รายงานวิจัยฉบับสมบูรณ์

โครงการการคัดเลือก *Streptomyces* sp. ที่สร้างเอนไซม์ *N*-acylhomoserine lactone acylase และศึกษาประสิทธิภาพในการเป็น quorum quenching เพื่อควบคุมแบคทีเรียก่อโรคในพืช

โดย ดร. สุรางค์ ชาญกำแหงเดชา และคณะ

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สัญญาเลขที่ MRG5180031

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คณะผู้วิจัย	สังกัด
1. ดร. สุรางค์ ชาญกำแหงเดชา	มหาวิทยาลัยมหิดล
2. ศ.ดร. วัฒนาลัย ปานบ้านเกร็ด	มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย และสำนักงานคณะกรรมการการอุดมศึกษา

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ชื่อโครงการ : การคัดเลือก *Streptomyces* spp. ที่สร้างเอนไซม์ *N*-acylhomoserine lactone acylase และศึกษาประสิทธิภาพในการเป็น quorum quenching เพื่อควบคุมแบคทีเรียก่อโรคในพืช

ชื่อนักวิจัย : ดร. สุรางค์ ชาญกำแหงเดชา ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

E-mail Address : surang.cha@mahidol.ac.th

ระยะเวลาโครงการ : 2 ปี

บทคัดย่อ :

แบคทีเรียแกรมลบหลายชนิดควบคุมการแสดงออกของยีนที่เกี่ยวข้องกับความรุนแรงและการก่อโรคโดยระบบ quorum sensing ซึ่งมี *N*-acylhomoserine lactone (HSL) เป็นสารสื่อสัญญาณ การรบกวนหรือทำลายระบบ quorum sensing (quorum quenching) สามารถทำได้โดยการทำลายสารสื่อสัญญาณด้วยเอ็นไซม์ acylhomoserine lactone acylase (HSL-acylase) ซึ่งพบได้ในแบคทีเรียหลายชนิดรวมถึงสเตรปโตมัยซีส จากการคัดเลือกสเตรปโตมัยซีส 475 สายพันธุ์ พบ 4 สายพันธุ์ได้แก่ SPM042, SPM042/3, SPM036, และ PS032 ที่แยกจากตัวอย่างดินสามารถย่อยสารสังเคราะห์ HSL ชนิด C_{10}HSL ได้มากกว่า 95% ในหนึ่งชั่วโมง โดย SPM036 ให้ค่ากิจกรรมเอ็นไซม์สูงสุดที่ 197.11±1.3 pmole/h เมื่อทดสอบกับแผ่นมันฝรั่งพบว่าเชื้อทั้ง 4 สายพันธุ์สามารถยับยั้งโรคเน่าที่เกิดจากเชื้อ *Pectobacterium carotovorum* จากนั้นโคลนยีนที่สร้าง HSL-acylase จาก SPM036 (*ahlS*) ด้วยเทคนิค inverse PCR เมื่อวิเคราะห์ลำดับนิวคลีโอไทด์พบว่ายีน *ahlS* ประกอบด้วย 2,415 นิวคลีโอไทด์ ซึ่งแปลรหัสให้กรดอะมิโนเป็นความยาว 804 หน่วย ทำการแสดงออกยีน *ahlS* ใน *S. lividans* TK21 โดยใช้ Integrative plasmids ชนิด pLT101 และ pSET152 ซึ่งทำให้ได้ exconjugants สองชนิดคือ L3 และ S14 ตามลำดับ exconjugants L3 และ S14 สามารถย่อย C_{10}HSL ได้ 1,854.27±36.0 และ 1,827.00±20.7 pmole/h ตามลำดับ เมื่อตรวจสอบจำนวนชุดของยีน *ahlS* ที่สอดแทรกในโครโมโซมของ exconjugant ด้วยวิธี Southern hybridization พบว่า exconjugants ทั้งสองมียีน *ahlS* เพียง 1 ชุด จากนั้นนำเอ็นไซม์ย่อย HSL บริสุทธิ์จาก S14 ไปวิเคราะห์บน SDS-PAGE พบว่าเอ็นไซม์ประกอบด้วยโปรตีนขนาด 88, 62 และ 22 กิโลดาลตัน ซึ่งโปรตีนทั้ง 3 ขนาดได้มาจาก *ahlS* จากการยืนยันด้วย N-terminal amino acid sequence ปฏิกิริยาระหว่างเอ็นไซม์บริสุทธิ์กับ C_{10}HSL เมื่อวิเคราะห์ด้วยโครมาโทกราฟีเหลวประสิทธิภาพสูงตรวจพบสาร HSL เอนไซม์ชนิดนี้ทำงานได้ดีที่ช่วง pH 7-8 อุณหภูมิระหว่าง 25-40 องศาเซลเซียส เอนไซม์บริสุทธิ์ย่อย C_{10}HSL ได้ 1,616.22±24.1 pmole/h/μg ที่อุณหภูมิ 30 องศาเซลเซียสในบัฟเฟอร์ pH 7.0 และ AhlS-acylase บริสุทธิ์สามารถลดการเปื่อยยุ่ยของเนื้อเยื่อพืชจาก *P. carotovorum* ได้

คำหลัก : เอ็นไซม์ HSL-acylase, quorum sensing, quorum quenching, แบคทีเรีย Streptomyces

Project Code : MRG5180031

Project Title : Screening of *Streptomyces* with *N*-acylhomoserine lactone acylase degrading activity and study on its role as quorum quenching for antagonizing phytopathogenic bacteria

Investigator : Dr. Surang chankhamhaengdecha

E-mail Address : surang.cha@mahidol.ac.th

Project Period : 2 years

Abstract:

Several Gram-negative bacteria employ *N*-acylhomoserine lactone (HSL) dependent quorum sensing (QS) to control expression of violence factors and pathogenicity. Inactivation of QS system, commonly known as quorum quenching, by enzymatic digestion of signal molecules, represents a promising strategy to control QS-dependent virulence. *Streptomyces* spp. produces several useful enzymes, including HSL-degrading enzyme, HSL-acylase. Out of 475 *Streptomyces* isolates, only 4 (0.75%) soil isolates (SPM042, SPM042/3, SPM036 and PS032) degraded a synthetic HSL (C_{10}HSL) greater than 95% within 1 h. SPM036 showed the highest activity of C_{10}HSL degradation at 197.11±1.3 pmole/h. The strain could attenuate soft-rot disease caused by *Pectobacterium carotovorum* in potato by *in vitro* pathogenicity assay. Cloning of HSL-acylase gene from SPM036 was accomplished by inverse polymerase chain reaction technique. HSL-acylase gene of SPM036 (*ahlS*) consists of 2,415 bp encoding 804 amino acid residues. Integrative plasmids pLT101 and pSET152 were used to expression an *ahlS* in *S. lividans* TK21, yielding L3 and S14 exconjugants, respectively. L3 and S14 had HSL-degrading activity at 1,854.27±36.0 and 1,827.00±20.7 pmole/h, respectively. A single copy of *ahlS* was integrated to chromosome of both exconjugants as detected by Southern hybridization. S14 produced three HSL-acylases of different molecular masses (88, 62, and 22 kDa) as analyzed by SDS-PAGE. All of HSL-acylases were derived from a cloned *ahlS* as determined by N-terminal amino acid sequencing. HPLC analysis showed that HSL was the reaction product of HSL-acylase with C_{10}HSL as a substrate. This enzyme was stable at the pH range of 7.0 to 8.0 and temperature range from 25°C to 40°C. The purified enzyme showed the highest activity at 1,616.22±24.1 pmole/h/μg at pH 7.0, 30°C. The substrate specificity analysis showed that the enzyme had a high affinity with long chain HSL (C_6 to C_{12} HSL). The purified HSL-acylase exhibited the ability to attenuate potato tissue maceration by *P. carotovorum*.

