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Enhancing the production of syringomycin E in *Pseudomonas syringae* pv *syringae* by random mutagenesis and molecular characterization of the *SyrB1* gene

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Syringomycin E (SRE) is a phytotoxin produced by *Pseudomonas syringae pv syringae* with high potential as a safe and effective therapy for the control of human fungal infections and as a preservative for the food industry. In this study, 27 strains of *P. syringae* pv *syringae* were isolated from plums, potatoes, apricots and peaches, of which *P. syringae* pv *syringae* 120 (PS120) showed the highest SRE production levels. Furthermore, random mutagenesis induced by ultraviolet (UV) radiation resulted in the generation of a *P. syringae pv syringae* mutant that produced 30% more SRE than the parental strain PS120. To elucidate molecular mechanism underlying the higher SRE production ability of the mutant strain, *syrB1* and *syrB2* genes, which are known to be involved in SRE production, were cloned and sequenced. The nucleotide and amino acid sequences analysis showed that UV radiation induced numerous mutations at the AMP binding site on adenylation domain of *syrB1*, while no mutation was detected in *syrB2* gene. Real-time polymerase chain reaction (PCR) results showed that expression of *syrB1* gene in mutant strain was six-fold higher than that of PS120 strain. Taken together, these results suggest that the mutations at AMP binding site and overexpression of *syrB1* were responsible for increased biosynthesis of SRE in *P. syringae pv syringae*.

Key words: Antifungal protein, syringomycin E, UV mutagenesis, *Pseudomonas syringae* pv *syringae, syrB1*.

INTRODUCTION

The widespread resistance to commonly used antimicrobials and an increasing public health concern about the presence of chemicals in the food-chain have stimulated search for novel, effective and safe

antimicrobial compounds. As a result, new resources and approaches are being explored to identify/develop the next generation of antimicrobials. Syringomycin has emerged as a new class of peptide presenting strong antimicrobial activity. For example, numerous studies have demonstrated the effectiveness of syringomycin E (SRE) against multiple filamentous fungal and yeast pathogens (Mazu et al., 2016; Im et al., 2003; Bender et al., 1999; De Lucca and Walsh, 1999; Fukuchi et al., 1992). The SRE can kill 95 to 99% of *Aspergillus* strains (*Aspergillus flavus, Aspergillus niger,* and *Aspergillus fumigatus*) and *Fusarium* (*Fusarium moniliforme* and *Fusarium oxysporum*) in concentrations from 1.9 to 7.8 µg/ml, but is non-toxic to mammalians and plant cells (De Lucca and Walsh, 1999; Takemoto et al., 2010). The SRE is also more effective at killing germinated spores of *Aspergillus* and *Fusarium* species than other peptides such as cecropin A, cecropin B and dermaseptin (De Lucca and Walsh, 1999). Kawasaki et al. (2016) showed that SRE inhibits 50 and 90% of *P. ultimum* oospore germination at 31.3 and 250 µg/ml, respectively.

In vitro growth inhibition experiments have further shown that SRE is toxic to a number of fungi such as *A. fumigatus* (opportunistic fungal pathogen) and *Mucor* species, *Trichophyton* species (parasitic skin fungus), responsible for causing fungal infections in humans (Muedi et al., 2011). In addition, the potential of SRE as an effective preservative for agricultural products has also been demonstrated; SRE was reported to be effective against a range of fungi involved in food spoilage including *Penecillium digitatum* in lychee, and *Aspergillus, Rhizopus* and *Fusarium* in orange, lychee, mango and dragon.

