performed in a total reaction volume of 50 µl containing 1× PCR buffer, 100 µM of each dNTP, 1.5 mM $MgCl_2$, 0.1 µM primers, 100 ng of DNA extract and 1 U of Taq DNA Polymerase (Cinagene, IRIB, Cat.no. SN-560011). The following temperature cycle was used: an initial denaturation step of 5 min at 95°C followed by 30 cycles of 1 min denaturation at 95°C, 1 min annealing at 56°C and 1 min 30 s extension at 72°C and a final extension step of 5 min at 72°C. The amplification yielded a product of ca. 1500 bp which was analyzed by electrophoresis on 0.8% agarose gel and then by staining with ethidium bromide (Sambrook et al., 2001). The resulted PCR products were sequenced by an BigDye Terminator and ABI Prism 3700 Genetic Analyzer (Macrogene, World Meridian Venture Center, Korea), and at least 400 bp were subjected to the BLAST analysis within the NCBI database. Though some sequence comparisons authorized identification of isolates at the species level, the only genus level was retained in this study for homogeneity. Additionally some phenotypic characteristics of bacterial isolates such as gram reaction, cell morphology were determined.

Inhibition of the pectinolytic activity of *P. atrosepticum* on potato tubers

The assay was performed on potato tubers (cv. Agria) as described by Lojkowska et al. (1995). Potato tubers were washed and surface sterilized by two consecutive incubations with sodium hypochlorite (1% chlorine deg.), extensively rinsed with sterile water. The tubers were dried under sterile conditions and then were sprayed using 70% ethanol. They were dried again and were kept for co-inoculation method.

Strains used in this assay were *P. atrosepticum* SM1 (as pathogen) and EM1, EM84, EM18 and EM12 as biocontrol agents (quenchers). Strains were cultured overnight at 27°C in LB medium, suspended and diluted in sterile 0.8% NaCl. Each tuber was inoculated with 20 µl of bacterial suspension including pathogen alone, pathogen with the quencher and quencher alone. three potato tubers were used for each combination of strains. The experiments were repeated twice. After inoculation, the potato tubers were incubated in a moist chamber (over 90% humidity) at 25°C. Two days after infection, the tubers were cut in the middle and the results were assessed by visual inspection and photographed.

RESULTS

Screening of bacterial isolates degrading N-AHLs

Degradation of synthetic N-AHL was evaluated using *C. violaceum* strain CV026 and *A. tumefasciens* strain NT1 for short chain and long chain carbon N- AHLs respectively. Among 138 bacterial strains isolated from 65 rhizosphere and root samples, eighteen isolates completely degraded 5 mgl^{-1} of C6 HSL after 24 h indicated by the absent of violacein induction in the CV026 biosensor (Figure 1). For characterization of the N-AHL degradation pattern of selected isolates, four synthetic unsubstituted N- AHSL (described in material and methods) and cell culture extract of *P. atrosepticum* strain SM1 as natural N-AHL were used. The detection of remaining N-AHL molecules was performed after 24 h in LB and mannitol supplemented AB buffered media (Table 1; Figure 2) . Results revealed that all tested strains degraded all N-AHLs types under same conditions with high or low degradation activity. In fact, the degradation properties of the various strains differed with respect to their substrate preferences. For instance, EM1, EM2, EM36-1 and EM84 strains completely degraded all N-AHL as well as culture extract of PaSM1 after 24 h. These isolates were identified as *Bacillus* sp., which had previously shown high N- AHL degrading activity (Dong et al., 2000, 2002). EM12, EM40 and EM67 strains also completely degraded 6mgl^{-1} C12 and C14-HSL after 20h. However, some remained of C6 and C8-HSL in cell culture supernatant indicating by slightly production violet pigment induced by CV026 biosensor, revealed that these strains could not completely degrade 5 mgl^{-1} of short chain N-AHLs. Additionally, all tested strains excluding EM12, EM40, EM67 and EM113 thoroughly degraded natural N-AHL produced by *P. atrosepticum* SM1. The results indicated that all isolates could degrade the various structures of AHLs however; the efficacy of each isolate could be varied compared to others.