Keywords : HSL-acylase, quorum sensing, quorum quenching, *Streptomyces*

1. Executive summary

Bacterial cells sense their population density through a sophisticated cell-cell communication system and trigger expression of particular genes when the density reaches a threshold. This type of gene regulation, which controls diverse biological functions including virulence, is known as quorum sensing (QS). *N*-acyl homoserine lactones (HSLs) are found in the quorum-sensing systems of many gram-negative bacteria. They are one family of the most characterized QS signal. These HSL molecules have critical roles in controlling a number of phenotypic traits, such as antibiotic production, biofilm formation, hydrolytic enzyme production and especially, the generation of virulence factors in some pathogenic bacteria. The inactivation of quorum sensing or quorum quenching has been gained an interest as the potential strategy to control the QS-dependent bacterial virulence. Two groups of HSL-degrading enzymes (also called as quorum quenching enzyme) classified according to the HSL cleavage site play role in QS signal interference. Both enzymes are produced by soil bacteria. The first group is HSL-lactonases, first identified in the *Bacillus cereus* group, inactivate HSLs by hydrolyzing their lactone rings and produce the corresponding acylhomoserine molecules. The second group is HSL-acylase, which inactivated HSL by hydrolyzing the amide bond of HSLs. In this process, homoserine lactone (HSL) is released as a product of these reactions, and the acyl chain is further metabolized. Consideration of the possibility to use as the biocontrol agent, we focus on isolation of HSL-degrading enzyme from *Streptomyces*, since this bacterium possesses the ability to synthesize and secrete a variety of secondary metabolites, as well as various extracellular hydrolytic enzymes. From the result of screening, 420 soil sample isolates, representing 34.3% of total soil isolates, contained HSL-degrading activity. Among these HSL-degrading enzyme producing isolates, the four soil isolates stood out for having high levels of HSL-degrading activity. Considering the quantitative assay soil-isolated SPM036 was selected as a potential producer of HSL-acylase which showed the highest HSL-degrading activity at 197.11 ± 1.3 pmole/h. Molecular identified by the 16s rDNA sequence, SPM036 was the most similarity to *Streptomyces parvus*. To study the HSL-acylase characteristics of SPM036, cloning of HSL-acylase gene (*ahlS*) was performed. Firstly, this gene was cloned by restriction enzyme cloning technique which showed none of the positive transformant. This may be due to high GC-content DNA of *Streptomyces*; therefore, targeting inserted fragments may form secondary structure and could not ligate into the cloning vector. Then the inverse PCR cloning technique was designed and the nucleotide sequence of *HSLS* gene was obtained by this method which proved useful for high GC-content gene cloning.

The HSL-acylase gene from SPM036 (*ahlS*) successfully cloned and heterologous expressed in *S. lividans* TK21 with the maximum activity recorded at 1845.76 ± 29.4 pmole/h. The HSL-acylase was purified using Ni-NTA (Nickel- nitrilotriacetic acid) and was later characterized.

The purified protein contains 88, 62, and 22 kDa which the N-terminal sequence of 62 kDa is the amino acid sequence of β-subunit of HSL-acylase. The enzyme showed the optimum activity at pH 7-8, the temperature range from 25 to 40 $^{\circ}$C, and long-chain HSL as substrate preference. Once C_{10}HSL was degraded by HSL-acylase, HSL was an end product which was verified by HPLC analysis. This result revealed that the acylase activity of the purified enzyme. For agricultural application, soft-rot symptom caused by QS signal of *P. carotovorum* was reduced by inoculation of the enzyme and the bacterial culture into a potato tuber. In conclusion, this quorum sensing inactivation by HSL-acylase offers an alternative tool to control bacterial infection dependent upon QS system.

2. เนื้อหาวิจัย

2.1 บทนำ

Cell-to-cell communication or gene regulation dependent upon cell density is recognized as quorum sensing (QS) (Fuqua, et al., 1994; Miller and Bassler, 2001; Whitehead, et al., 2001; Winans, 1998). In this process, each cell secretes QS signal molecules into confined environment. Until these molecules reach a threshold concentration as the cells increase, the signal is bound by its cognate transcriptional regulator. The signaling compound plays a role in monitoring the expression of targeting gene(s) (Kell, et al., 1995; Williams, et al., 1999). These specific genes relying on quorum sensing are involved in production of extracellular hydrolytic enzymes (Jones, et al., 1993), biofilm formation (Davis, et al., 1998; Wei and Zhang, 2006), bioluminescence (Sitnikov, et al., 1995), plasmid transfer (Piper, et al., 1993), swarming motility (Eberl, et al., 1996), and antibiotic production (Bainton, et al., 1992). These phenotypes provide bacterial communities with ability to adapt themselves to changing environment; and cause pathogenicity to their respective hosts.

Over last decades, quorum quenching or the inactivation of quorum sensing has been gained an interest as the potential strategy to control the QS-dependent bacterial virulence. This strategy is focused on controlling of QS phenotypes without interfering of cell proliferation. In order to antagonize QS process in Gram-negative bacteria which employ *N*-acylhomoserine lactones (AHL) as signaling molecules, targeting at signaling synthesis, dissemination, and reception have been reviewed (Zhang, et al., 2003). Inhibition of AHL production is accomplished by using S$'$-adenosylmethionine (SAM), 5$'$-methylthioadenosine (MTA) analog (Parsek, et al., 1999) and triclosan (Hoang and Schweizer, 1999). As well as free AHL molecules could be digested by enzymatic reactions from AHL-lactonase and AHL-acylase, respectively. The former enzyme has ability to cleave a lactone ring and another is able to hydrolyze an amide bond connecting between an acyl side chain and the lactone ring (Leadbetter and Greenberg, 2000; Leadbetter, 2001). In addition, halogenated furanones from Delisea pulchra serve to degrade transcription regulator (Manefield, et al., 1999). Meanwhile other substances from natural products and chemicals such as garlic extracts, *N*-acylcyclopentylamides, 4-nitro-pyridine-N-oxide (4-NPO), patulin and pennicillic acid could inactivate QS with unclear mechanism (Ishida, et al., 2007; Morohoshi, et al., 2007; Rasmussen, et al., 2005a; Ramussen, et al., 2005b).

In searching for novel quorum quenching bacteria from soil samples, Streptomyces spp. were of interested since this bacterium possesses the ability to synthesize and secrete a variety of secondary metabolites, as well as various extracellular hydrolytic enzymes (Cho, et al., 1998; Strickler, et al., 1992). We obtained an isolate that strongly inactivated autoinducing activity and reduced the severity of plant soft-rot. Cloning and expression of the *ahlS* gene in *S. lividans*, the analysis of the substrate specificity of the purified enzyme and the effect of the purified enzyme on *P. carotovorum* pathogenicity, underlined the potential of enzyme as a novel biological control agent for certain bacteria.

2.2 วัตถุประสงค์

The objectives of this study are (i) to isolate and identify *Streptomyces* spp. with HSLs degrading activity; (ii) to clone and characterize the gene for quorum quenching enzyme and enzyme purification and; (iii) to explore their antagonistic activity against the plant pathogenic bacteria via QS-regulatory process.

2.3 วิธีทดลอง

2.3.1 Sample correction

Total of 15 samples of soil under various fruit trees were collected from 15 locations in Rayong and Samutsakorn provinces. Soil samples were collected at a depth of 4 cm. The samples were used for the isolation of *Streptomyces* spp.

Three hundred and ninety seven soil isolated *Streptomyces* strains were obtained from *Streptomyces* culture collection of Professor Watanalai Panbangred, Department of Biotechnology, Mahidol University. These isolates were screened from soil samples in shrimp ponds from Samutsakorn Province, in orchards from Bangkok, Chanthaburi, Prachinburi, Chonburi and Nuangbualumpoo provinces.

2.3.2 Isolation of soil *Streptomyces* from soil samples

One gram of each soil sample was suspended in 9 ml of 0.85% normal saline solution. The soil suspension was serially diluted (ten folds) and 0.1 ml of the appropriate dilutions were spread on Pridham’s agar and Water-proline agar supplemented with 25 µg/ml nalidixic acid and 50 µg/ml cycloheximide. The inoculation plates were incubated at 28°C for 4-14 days. Colonies with characteristics of *Streptomyces* were selected.

2.3.3 Identification of HSL-degrading *Streptomyces*

The 23 *Streptomyces* strains primarily isolated from the screening and 452 strains from Professor Watanalai Panbangred culture collection were determined for their HSL-degrading activity. The test system was performed by using *A. tumefaciens* NTL4 (pZLR4) as the indicator strain. pZLR4 harbors *traR* gene and its product TraR protein when binding with HSLs, induce the expression of *lacZ* gene fused with *traG* gene on the same plasmid. *A. tumefaciens* NTL4 normally does not make its own HSL; consequently the *lacZ* reporter fusion is not expressed. The TraR protein will activate the expression of *PtraG-lacZ* fusion when TraR is induced by HSL. β-galactosidase from *lacZ* expression will digest 5-bromo-4-chloro-3-indoyl-D-galactopyranoside (X-gal) on the plate and cause blue zone which determines the amount of HSL residue by comparing with blue zone of known HSL concentration. The *Streptomyces* strains were cultured in 5 ml of 301 broth and incubated at 28°C with shaking at 180 rpm for 6 days. Then cell-free supernatant of cultures was collected and determined for their HSL-degrading activity. The supernatant was mixed with C_{10}HSL (20 µM final concentration) and incubated at 28°C for 1 h with gentle shaking. All reaction mixtures were heated at 95°C to stop reaction. Ten microliters of reaction mixture were loaded into the well of a bioassay plate. This plate is composed of AB minimal medium and 40 µg/ml of X-gal overlaid with 5 ml soft agar (0.7% agar) seeded with 2.5 ml of overnight culture of *A. tumefaciens* NTL4 (pZLR4), as an indicator strain for C_{10}HSL detection. The bioassay plates were incubated at 28°C for 16 h for allowing blue color development. The supernatant from HSL-degrading enzyme producing strain degraded C_{10}HSL and yielded a colorless area around the hole of a bioassay plate whereas HSL-degrading enzyme nonproducing strain yielded a blue zone. The amount of HSL residues leftover in the reaction mixture was determined by comparing with blue zone of known concentrations HSL.