Syringomycin E, a natural phytotoxic, is a small cyclic lipodepsinonapeptide (ca. 1,200 Da) produced by the plant bacterium *P. syringae pv. syringae* (Guenzi et al., 1998; Raaijmakers et al., 2006). *P. syringae pv. syringae* also produces the large cyclic lipodepsipeptide, syringopeptins (Bensaci et al., 2011). The syringomycin gene cluster in *P. syringae pv. syringae* contains four genes involved in the biosynthesis of syringomycin E, including *SyrB1, SyrB2, SyrC* and *SyrE* (Guenzi et al., 1998); of which expression levels are regulated by *salA, syrG,* and *syrF* (Wang et al., 2006; Vaughn and Gross, 2016). *SyrB1* and *syrB2* function in generating one of the unusual amino acids of SRE scaffold, 4-Cl-$_L$-Thr residues. *SyrB1* gene with approximately 2 kb in length encodes for a 66 kDa enzyme consisting of an adenylation (A) domain which is responsible for amino acid selection and activation, and a thiolation (T) domain which is responsible for thioesterification of the activated substrate (Bender et al., 1999). The A domain catalyzes the activation and binding of L-Thr to phosphopantetheninyl in T domain, where it undergoes halogenation catalyzed by SyrB2 to create 4-Cl- thr-S- SyrB1 (Vaillancourt et al., 2005). Next, SyrC enzyme transfers the 4-Cl-Thr group from SyrBs1 to SyrE to create the complete SRE (Singh et al., 2007). Syringomycin is a cyclic lipodepsinonapeptide composed of a 3-hydroxy fatty acid tail. SRE, which contains a 3-hydroxy dodecanoic acid tail, is the major form produced by *P. syringae pv.syringae* strain B301D (Bender et al., 1999). Syringomycin is synthesized via a nonribosomal peptide synthetase system (Bender et al., 1999; Zhang et al., 1995). The syringomycin gene cluster is located in a 55 kb DNA region in the genome of B301D strain containing genes that are responsible for biosynthesis, regulation, and secretion of syringomycin (Lu et al., 2002; Quigley et al., 1993; Zhang et al., 1995, 1997).

The lower production efficiency of SRE by *P. syringae pv. Syringae* has been the major barrier to its cost-effective production and industrial application. Currently, much effort is focused on finding new ways to improve SRE production efficiency and develop SRE-based fungicides. Hence, the key objectives of this study was to determine if exposure to UV could be used to generate *P. syringae pv. syringae* mutants with enhanced SRE production efficiency and to examine molecular mechanisms, especially the role of *syrB1* and *syrB2* genes.

MATERIALS AND METHODS

Bacteria isolation

P. syringae pv. *syringae* was isolated from stone fruit trees such as plums, apricots or peaches, and potatoes in Hanoi, Vietnam according to the method of Mohammadi et al., (2010). Briefly, biological samples were surface-sterilized by 70% ethanol for 2 min before incubation in distilled water with shaking for 2 h. 100 µl of suspension were plated on CMB agar supplemented with 5% sucrose. Colonies that produced fluorescence under UV light were picked and grown on King's B media; gram stained and identified by standard biochemical assays for *Pseudomonas* species. Identification methods include catalase, oxidase, arginine dihydrolase, gelatin hydrolase and nitrate reductase assays together with levan production. Strains with correct biochemical activity were finally confirmed as *P. syringae* by 16S rDNA analysis.

Syringomycin production bioassay

The ability to produce SRE of *P. syringae pv syringae* was determined as described previously (DeVay and Gross, 1977). In brief, bacterial cells were grown overnight in nutrient broth yeast (NBY) medium at 25°C. The cells were harvested by centrifugation, washed with sterile deionized water, and adjusted to a final cell density of ~2×10^8 CFU/ml. Five microliters of the bacterial suspension was inoculated to the previously prepared PDA plates

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Table 1. PCR primer sequences.