Identification of the AHL degrading isolates

To Identify the bacterial species, 16s rRNA region were amplified by PCR using pA and pH primers (Figure 3). DNA sequences were compared to those found in the DNA Data Bank using the online FASTA search engine (http://www.ncbi.nlm.nih.gov). All the sequences

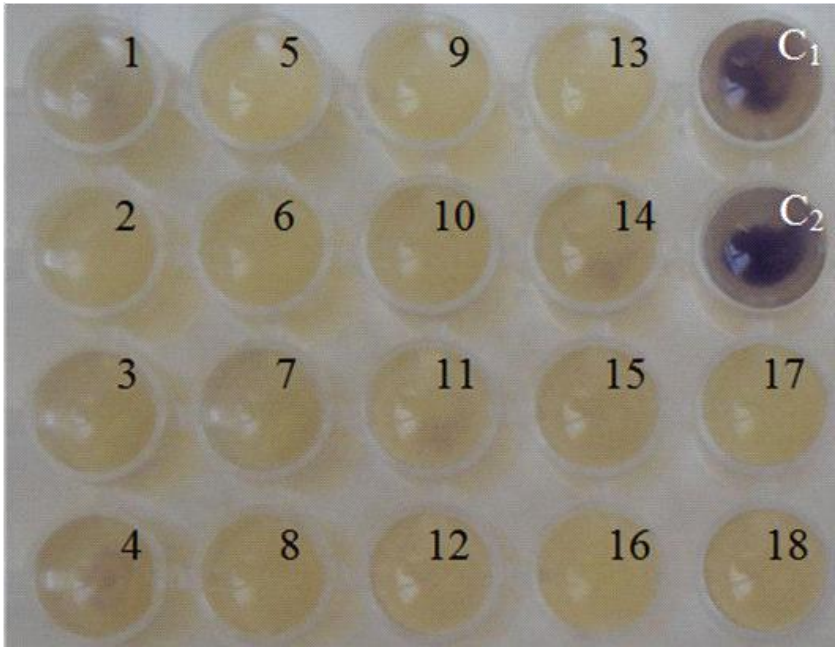

Figure 1. Detection of N- AHL-degrading isolates. The N-AHL-degrading isolates were detected as they ability to inhibition the synthesis of violacein by *Chromobacterium violaceum* CV026 in the presence of C6- HSL at 6mgL^{-1}. The eighteen N-AHL-degrading isolates are numbered from 1 to 18. C_1 and C_2 (control): degradation assay performed without bacteria. Numbers 1 to 18 respectively are strains: EM1, EM2, EM36-1, EM84, EM93, EM60, EM73, EM85, EM133, EM37, EM113, EM101, EM18, EM22, EM128, EM40, EM12 and EM67. The picture was taken after 24 h incubation.

exhibited similarity between 80 to 100% with known genera. Alignment with the Gene Bank Data base indicated that the isolates belong to four different genera. Nine isolates including EM1, EM2, EM36-1, EM60, EM84, EM85, EM93, EM73 and EM133 showed high similarity to *Bacillus* species. In the genus *Bacillus*, the *Bacillus cereus* group, which includes *Bacillus anthracis*, *B. cereus* and *Bacillus thuringiensis*, showed AHL degrading activity and carried lactonase gene; *aiiA* (Dong et al., 2000). The aiiA gene responsible for AHL degrada-tion in *Bacillus* sp. and it appears to be widely distributed among *Bacillus* strains (Dong et al., 2000, 2002; Lee et al., 2002). Isolates EM12, EM40 and EM67 showed 95% similarity to *Pseudomonas* species, have been found to degrade AHLs. In this research, we isolated three strains of *Arthrobacter*, which included EM18, EM22 and EM128, as AHL degrading bacteria from potato rhizosphere. Two of the strains, EM101 and EM113, were identified as *Streptomyces*, a species that has been already described as capable of N-AHL degradation with long chain degradation preference (Park et al., 2005). The genus *Mesorhizobium* is a nitrogen-fixing symbiotic bacterium that cans degrade AHLs and related compounds (Funami et al., 2005). In this research, we isolated one strain, EM37, with AHL degrading activity, which showed high similarity to *Mesorhizobium* species.

Biocontrol of *P. atrosepticum* by AHL degrading bacteria

The biocontrol activity of tested isolates (Table 1) was performed against PaSM1, in which the virulence is regulated by QS with 3oxo-C8HSL as an essential signal (Smadja et al., 2004). Inoculation of potato tubers with *P. atrosepticum* SM1 resulted in extensive tissue macera-tion. Co-inoculation of PaSM1 with AHL degrading bacteria provide substantial reduction in tissue maceration compared to the pathogen alone (Figure 4). The biocontrol activity of the *Bacillus* sp. EM84 was more effective than antagonistic activity of the other tested isolates. Co- inoculation of *Bacillus* sp. EM84 as quencher produced a significant reduction of tuber maceration compared to the tuber rot area when potato tubers were inoculated with the pathogen alone as well as other tested bacteria. Additionally, the ability of PaSM1 to macerate potato tubers tissue was attenuated by EM18 (known as *Atrhrobacter* sp.) when co-inoculated with PaSM1 at 10^6 cfu per ml (Figure 4).