2.3.4 Bioassay for quantitatively determination of HSL-degrading activity

Isolates that showed strong HSL-degrading activity on bioassay plate (HSL remained in reaction mixture less than 6.25 pmole) were quantitatively determined for their activity. Minimal medium agar (25 ml) supplemented with 40 µg/ml of X-gal was cut into agar slices every 0.5 cm on the plate. Culture supernatant of each tested strain was collected and 40 µl of each was mixed with equal volume of 40 µM C_{10}HSL and incubate at 28ºC for 1 h. The reaction mixture (5 µl) was spotted on one end of an agar slice, and then the culture of the *A. tumefaciens* NTL4 (pZLR4) ($OD_{600} \approx 0.4$) was spotted (0.6 µl for each spot) at progressively further distances from the loaded reaction mixture (with an interval of 0.5 cm) (Figure 1). The plates were incubated at 30ºC overnight. The distance from the last induced blue colony to the origin of the loaded sample in each agar slice was measured. The distance of blue colonies is in inverse proportion to the autoinducer-degrading activity. All bioassay experiments were performed in triplicate unless otherwise stated. To calculate HSL-degrading activity, the amount of initial substrate was subtracted by the leftover of HSL yielding the amount of digested HSL. The amount of leftover HSL was derived from substitution of measured blue colony into the equation of relationship between distance of blue zone and known amount of HSL.

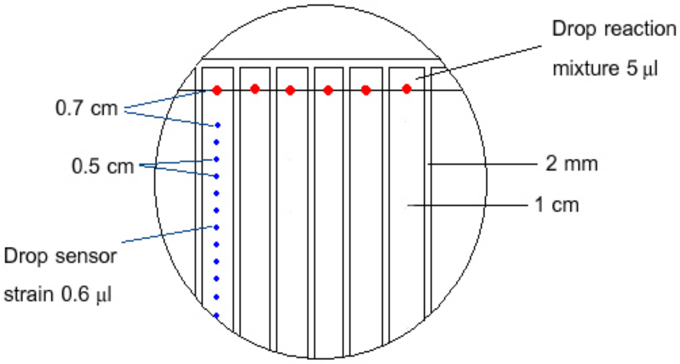


Figure 1 The pattern of a bioassay plate for quantitative determination of HSL-degrading activity

2.3.5 In vitro pathogenicity assay

To test the efficiency of soft-rot disease attenuation, potato was used as a host plant. Fresh tuber was soaked in 5% hypochlorite and washed with distilled water then sliced into a chip about 5 mM thickness. Inoculum was prepared by mixing twenty microliters from of overnight culture of *P. carotovorum* in NB broth at 30^{O}C and twenty microliters from 6 days culture of tested *Streptomyces* in 301 broth at 28^{O}C. This inoculum was further incubated for 4 h. Then ten microliters of inoculum was mounted on 5 mM thickness potato chips. All chips were incubated in sterile boxes containing sterile wet cotton to maintain humidity. After 48 h of incubation at 30^{O}C. lesion area was observed and maceration was measured comparing to the control. Each treatment was tested as triplicate.

2.3.6 Strain identification of HSL-degrading *Streptomyces*

The isolate with the highest HSL-degrading activity was identified using 16S rRNA gene analysis. The 16s rRNA gene was sequenced using the universal primers UFUL (5′-GCCTAACACTGCAAGTCGA-3′). The 16s rRNA gene sequence was aligned and compared with other 16s rRNA genes in the Genbank by using the BLAST facility of the National Center for Biotechnology Information and the sequence-matching facility of the Ribosomal Database project (www.ncbi.nlm.nih.gov).

2.3.7 Cloning of HSL-acylase gene

2.3.7.1 Amplification of acylase specific fragment and DNA sequencing

The central catalytic domain of acylase gene from genomic DNA of SPM036 was amplified by using primers AHL-F (5′-GGTCGAGGTTCTTCGGACC-3′) and AHL-R3 (5′-CGCTGTCGTTGG AGTTCTCCAC-3′). The PCR primers were designed on the basis of the most conserved amino acid sequences of HSL-acylase and acylase homologues. AHL-F and AHL-R3 can amplify three different acylase genes including HSL-acylase, penicillin amidase, and cyclic lipopeptide acylase which had the same PCR product size of 1,266 bp. However each gene can be distinguished from each other by using restriction enzymes analysis as shown in Table 1. The PCR product with partial gene sequence of HSL-acylase was then subcloned to pGEM-T easy, sequenced and was used as probes for Southern blot hybridization.

Table 1 Restriction endonuclease cutting sites within three genes that can be amplified by AHL-F and AHL-R3 primers

Gene	Restriction enzyme	Cutting site(s)	Expected cutting fragment(s) of PCR product
HSL-acylase	*Bam*HI	-	1
	*Sal*I	-	1
Penicillin amidase	*Bam*HI	2	3
	*Sal*I	2	3
Cyclic lipopeptide acylase	*Bam*HI	-	1
	*Sal*I	1	2

2.3.7.2 Inverse PCR cloning

By using an HSL-acylase specific fragment as a probe, the *Bam*HI digested fragments harboring HSL-acylase gene were identified by Southern blot analysis. Cloning of complete coding region of HSL-acylase gene was accomplished through inverse polymerase chain reaction (iPCR). The technique involves circularization of *Bam*HI restricted fragments and amplification using primers SeqF and SeqR that prime the DNA synthesis directed away from the core region of a known sequence (Figure 2). Sequence analysis of the 5′ and 3′ flanking domains of the known core region enabled the design of primers CDS_F0 and CDS_R0 with the usual forward/reverse orientation and additional cloned was generated from native genomic DNA using a high fidelity DNA polymerase. The amplified coding region of HSL-acylase encoding gene (*ahlS*) was subcloned in pGEM-T easy yielding pGEM-AHLS. The *ahlS* gene was further analyzed by restriction cutting, DNA sequencing and sequence analysis by Vector NTI program. Primers sequences were shown in Table 2.

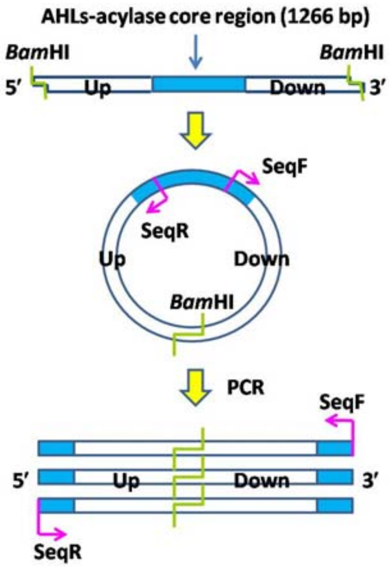


Figure 2 Inverse PCR technique was used for amplification of flanking regions of HSL-acylase core region.