Gene	Primer	Sequence	Product size (kb)
16SrRNA	27F	5'-AGAGTTTGATCMTGGCTCAG-3'	1.4
	1492R	5'-CGGTTACCTTGTTACGACTT-3'	
SyrB1	Forward	5'-GTTAGTCGGTGCTGAAATGC-3'	2.2
	Reverse	5'-CATTTCTTCTGGCGAGTAAGC-3'	
SyrB2	Forward	5'-GAGCAAGCTCAAGACACATTAC-3'	1.3
	Reverse	5'-GCATCGGAGGAAATAGTCATGG-3'	

and incubated at 25°C for 4 days. The inoculated plates were overlaid with *Geotrichum candidum* VTCC-Y-0483 as an indicator organism and were further incubated for 24 h at 25°C in the dark. Clear zones of fungi growth inhibition around bacterial colonies were considered as an indicator of syringomycin production. One unit of antifungal activity was defined as the amount of the final dilution of cell-free extract in 1 mM HCl required to completely inhibit the growth of *G. candidum* in the area where a 10 µl droplet was applied on PDA plate (Sinden et al., 1971). Each assay was repeated independently three times with three plates per replicate.

The presence of syringomycin in *P. syringae* fermentation broth was confirmed by HPLC and LC-MS as previously described (Singh et al., 2007). Briefly, cells from 24-h cultures in NBY media at 25°C were pelleted by centrifugation, and supernatant extracted with water-saturated *n*- butanol at pH 2 before purification by cation-exchance chromotography and analysis by HPLC. HPLC was carried out with 20 µl of sample loaded onto a octadecylsilane bounded silica (5 µm) column (25 cm × 4.6 mm). The mobile phase, a mixture of 50 volume of 0.2% (w/v) acetic acid in water, 25 volume of acetonitrilc and 25 volume of methanol, was filtered and de-gased. HPLC was performed at a flow rate of 1 ml/min and observed at 240 nm. LC-MS analysis was performed on an LCMS-QP8000 a spectrometer (Shimadzu, Japan) with a Vydac C18 LC-MS column (Thermo Fisher Scientific, USA).

Random mutagenesis

P. syringae pv syringae parental strain was cultured at 180 rpm at 26°C on nutrient broth (NB) medium for 16 h. Biomass was collected by centrifugation; pellet was re-suspended in sterile distilled water to an OD of 0.5 to 0.6 and subjected to a UV light treatment with power of 40 W at various time doses. After 48 h culturing at 26°C, cells were plated to count the surviving colonies.

DNA preparation

P. syringae pv *syringae* mutant and parental strains were grown for 48 h at 28°C in SRM medium and collected by centrifugation at 16128 rpm for 2 min. Total DNA extraction was performed using PureLink™ Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer's instructions. DNA was stored at -20°C until used.

Cloning and sequencing of 16S rRNA, syrB1 and syrB2 genes

The 16S rDNA, syrB1 and syrB2 genes were amplified by PCR with the specific primer pairs (Table 1) using Thermo cycler engine (AB system). The reaction mixture (25 µl) containing 12.5 µl of master mix (Thermo Fisher Scientific, USA) 10 pmol of each forward and reverse primers and 2 µl total DNA. The PCR conditions were as follows: 94°C for 3 min, followed by 40 cycles of 94°C for 30 s, 56°C for 40 s, 72°C for 1 min 30 s, and a final extension at 72°C for 7 min samples were then stored at 4°C until used. The PCR products were purified using the GENCLEAN® II Kit (Q-BIO gene, Carlsbad, CA, USA), and directly cloned into a TOPO TA cloning vector (Invitrogen, USA) according to the manufacturer's instructions and sequenced with ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, USA) using M13 forward primer and M13 reverse primer.

Real-time RT-PCR assay

Total cellular RNA was extracted using the TRIzol reagent (Invitrogen, USA) as per the manufacturer's instructions. The extracted RNA was treated with DNase I at 37°C for 3 h to remove DNA. Reverse transcription was carried out with the Superscript TM III First-Strand Synthesis System (Invitrogen, USA) according to the manufacturer's instructions. In detail, 500 ng mRNA was used as template for reverse transcription reaction. The cDNA was then used as a template for qualitative real-time PCR analysis using specific primers (Table 2). As a control for the input RNA, level of a housekeeping gene 16S rRNA was also assayed. The data was analyzed by LightCycler 2.0 software (Roche, USA).