DISCUSSION

The microenvironment of the rhizosphere is relatively rich

Table 1. Properties of N-AHL degrading bacteria isolated from potato rhizosphere.

strains	*rrs* sequencing identification	Genebank Acc. no.	Gram	Colony and cell morphology	Degradation ability[a]					Effect on maceration ability of PaSM1[c]
EM1	*Bacillus* sp.	EU977693.1	+	White, rod shape, motile	++	++	++	++	++	++
EM2		HM748447.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM36-1		HM776218.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM84		HM748447.1	+	White, rod shape, motile	++	++	++	++	++	++
EM93		EU240440.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM60		FJ866758.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM73		AY948211.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM85		D26185.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM133		HM188452.1	+	White, rod shape, motile	++	++	++	++	++	Nd
EM37	Mesorhizobium	AF410896.1	-	white, rod shape, motile	++	++	++	++	++	Nd
EM113	Streptomyces sp.	HM748050.1	+	White, like filamentous, non-motile	+	+	++	++	+	Nd
EM101		GQ036453.1	+	White, like filamentous, non-motile	+	+	++	++	+	Nd
EM18	*Arthrobacter* sp.	AY444858.1	+	White to grayish, rod, non-motile	++	++	+	+	++	++
EM22		AY731366.1	+	White to grayish, rod, non-motile	++	++	+	+	++	Nd
EM128		AY635865.1	+	White to grayish, rod, non-motile	++	++	+	+	++	Nd
EM12	*Pseudomonas* sp.	AJ969084.1	-	White, rod, fluorescent on King-B, motile	+	+	++	++	+	+
EM40		HM134250.1	-	White, rod, fluorescent on King-B, motile	+	+	++	++	+	Nd
EM67		AY303294.1	-	White, rod, fluorescent on King-B, motile	+	+	++	++	+	Nd

a: AHL degrading ability was performed as described in methods.
b: *P. atrosepticum* strain PaSM1 used as natural N-AHL production source, +: low degrading ability, ++: high degrading ability
c: effect on potato tubers maceration ability of *P. atrosepticum* SM1, +: weak, ++: strong, Nd.: not determined.

in nutrient substances exuded by the plant and, therefore, is inhabited by many different bacterial species. The composition of specific root exudates varies depending on the plant species, genus, cultivar, and growth stage and determines the microbial communities that colonize the roots (Savka et al., 2002). The first aim of this work was isolating and identifying bacterial population of potato rhizosphere that degrades N-AHLs molecules. Out of 138 isolates, which analyzed in this research, only eighteen strains were capable of degrading N-AHLs. Two explanations may be proposed to account for disability of the other isolates not degrading the QS signaling molecules. Firstly, they might exhibit very slow degradation that was not revealed by our experimental procedures. Second, they may have grown at the expense of some of the N-AHL degradation products generated and released into media by the true degrader, as reported for the couple *Arthrobacter/Variovorax* (Flagan et al., 2003; Uroz et al., 2003).

Bacteria that inactivate N-AHLs signal molecules are taxonomically diverse (α,β, protobacteria, firmicutes and actinobacteria) and may represent 10 to 20% of total cultivable bacteria in soil (Dong et al., 2000; Jafra et al., 2006) . This work allowed the isolation of many more strains degrading N-AHL than described in previous reports. Out of 138 analyzed isolates, eighteen N-AHL degrading isolates were finally identified using a polyphasic approaches (ribotyping, Gram determination, morphology examination, ect.). They fell within the genera belonging to the α-Proteobacteria (that is *Mesorhizobium*, Funami et al., 2005), the -