Table 2 List of primers for sequencing and analysis of inverse PCR product

Name of Primer	**Sequence**	**Length (bp)**	**Tm (^{o}C)**	**Length of PCR products (bp)**
SeqF	5′-TCGACGGTTCGCGGAAGGAC-3′	20	56.6	1,792
SeqR	5′-TTCAGCAGCTTCTCCACCGT-3′	20	49.4	
CDS_F0	5′-GTGCTGCTGCGTACACCTGTCAT-3′	23	53.8	2,666
CDS_R0	5′-CTACCGCCGCTCGTGCAC-3′	18	51.9	

2.3.8 Expression *ahlS* in *S.lividans* TK21

The AHL-acylase coding gene (*ahlS*) with C-terminal 6XHis fused was amplified with C-terminal 6X His fused from SPM036 by PCR using primers CDS_F1_BamHI RBS2 (5′-cggttcaaGGATCCgtgccacccgcaccgttgggaggaacaccttg-3′), CDS_F0 (5′-gtgctgctgcgtacacctgtcat-3′), and CDS_R0_his_BamHI (5′gaaGGATCCctaGTGGTGGTGGTGGTG GTGccgccggttttgcacccgc-3′)

(upper case letters indicate the site for *Bam*HI and GTG is a reverse complement of his tag sequences). The PCR fragments were digested with *Bam*HI and were further cloned into two *Streptomyces* vectors which were pLT101 and pSET152 leading to the pLTH and pSTH constructs, respectively. These recombinant plasmids were firstly cloned and screened in *E. coli* DH5α transformant and then the selected recombinant plasmids were transferred to *E. coli* ET12567 (pUZ8002) for conjugal transfer the constructed plasmids to *S. lividans* TK21. Intergeneric conjugation between *E. coli* and *Streptomyces* was performed. Each exconjugant was tested for their AHL-degrading activity as described previously. The secreted proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A copy number of *ahlS* gene inserted in chromosome of exconjugants was determined by Southern blot hybridization where the 1.2 kb of *ahlS* core region was used as a probe.

2.3.9 Purification of HSL-acylase from in *S.lividans* TK21 exconjugant

S. lividans TK21 exconjugant harboring *ahlS* gene was cultured in 301 medium. After 3 days of incubation with shaking at 30^{O}C, the bacterial culture was filtrated by Whatman filter membrane no.541 (GE healthcare, USA). Then the target protein was precipitated at 40-60% of ammonium sulfate at 0^{O}C for 1 hr. The protein pellet was harvested by centrifugation at 12,000 rpm (17,000 × g) for 10 minutes at 4^{O}C. The pellet was resuspended in 20 mM sodium phosphate buffer pH 7.0 and dialyzed against the same buffer.

The dialysed fraction was further purified by Ni-NTA resin (Millipore) gravity column. The column was equilibrated by loading with 2 ml of NPI-10 (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole pH 7.5). After equilibration of column, 80 μg of the crude protein sample was loaded. The unspecific binding proteins were washed with three times of 3 ml of NPI-40 (50 mM NaH_2PO_4, 300 mM NaCl, 40 mM imidazole pH 7.5). The bound protein was eluted with 2.5 ml of NPI-100 (50 mM NaH_2PO_4, 300 mM NaCl, 100 mM imidazole pH 7.5) twice. Imidazole in fraction was removed by PD-10. The samples were concentrated by using membrane concentrator with molecular weight cut off 10 kDa. Each fraction of flow-through, washing and eluting was collected and analyzed on SDS-PAGE.

2.3.10 Evaluation of purified HSL-acylase

2.3.10.1 Determination of optimum temperature on HSL-acylase activity

Effect of pH on enzyme activity was determined at 30^{O}C in buffers of which pH ranges from 5.0 to 9.0. Then HSL-acylase activity was measured as previously described.

Effect of temperature on HSL-acylase activity was determined from 0-60°C in 0.1 M Tris-HCl pH 8.0. After that the residual enzyme activity of each tested temperature was measured according to bioassay analysis as previously described.

2.3.10.2 Substrate specificity of HSL-acylase

The substrate specificity analysis was performed using bioassay system based on the biosensor strains *C. violaceum* CV026 and *A. tumefaciens* NTL4 (pZLR4). *C. violaceum* CV026 was used in the detection of short-chain acyl-homoserine lactones (C_4HSL, C_6HSL and 3-oxo-C_6HSL) whereas *A. tumefaciens* NTL4 (pZLR4) was used for long-chain acyl homoserine lactones (C_8HSL, C_{10}HSL, C_{12}HSL, and 3-oxo-C_8HSL). The reaction mixture was carried out in 40 μl of 20 mM sodium phosphate at pH 7.0 containing 1 μg of HSL-acylase and 0.4 mM of substrates. Incubation was at 30°C for 1 h followed by stop reaction at 95°C for 5 min. Ten microliters of reaction mixture was dropped on bioassay plates and were incubated for overnight. For short-chain acyl-homoserine lactones (C_4HSL, C_6HSL, 3-oxo-C_6HSL) detection, samples were dropped on strips of LA plate spotted with 0.6 μl of *C. violaceum* (CV026) at OD_{600} of 0.4 and the violacein production by this strain was induced by AHL leftover in the reaction mixture. For long-chain acylhomoserine lactone (C_{10}HSL, C_{12}HSL, 3-oxo-C_8HSL), samples were dropped on AB minimal agar bioassay plate containing *A. tumefaciens* NTL4 (pZLR4). β-galactosidase production was induced by the HSL leftover. X-gal degraded by β-galactosidase displays blue colonies of *A. tumefaciens* NTL4 (pZLR4) on the bioassay plate. β-galactosidase and violacein production shown on plates was used to calculate HSL-acylase activity based on the standard curve of substrate concentration.

2.3.10.3 Identification of reaction product of HSL-acylase digestion by high performance liquid chromatography (HPLC)

AHLs digestion was prepared in 700 μl of 20 mM Sodium phosphate pH 7.0 containing 13 μg of purified HSL-acylase and 200 mM of C_{10}HSL. The reaction mixture was incubated at 30°C overnight. Then supernatant was extracted three times by equal volume of ethyl acetate. The combined organic phase was dried in a rotary evaporator and the residue was resuspended in 50 μl of methanol. For HPLC (Agilent Technologies, Japan) analysis, five microlitre (dissolved in methanol) was introduced onto a CAPCELL PAK C18 UG 80 S5 column, 4.6 mm. I.D.× 250 mm (Shiseido, Japan), the flow was 1.0 ml/min. Mobile phase was performed by a linear gradient of 10% to 90% acetonitrile for 22 minute and additional 5 minutes at 90% acetonitrile. The active soluted was detected at UV 210 nm by photodiode array detector.Total run time was 35 minute. Spectrum of each peak was detected at UV 200 to 280 nm.

2.3.11 Effect of HSL-acylase on *P. carotovorum* pathogenicity

To investigate the effect of HSL-acylase on the development of plant soft-rot disease caused by *P. carotovorum*, *in vitro* pathogenicity test was performed. In this study potato was used as the host plant of *P. carotovorum*. Fresh plants were surface sterilized by immersion in 5% hypochlorite solution for 10 min, and then were washed with distilled water and dried in a biosafety cabinet. The inoculums were prepared by culturing *P. carotovorum* in LB medium at 30°C for overnight. Thirty microliters of the culture at 10^5 fold dilution of OD_{600} of 1 was mixed with 90 μl of purified HSL-acylase, respectively. Then 40 μl of these mixtures were inoculated into each tuber by using 200 μl pipette tips. All tubers were wrapped with an aluminum foil alternating with a damp paper towel. Potato tubers were kept in a box and incubated at 30°C for 3 days. After incubation, the lesion of inoculated tissue was observed and measured the maceration weights compared to the control. Each treatment was performed in triplicate. The decrease or limitation of maceration weight indicated quorum quenching efficiency of HSL-acylase, which could control *P. carotovorum* infection.

2.4 ผลการทดลอง

2.4.1 Screening and isolation of *Streptomyces* spp.

A total of 23 *Streptomyces* isolates were obtained from fifteen soil samples collected from Rayong and Samutsakorn provinces (Table 3). The samples were collected from shading areas under the trees and *Streptomyces* from these samples were screened on Pridham's agar and Water proline agar supplemented with nalidixic acid (25 μg/ml) and cyclohexamide (50 μg/ml). *Streptomyces* isolates showed filamentous growth, spore chain, and several types of convex and margin colonies on media agar. The other 379 soil and 55 endophytic isolates were kindly provided by Prof. Dr. Watanalai Panbangred.

Table 3 *Streptomyces* isolated from soil

Sample	Sources	Number of isolates	Names
	Rayong		
1	Rambutan tree	2	RA001, RA002
2	Durian tree	2	RA003, RA004
3	Longkong tree	2	RA005, RA006
4	Sugar apple tree	-	
5	Mangosteen tree	1	RA007

Table 3 *Streptomyces* isolated from soil (continue)

Sample	Sources	Number of isolates	Names
6	Longan tree	-	
7	Mango tree	2	RA008, RA009
8	Rose apple tree	1	RA010
9	Lemongrass tree	1	RA011
	Samutsakorn		
10	Coconut tree	1	SS001
11	Leech lime tree	3	SS002, SS003, SS004
12	Jackfruit tree	3	SS005, SS006, SS007
13	Plu tree	1	SS008
14	Tamarind tree	2	SS009, SS010
15	Betel nut tree	2	SS011, SS012
	Total	23	

2.4.2 Isolation of HSL degrading *Streptomyces*

After incubation of the reaction mixture of culture supernatant of *Streptomyces* isolate and the synthetic autoinducer C_{10}HSL for 1 hour, its residual autoinducing activity was determined by comparing with the standard plate of C_{10}HSL. The ability to degrade C_{10}HSL was variable, ranging from 1.25 to 200 pmole/h (Table 4). Among these 138 isolates, four strains, SPM042, SPM042/3, SPM036, and PS032 were able to reduce C_{10}HSL greater than 96.9%.