RESULTS

Screening of *P. syringae* strains for SRE production

In this study, a number of bacterial strains were isolated from leaf tissues of plums, peaches, apricots, tomatoes and screened for production of syringomycin as described earlier. Among them, 120 strains were *P. syringae pv syringae* strains as indicated by biochemical assays and 16S rDNA analysis (data not shown). 14 strains produced antifungal metabolites characteristics of syringomycin and PS120 strain showed the highest amount of antifungal activity (36 U/ml) (Table 3).

Characterization of antifungal compounds produced by strain PS120

The antifungal compounds of PS120 were extracted and purified and analyzed by SDS-PAGE; similarly extracted metabolites from a non-syringomycin producing strain

Table 2. Real-time PCR primer sequences.

Gene	Primer	Sequence	Product size (bp)
SyrB1(PS120)	Forward	5'-GAGCACATCGTGCGTGACAG-3'	154
	Reverse	5'-TCGATACGGGTGTCTTCAGC-3'	
SyrB1(PS120-15)	Forward	5'-TGGTTATTACACAGGAGCCTTGG-3'	260
	Reverse	5'-CGACAATTTCCTGAGCGCAC-3'	
SyrB2(PS120)	Forward	5'-TTAACTGCGGAACAGCGTGC-3'	179
	Reverse	5'-CATAGTTGGCGATGTTGGTAC-3'	
SyrB2(PS120-15)	Forward	5'-TTAACTGCGGAACAGCGTGC-3'	189
	Reverse	5'-CGGTCATAGTTGGCGATGTTG-3'	
16SRNA	Forward	5'-ACACATGCAAGTCGAACGAG-3'	100
	Reverse	5'-CGTCCGTTTCCAGACGTTAT-3'	

Table 3. Syringomycin production levels of the isolated *P. syringae* strains.

S/N	Strains	Antifungal activity (U/ml)	No.	Strains	Antifungal activity (U/ml)
1	PS13	17	8	PS57	12
2	PS41	13	9	PS17	15
3	PS28	10	10	PS89	27
4	PS53	20	11	PS52	34
5	PS120	36	12	PS15	15
6	PS54	17	13	PS25	25
7	PS32	26	14	*P. syringae* ATCC55389	31

were used as a negative control. Fifteen micrograms of the purified compound and an equal amount of standard SRE (Sigma, USA) were separated on 12% polyacrylamide gel, and then stained with Coomassie-blue. The results showed that the purified compound was a protein with MW similar to that of the standard SRE (Figure 1). To determine whether this compound was syringomycin, its antifungal activity was examined against *G. candidum* strain VTCC-Y-0483 using a standard plate bioassay. It was observed that this compound produced large zone of inhibition against *G. candidum*. In contrast, no zone of inhibition was observed with the compound extracted from non-syringomycin producing strain (Figure 2).

Further analysis of the purified compound by HPLC revealed that it has the same retention time as the standard SRE (Sigma, USA). The standard SRE appeared at 4.283 min, while that from *P. syringae pv syringae* PS120 was at 4.281 min (Figure 3), indicating the purified compound was SRE. The identity of the purified compound was further analysed using ESI mass spectroscopic analyses. It was found that the purified compound generated MH+ species with molecular weights of 1224 to 1226 and major fragmentation species with molecular weight of 613 and 614 (Figure 4) that are

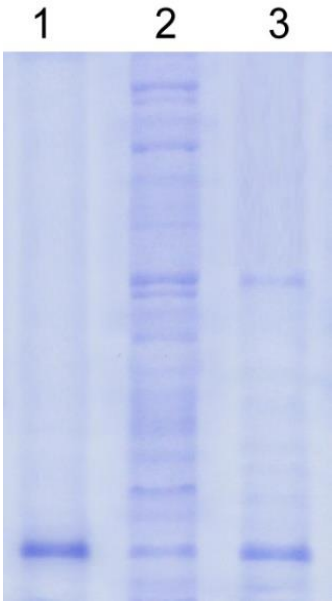

Figure 1. SDS-PAGE gel electrophoresis of SRE product. 1, standard SRE; 2, fermentation supernatant; 3, purified SRE of PS120 strain.