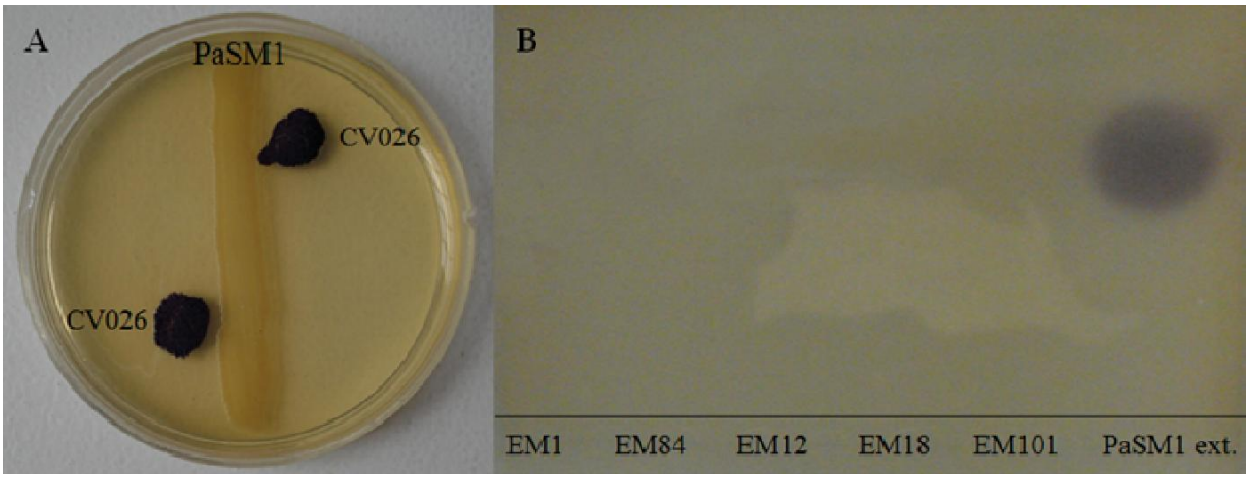

Figure 2. (A) N-AHL production by *Pectobacterium atrosepticum* strain PaSM1. Test strain was streaked as a homogeneous line on LB medium and biosensor strain, *Chromobacterium violaceum* CV026, was spotted at 10 mm from the test strain. Development of violet pigment in CV026 colonies revealed the production of violacein by CV026 as well as production of N-AHL by PaSM1. (B) Thin layer chromatogram for degradation of N-AHL produced by *P. atrosepticum* PaSM1. (EM1 and EM84: *Bacillus* sp.; EM12: *Pseudomonas* sp.; EM18: *Arthrobacter* sp.; EM101: *Streptomyces* sp.; PaSM1: natural AHL extracted from culture media containing *P. atrosepticum* PaSM1).

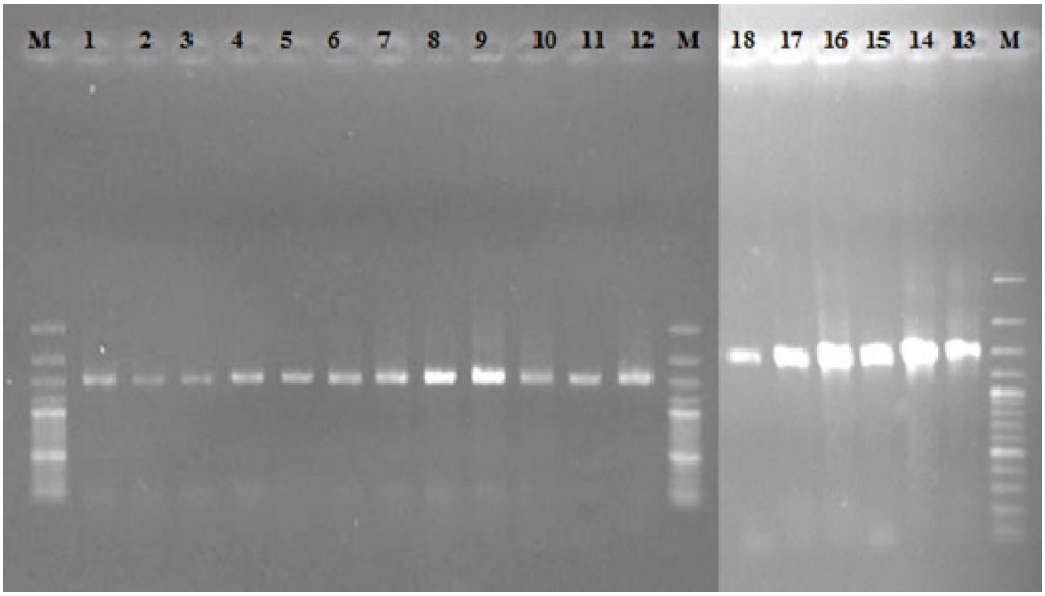

Figure 3. PCR amplification of 16s rRNA region (about 1500 bp) from AHL degrading rhizobacteria using universal primers pA/pH. Numbers 1 to 18 respectively are strains: EM1, EM2, EM36-1, EM84, EM93, EM60, EM73, EM85, EM133, EM37, EM113, EM101, EM18, EM22, EM128, EM40, EM12 and EM67; M: 100bp DNA Ladder.