Table 4 HSL-degrading activity of *Streptomyces* isolates

HSL degrading activity (pmole/h)	Sources of isolates			
	Soil		Endophyte	
	Number of isolates	Percentages	Number of isolates	Percentages
0	276	65.7	47	85.5
1.25—100	84	20.0	8	14.5
≥100 — 150	24	5.7	-	-
≥150 — 175	16	3.8	-	-
≥175 — 200	20	4.8	-	-

2.4.3 Bioassay for quantitatively determination of HSL-degrading activity

Isolates with ability to digest C_{10}HSL greater than 193.75 pmole/h (96.9%) were determined their quantitative HSL-degrading activity. These isolates were SPM042, SPM042/3, SPM036, and PS032. After 6 days of incubation with shaking, cell-free supernatant of each strain was indirectly analyzed for HSL-degrading activity against 200 pmole of C_{10}HSL according to the previously described method. HSL-degrading activity of each isolate was shown in Table 5. All of them displayed a slightly difference of crude enzyme activity and could be selected for gene cloning.

Table 5 Quantitative HSL-degrading activity of *Streptomyces* isolates

Name of isolates	HSL-degrading activity (pmole/h)
SPM042	196.69 ±0.9
SPM042/3	196.54 ± 0.8
SPM036	197.11 ± 1.3
PS32	196.15 ± 0.9

2.4.4 Effect of HSL-degrading *Streptomyces* on pathogenicity of *P. carotovorum*

In vitro pathogenicity assay of inoculums mixture between *P. carotovorum* and each *Streptomyces* isolate*,* SPM042, SPM042/3, SPM036 and PS032, showed the significant reduction of watery rotten lesions on potato slices (Figure 3). However, SPM036 showed the highest ability to reduce soft-rotting effect of *P. carotovorum* and was able to grow rapidly in culture medium and produced low concentrations of polysaccharides. Therefore SPM036 was chosen for further studies.

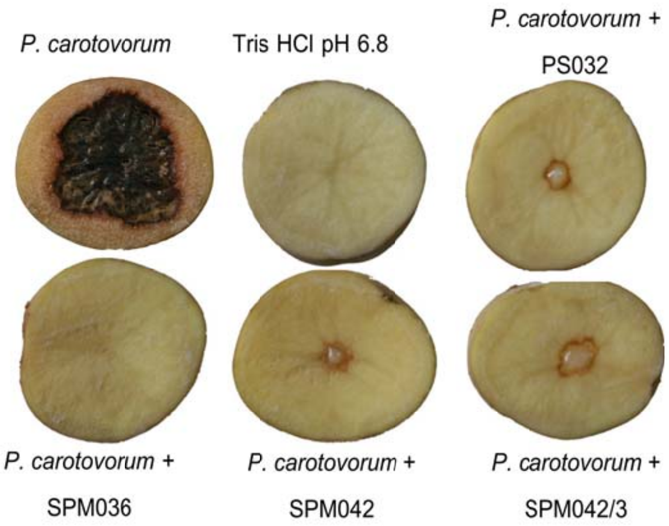


Figure 3. After incubation of tested-inoculums on potato slices, *P. carotovorum* causes lesion on potato chips. The watery rotten lesion was reduced when incubated this pathogen with culture broth of SPM042, SPM042/3, SPM036 and PS032 before inoculation to potato slices.

2.4.5 Strain identification

The isolate SPM036 was identified its strain by 16S rRNA gene. This gene was sequenced by UFUL primer. The 16S rRNA gene sequence of strain SPM036 showed the most similarity with *Streptomyces parvus.*

2.4.6 Inverse PCR cloning

AHL-F and AHL-R3 primers were used for amplification of 1.2 kb of HSL-acylase specific fragment. Genomic DNA of *Streptomyces* SPM036 was used as a template. A PCR product was distinctly shown in Figure 4. The reaction was precipitated and PCR products were cut by *Bam*HI and *Sal*I to preliminary verify HSL-acylase fragment. The product was a single band after cutting by both restriction enzymes. Sequence of this 1.2 kb fragment was determined by DNA sequencing. Result of DNA sequencing showed that the product was 96% identities to *ahlM* gene which is an HSL-acylase in *Streptomyces* sp. strain M664 (accession number AAT68473).

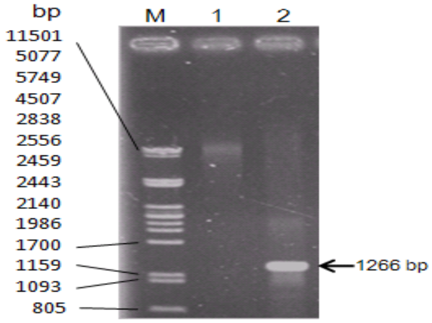
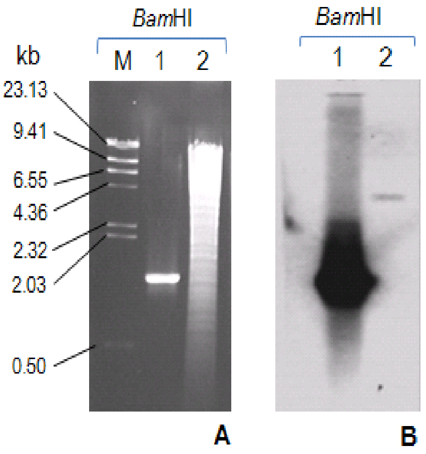


Figure 4 Agarose gel electrophoresis of the PCR products amplified from genomic DNA of *Streptomyces* SPM036 by AHL-F and AHL-R3 primers (lane 2). Lane M was λ/*Pst*I marker, lane 1; negative control.

Southern blot analysis of SPM036 chromosomal DNA digested with *Bam*HI using 1.2 kb HSL-acylase specific probe revealed a band of about 4 kb (Figure 5). This DNA band was purified from agarose gels, circularized to be circular DNA template for inverse PCR amplification. The 3.4 kb fragments were obtained from inverse PCR amplification. From sequence analysis by Vector NTI program showed that the start codon (TTG) was located at 387 bp downstream from SeqR primed position and the stop codon (TAG) was located at 981 bp upstream from SeqF primed position (Figure 6). By using primers designed for usual forward/reverse orientation, the coding region of HSL-acylase gene (*ahlS*) from SPM036 was successfully amplified and subcloned in pGEM-T easy vector. The *ahlS* consists of an open reading frame of 2,415 bp that encodes a protein with 804 amino acid residues. The nucleotide sequence of *ahlS* gene was deposited in Genbank under the accession number of JX484296.



hybridized band of
HSL-acylase specific fragment

Figure 5 Agarose gel electrophoresis of HSL-acylase specific fragment (lane1) and *Bam*HI digested genomic DNA of SPM036 (lane 2) were fractionated (A), blotted and then probed with a digoxigenin-labled 1.2 kb HSL-acylase specific fragment (B).

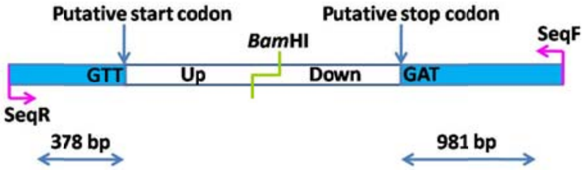


Figure 6 Schematic of putative start and stop codons of *ahlS* on inverse PCR products.

2.4.7 Expression and purification of HSL-acylase

The HSL-acylase encoding fragments were ligated into pSET152 and pLT101 to obtain pSTH and pLTH (Figures 7, 8), respectively. Recombinant plasmids were transfromed into *E. coli* ET12567 (pUZ8002). Plasmid pLTH and pSTH were transformed from these *E. coli* transformants to *S. lividans* TK21 through conjugation method. The exconjugants LTH3401-3 (L3) and STH11-4 (S14) of both recombinant plasmids showed the ability to degrade C_{10}HSL at 1,854.27 ± 36.0 and 1,827 ± 20.7 pmole/h, respectively (Table 6). A copy number of *ahlS* gene integrated in exconjugant L3 and S14 chromosomal DNA was determined by Southern hybridization (Figure 9). *Pvu*II, *Sac*I and *Xho*I restriction enzymes which are unable to cut inside *ahlS* were used to digest a total DNA and have different cutting sites on pSET152 and pLT101. When restriction fragments were hybridized with 1.2 kb *ahlS* probe, only one hybridized band was detected in both L3 and S14 digested chromosomal DNA. An integration of *ahlS* into *S. lividans* is occurred due to the homologous recombination between *attP* site of conjugative plasmid and *attB* site of host chromosomal DNA.