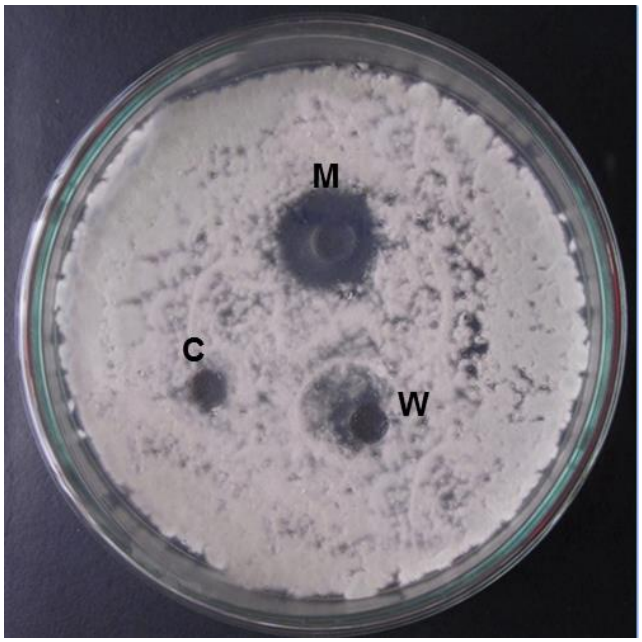

Figure 2. Plate bioassays for syringomycin production by *P. syringae pv. syringae* parental strain PS120 and mutant strain PS120-15. Indicator strain *G. candidum* VTCC-Y-0483 was overlaid on pre-inoculated bacterial suspension and zones of growth inhibition were observed after 24h at 25°C. C, the compound extracted from non-syringomycin production strain as negative control; W, the compound extracted from PS120; M, the compound extracted from PS120-15 mutant.