Proteobacteria, *Pseudomonas* (Molina et al., 2003), the low-G+C Gram-positive bacteria, *Bacillus* (Dong et al., 2002; Lee et al., 2002) and the high-G+C Gram- positive bacteria, *Streptomyces* and *Arthrobacter* (Park et al., 2003). The isolates degrading N-AHL were essentially strains of *Bacillus* sp. (about 50%), while four other genera were isolated at much lower frequencies (*Arthrobacter* 16%; *Streptomyces* 12%; *Mesorhizobium* 6% and *Pseudomonas* 16%) . Previous studies revealed that *Bacillus*, *Pseudomonas* , *Arthrobacter* , *Streptomyces* and *Mesorhizobium* isolates possess AHL degrading pro-perties (Cirou et al., 2010). All N-AHL degrading isolates completely degrade N-AHL in crude culture extract of PaSM1. 3-oxo-C8HSL signal molecule is an essential

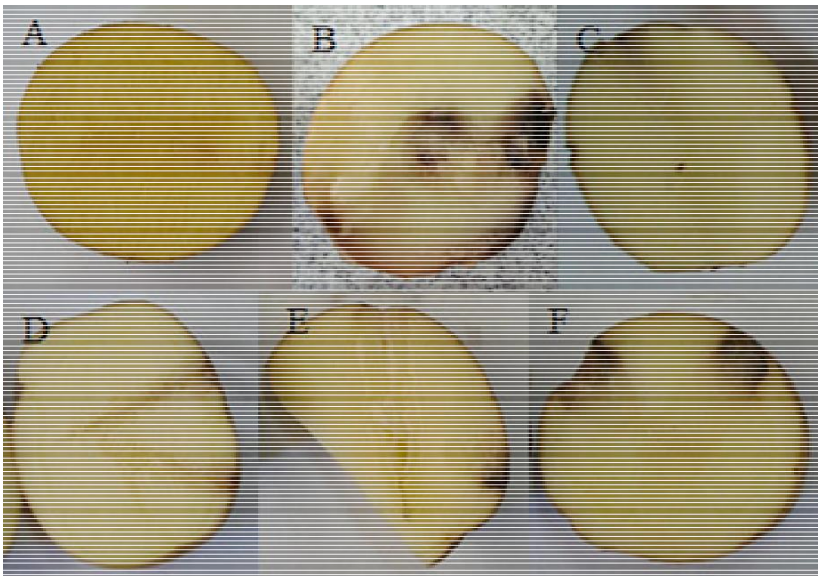

Figure 4. Biocontrol activity of AHL degrading rhizobacteria against plant tissue maceration activities in *Pectobacterium atrosepticum* strain SM1. A, negative control consisting of a tuber treated with 0.8% NaCl; B, Inoculation of 20 µl of PaSM1 alone at about 10^6 c.f.u per tuber; C,D,E,F, co-inoculation of Pa-SM1 at about 10^6 cfu per tuber with EM84 (*Bacillus* sp.), EM18 (*Arthrobacter* sp.), EM1 (*Bacillus* sp.) and EM12 (*Pseudomonas* sp.).

element for QS regulatory system in *P. atroseptium*, which regulate production of virulence deter-minant such as extracellular enzymes was interrupted in quenching experiments.

The final goal of designation of this research was evaluation of the potential antagonistic activity of tested isolates against *P. atrosepticum* SM1 on potato tubers. To set up quenching experiments we used *Bacillus* sp. strains EM1 and EM84, *Artherobacter* sp. strain EM18 and *Pseudomonas sp.* strain EM12 as interfering agents, since during degradation assays these were demonstrated to be the most efficient N-AHL- degrading activities. In quenching experiment, all isolates especially *Bacillus* sp. EM84 inhibited growth of pathogen and effectively reduced plant tissue maceration.

Recently, several authors have proposed to target the QS regulation system to develop innovative approaches to flight plant, animal or human pathogens that rely upon this mechanism to control the expression of pathogenicity determinants (Dong et al., 2007). N-AHL degrading rhizobacteria could be potentially used for biological control of AHL producing plant pathogenic bacteria. The success of their application will be depending on population densities of the antagonists and the efficiency with which the AHLs are inactivated. There-fore, identification and evaluation of antagonistic ability of these AHL-degrading agents is the first step of this procedure, which also introduced in recent years. Dong et al. (2002) and Morohoshi et al. (2009) reported a possibility for attenuating the virulence of *P. carotovorum* on potato introducing of Quorum sensing interfering bacteria. Those studies, as well as the data presented in this report, suggest a promising strategy for the biocontrol and prevention of infectious diseases through AHL signal degradation.

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