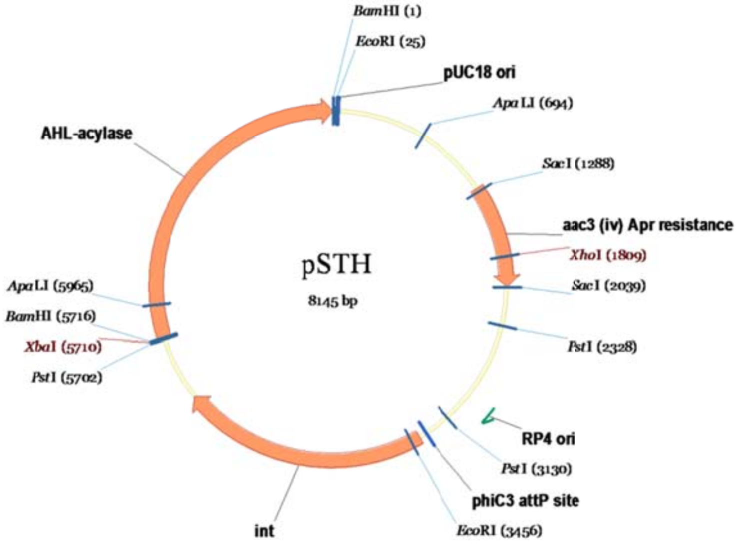


Figure 7 Restriction map of pSTH recombinant plasmid; pSET152 harboring *ahlS* gene fused with 6x histidines.

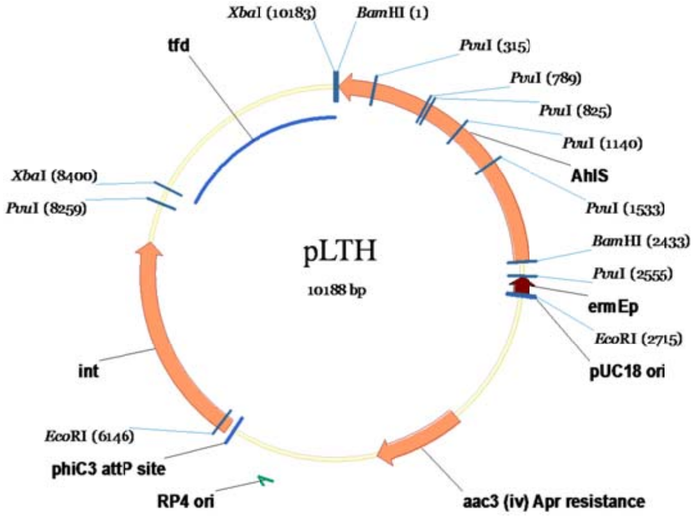


Figure 8 Restriction map of pLTH recombinant plasmid; pLT101 harboring *ahlS* gene fused with 6x histidines.

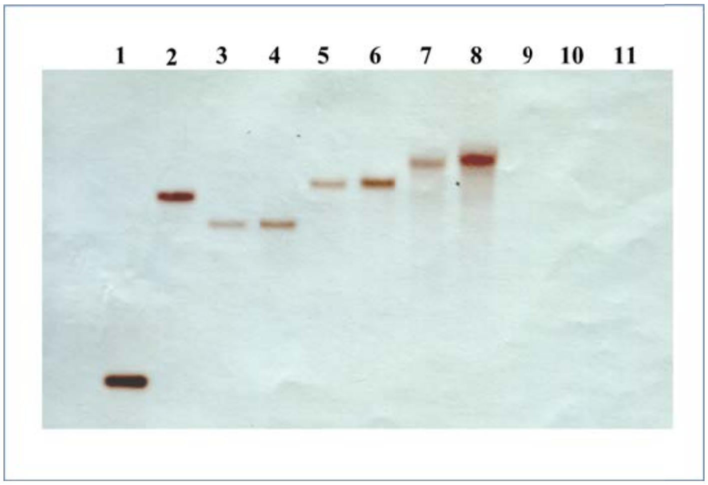


Figure 9 Southern blot analysis of chromosomal DNA from *S. lividans* TK21::*ahlS* exconjugants. Lane 1, positive control, *ahlS* core region (1.2 kb); lane 2, SPM036 DNA cut by *Pvu*II (2.8 kb); lanes 3, 5 and 7, L3 exconjugant DNA cut by *Pvu*II, *Sac*I, and *Xho*I, respectively (2.6, 2.9, and 4.4 kb); lanes 4, 6 and 8, S14 exconjugant DNA cut by *Pvu*II, *Sac*I, and *Xho*I, respectively (2.6, 2.9, and 4.4 kb); lane 9, *S. lividans* TK21 DNA cut by *Pvu*II; lane 10, *S. lividans* (pLT101) DNA cut by *Pvu*II; and lane 11, *S. lividans* (pSET152) DNA cut by *Pvu*II.

Table 6 HSL-acylase activity of *S. lividans* TK21 exconjugants harboring *ahlS*

Exconjugant	HSL acylase activity ± SD(pmole/h)
LTH3401-1 (L1)	1845.76 ± 29.4
LTH3401-2 (L2)	182.56 ± 3.8
LTH3401-3 (L3)	1852.77 ± 10.3
LTH3401-4 (L4)	120.95 ± 15.2
LTH3401-5 (L5)	187.71 ± 9.0
LTH3401-6 (L6)	196.43 ± 5.1
STH11-1 (S11)	182.75 ± 2.6
STH11-2 (S12)	182.62 ± 1.9
STH11-3 (S13)	780.49 ± 0.3
STH11-4 (S14)	1825.76 ± 20.7
STH11-5 (S15)	783.49 ± 0.3
STH11-6 (S16)	189.49 ± 7.9
STH21-1 (S21)	106.22 ± 9.2
STH21-2 (S22)	58.62 ± 18.4
STH21-3 (S23)	36.97 ± 24.4
STH21-4 (S24)	64.53 ± 18.9
STH21-5 (S25)	63.17 ± 12.0
STH21-6 (S26)	180.60 ± 1.8
STH51-1 (S51)	144.29 ± 9.1
STH51-2 (S52)	88.51 ± 27.2
STH51-3 (S53)	137.41 ± 7.8
STH51-4 (S54)	184.91 ± 3.3
STH51-5 (S55)	182.23 ± 0.5
STH51-6 (S56)	186.22 ± 5.5

* Activity was calculated from triplicate experiments based on standard equation of distance of blue colony in centimeters against known amount of C_{10}HSL.

Recovery of HSL-acylase from culture supernatant of S14 was performed by ammonium sulfate precipitation. Each fraction of 40%, 40-50%, and 50-60% ammonium sulfate precipitated proteins was determined for HSL-acylase activity and the profile of precipitated proteins were analyzed on SDS-PAGE. HSL-acylase activity was detected in all fractions and highest in the fraction precipitated with 50-60% salt (Table 7). Protein profile on SDS-PAGE demonstrated a 62 kDa protein band at highest density in the pellet of 50-60% of ammonium sulfate fraction (Figure 10).

Table 7 HSL-acylase activity of each precipitated-protein fraction

Ammonium sulfate (%)	HSL acylase activity (nmole/h/L)
0 - 40	73.10
40 - 50	99.54
50 - 60	129.14

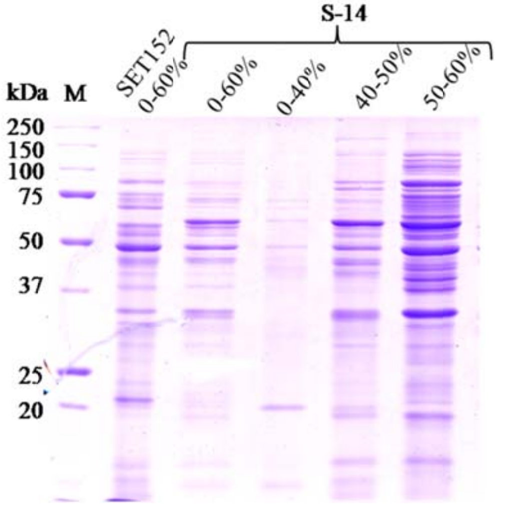


Figure 10 Ammonium sulfate precipitation of the crude protein from S14 (*S. lividans* TK21::*ahlS*) at varied percentage of salt, precision plus was used as a molecular marker. Arrow indicates the 62 kDa band of HSL-acylase. Crude proteins of SET152 (*S. lividans* TK21::pSET152) at 60% of ammonium sulfate precipitation was used as a control.