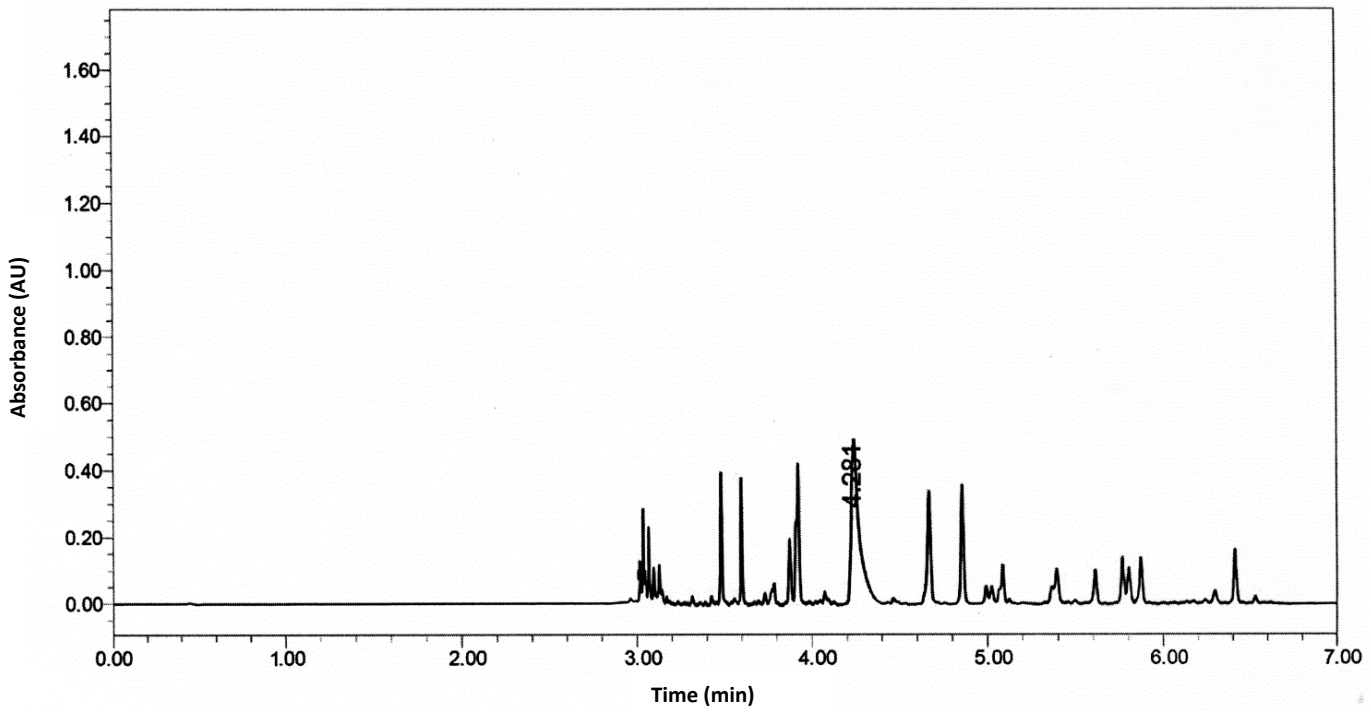

Figure 3. HPLC analysis of purified syringomycin. Purified fermentation broth of *P. syringae pv syringae* PS120 was analyzed using HPLC and showed a major peak among others at retention time of 4281 min, matched retention time of standard SRE at 4283 min, indicating the presence of syringomycin in fermentation broth.