HSL-acylase with six histidine residues fused at the C-terminus was purified from S14 exconjugant under native condition. To optimize pH for purification, pH at 7.5 and 8.0 were tested. The result showed that pH at 7.5 was able to retrieve HSL-acylase better than that of pH 8.0. HSL-acylase was then eluted by 100 mM imidazole at pH of 7.5. From this purification, eluted fraction contained three different molecular mass weight proteins of 88, 62, and 22 kDa as analyzed by SDS-PAGE. The largest protein band of 88 kDa corresponds to a mature molecular weight of *ahlS* ORF. The two smaller proteins supposed to be the proteolytic modification products of the 88 kDa mature protein. The 62 kDa protein was blotted on PVDF membrane and N-terminal amino acid sequences were analyzed. These amino acid residues are S-N-A-V-A-F which is the deduced amino acid sequences of *ahlS* gene next to the signal peptide cleavage site. This result, therefore; indicated that 62 and 22 kDa proteins were derived from proteolytic cleavage at the N-terminal region of the mature 88 kDa protein (Figure 11).

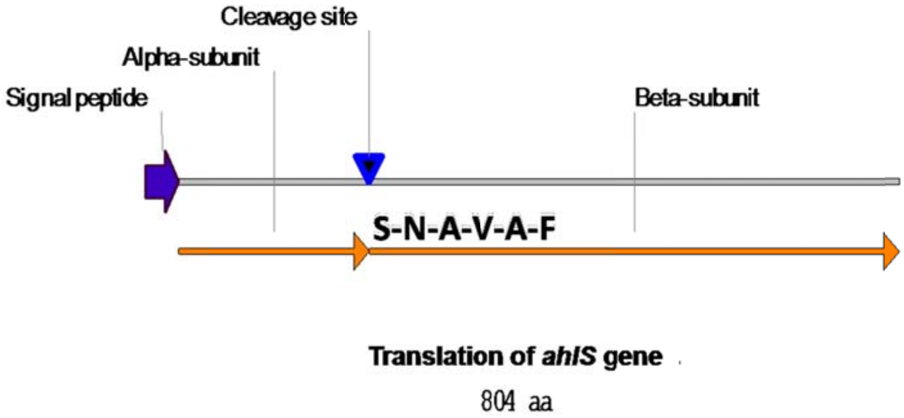


Figure 11 The typical polypeptide organization of HSL-acylase encoded by *ahlS* gene.

2.4.8 Evaluation of purified HSL-acylase

2.4.8.1 Determination of optimum pH and temperature for HSL-acylase activity

Purified HSL-acylase 0.25 μg was mixed with 0.4 mM final concentration of C_{10}HSL in buffer at pH 5-9. The reaction mixtures were incubated at 28^{O}C for 30 min and stop reaction by heating at 95^{O}C. Then 10 μl (equal to 4,000 pmole without digestion) of the mixtures were dropped on bioassay plates to determine relative HSL-acylase activity comparing to reactions containing only C_{10}HSL. Purified HSL-acylase had the highest relative activity at pH 7.0. (Figure 12).

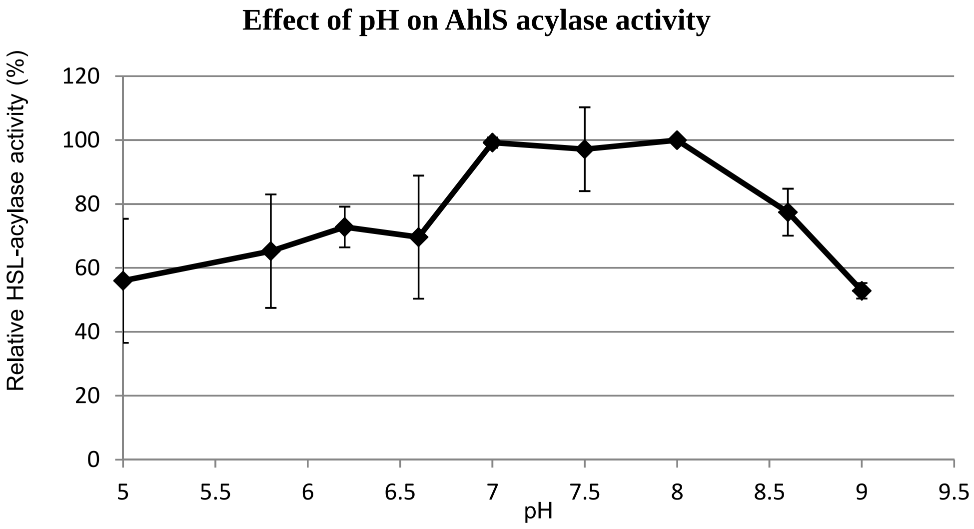


Figure 12 Determination of optimum pH on HSL-acylase activity. Purified enzyme shows the high efficiency to degrade substrate at the less alkaline condition of the mixtures.

To study the effect of temperature against HSL-acylase activity, the reaction mixtures were carried out in 0.1 M Tris-HCl (pH 7.0) containing 0.25 μg of HSL-acylase and 0.4 mM of C_{10}HSL. Incubations were at 4, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60°C. The result displayed a range of optimum temperatures. The enzyme retained more than 95% of the highest activity at 25-40°C. HSL-acylase was totally inactivated at 60°C (Figure 13).

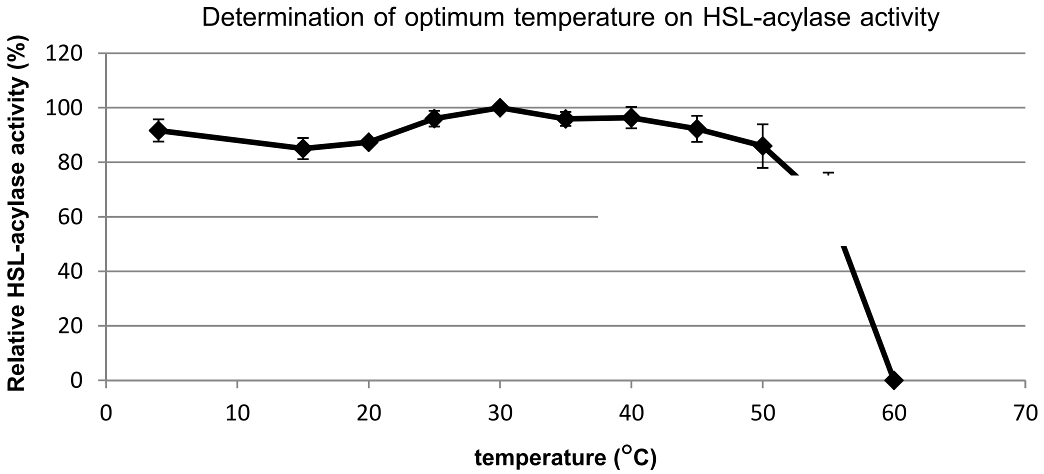


Figure 13 Determination of optimum temperature on HSL-acylase activity, HSL-acylase and C_{10}HSL were mixed in 0.1 M Tris-HCl pH 7.0. The mixture was incubated at temperature in a range of 4 to 60°C. Aliquot of reaction was verified C_{10}HSL leftover on a bioassay plate.

2.4.8.2 Substrate specificity of AhlS-acylase

Determination of HSL-acylase activity against different HSLs substrates was performed based on bioassay system. Purified HSL-acylase from *S. lividans*::*ahlS* inactivated both short chain HSLs which are C_4HSL, C_6HSL, and 3-oxo-C_6HSL and long chain HSLs which are 3-oxo-C_8HSL, C_{10}HSL, and C_{12}HSL (Table 8). The study showed higher relative HSL degrading activity toward long chain HSL in which C_{10}HSL was the most preferred substrate. The activity of this enzyme was not dependent on the substitution of oxo-group on the third carbon atom (Table 8) as there was no significant difference between two substrates, C_6HSL and 3-oxo-C_6HSL ($P \geq 0.05$).

Table 8 Substrate specificities of HSL-acylase against a wide range of AHLs based on bioassay system of *C. violaceum* (CV026) and *A. tumefaciens* NTL4 (pZLR4)

Substrate	Relative HSL-acylase activity (%)			Average	SD
C_4HSL	0.00	0.00	0.00	0.00	0.00
C_6HSL	17.60	12.37	37.48	22.48	13.3
3-oxo-C_6HSL	15.63	40.07	18.41	24.70	13.4
3-oxo-C_8HSL	37.20	46.99	50.92	45.04	7.1
C_{10}HSL	100.00	100.00	100.00	100.00	0.0
C_{12}HSL	88.92	89.27	88.25	88.81	0.5

2.4.8.3 *In vitro* pathogenicity assay of HSL-acylase

To test the ability of purified HSL-acylase to attenuate maceration from *P. carotovorum*, the potato tuber virulence assay was used for investigation. After inoculation of *P. carotovorum* supplemented with 2 μg of HSL-acylase, soft-rot weight was reduced significantly difference ($P<0.05$) as shown in Table 9. Statistical analysis using T-test was shown in Table 10. In the absence of this enzyme, macerated tissue was appeared when inoculated *P. carotovorum* at 10^3 cells per tuber. *P. carotovorum* at 10^3 cells per tuber caused potato tuber maceration whereas adding of 2 μg of HSL-acylase prior inoculation to potato tuber reduced maceration effect of this plant pathogen. NSS or HSL-acylase did not cause any symptoms on potato tubers.

Table 9 Virulence assay of *P. carotovorum* on potato tubers

Inoculum*	Soft rot weight (g)[†]
P. carotovorum	3.27 ± 0.26[a]
P. carotovorum adding 2 μg of HSL-acylase	1.83 ± 0.47[b]
0.85% NSS	0
2 μg of HSL-acylase	0

*The *P. carotovorum* inoculum equals to 1000 colonies

**Different superscripts indicate statistically significant difference at a confidence level of 95%.

[†] Soft rot weights were measured after inoculation for 72 h; data shows mean of four replicates

Table 10 Statistical analysis of soft-rot weight using T-test

Inoculum	t	df	Sig (2-tail)	95% Confidence interval of the difference	
P. carotovorum	24.970	3	0.000	2.8511	3.6839
P. carotovorum adding 2 μg of HSL-acylase	7.714	3	0.005	1.0736	2.5814

2.4.9 Verification of HSL-degraded products by HPLC

To investigate the product arising from enzymatic hydrolysis of C_{10}HSL, the digested products were determined by HPLC. The analysis of HPLC chromatograms showed both peaks of HSL and C_{10}HSL substances as standard controls (Figures 14 A and B, respectively). Another high single peak was found in buffer and HSL-acylase enzyme (Figure 14 D). As for AHL-acylase-degrading reaction, two distinct peaks with a retention time of 2.8 and 25.6 min were detected, respectively (Figure 14 C).

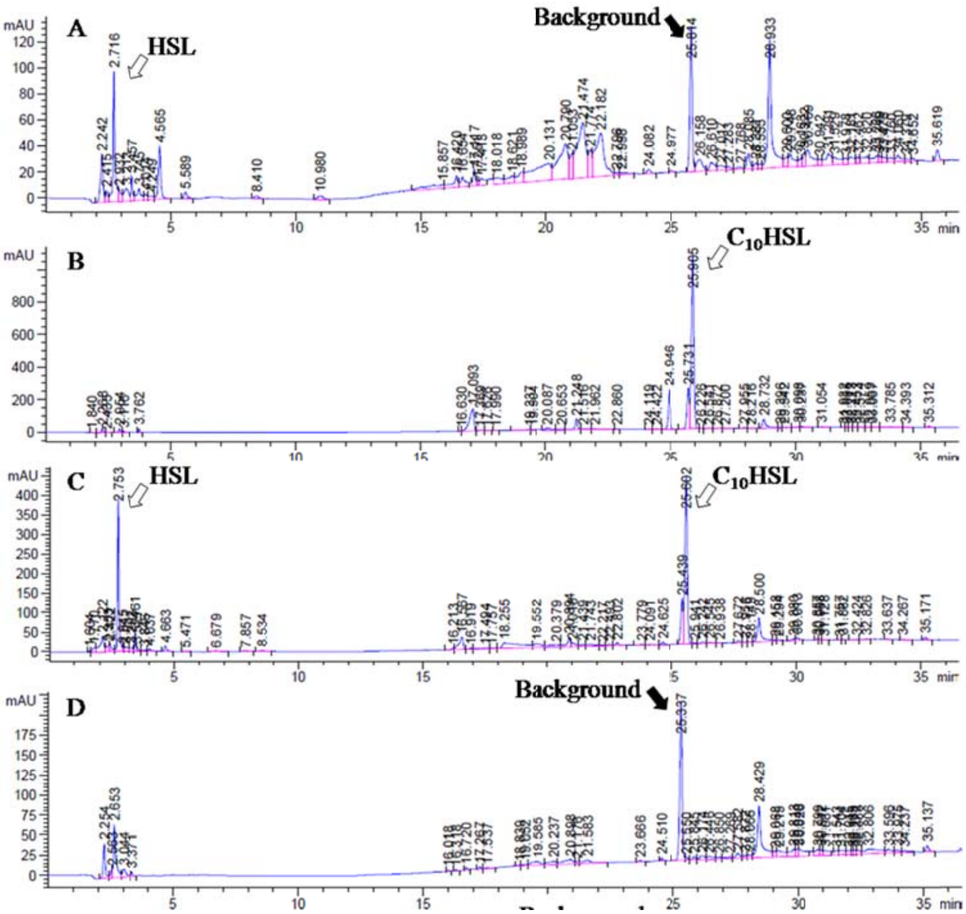


Figure 14 The HPLC chromatogram of decanoylhomoserine lactone (C_{10}HSL) deacylated products; A) homoserinelactone (HSL) or standard C_{10}HSL-deacylated product; B) standard C_{10}HSL; C) the reaction mixture of C_{10}HSL and HSL-acylase; D) buffer.

2.5 สรุปและวิจารณ์ผลการทดลอง ข้อเสนอแนะสำหรับงานวิจัยในอนาคต

From this study HSL-acylase from SPM036 showed a potential to attenuate pathogenicity of *P. carotovorum*. Several studies have shown the effectiveness of QS targeting strategy in controlling virulence of *P. aeruginosa*, *Enterobacteriaceae*, *B. cepacia*, *P. carotovorum*, *Serratial iquefaciens*, *Agrobacterium tumefaciens*, as well as aquatic consortia. These results offer an alternative strategy to control plant pathogen using QS-degrading enzyme from *Streptomyces*. Due to its ability to degrade long chain HSL with moderate activity toward short chain HSL, the quorum quenching enzyme from *Streptomyces* SMP036 might be useful to control several plant and human pathogens. However, more in-depth in vitro and also in vivo investigations are required.

3. Output จากโครงการวิจัยที่ได้รับทุนจาก สกว.

3.1 ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

- Chankhamhaengdecha S, Hongvijit S, Srichaisupakit A, Charnchai P, and Panbangred W. (2013) Endophytic actinomycetes: a novel source of potential acyl homoserine lactone degrading enzymes. BioMed Research International. (Impact factor = 2.880, Q1)

3.2 การนำผลงานวิจัยไปใช้ประโยชน์

- เชิงวิชาการ มีนักศึกษาทำงานในโครงการวิจัยจบระดับปริญญาโท

3.3 กิจกรรมอื่นๆ ที่เกี่ยวข้อง

- การเสนอผลงานวิจัยแบบ Poster presentation และบทความตีพิมพ์จากการเสนอผลงาน

Hongvijit S., Panbangred W., and Chankhamhaengdecha Surang. Screening of quorum quenching Streptomyces spp. for quorum-sensing control of phytopathogenic bacteria. The 23rd Annual meeting of the Thai Society of Biotechnology. Feb 1-2, 2012 Imperial Queen's Park Hotel Bangkok, Thailand

Chankhamhaengdecha S., Srichasupakit A, Panbangred W., Identification of N-acyl homoserine lactone acylase from Streptomyces sp. and its application for quenching quorum sensing. dependent phytopathogenic bacteria. TRF-CHE meeting Oct 15-17 2552 เพชรบุรี

Hongvijit S., Srichasupakit A, Chankhamhaengdecha S., Panbangred W., and. Detection and analysis of N-acyl homoserine lactone acylase from Streptomyces sp. The 4th AG-BIO/PERDO Graduate Conference on Agricultural Biotechnology and UT-KU Joint Seminar. Dec 9-10, 2010 Nakhon Pathom.