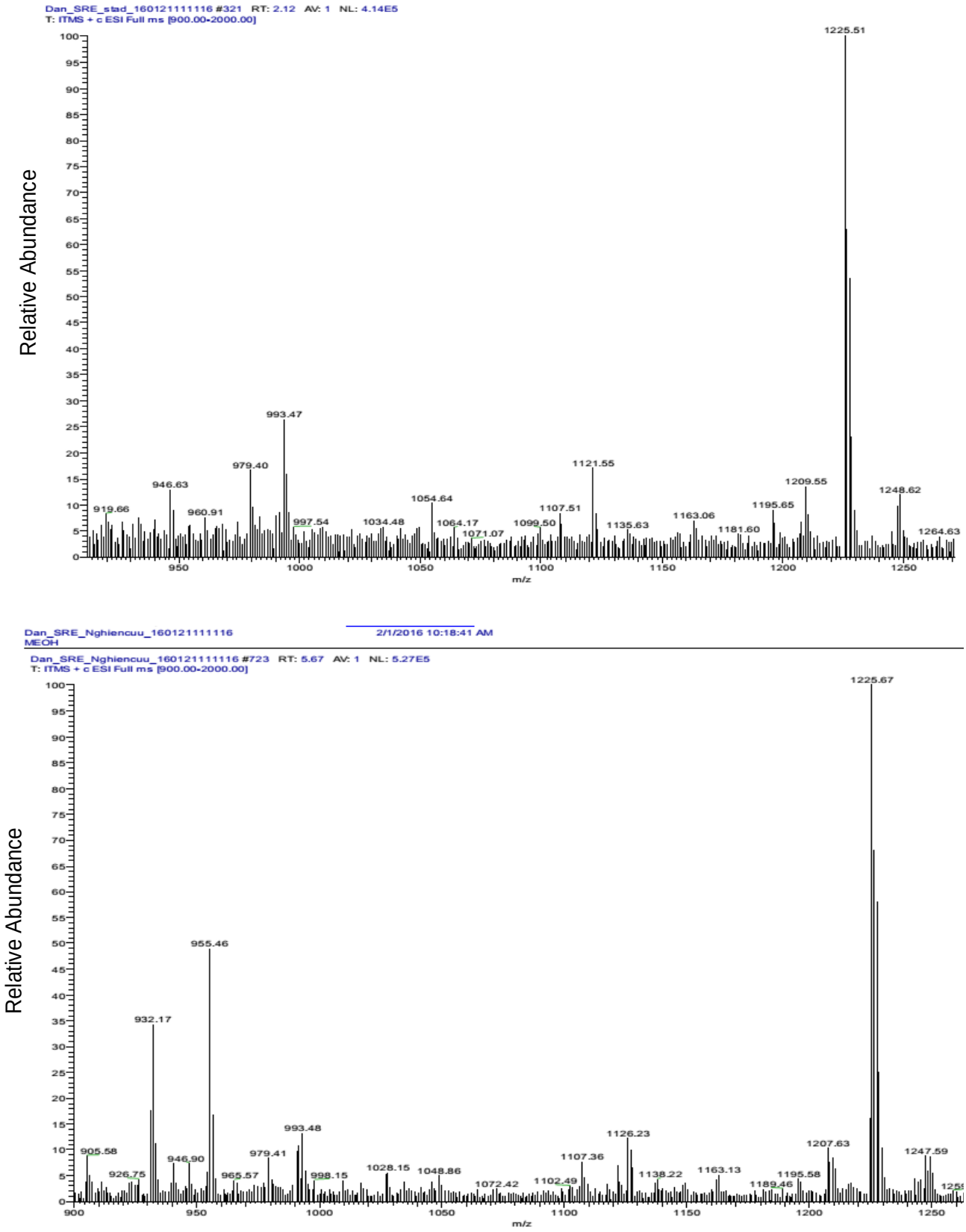

Figure 4. ESI mass spectroscopic analysis of the purified syringomycin. Purified compounds gave MH+ species with molecular weights of 1224-1226 and major fragmentation species with molecular weight of 613 and 614, compatible with the proportion of 2 isoforms of chlorine (^{35}Cl and ^{37}Cl), which are characteristics of SRE.

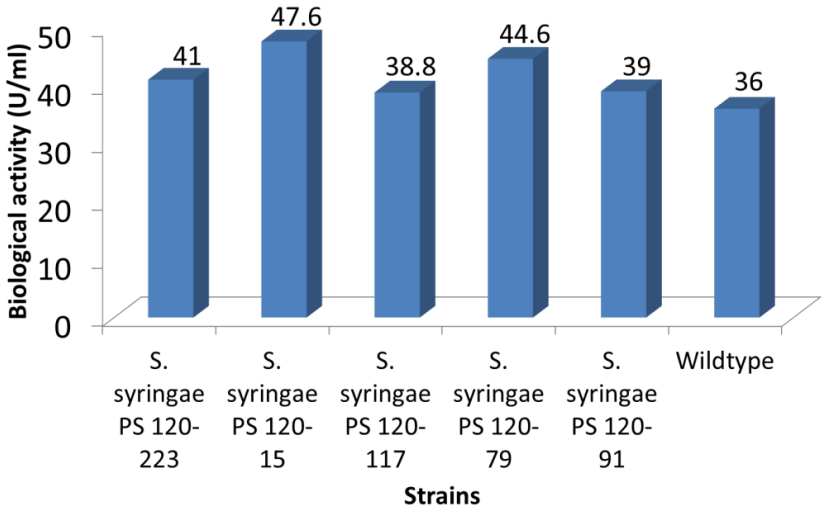

Figure 5. Biological activity of SRE from top five mutants. Among all mutants, five trains PS120-223, -15, -117, -79 and -91 showed the highest increase in SRE production, with 20 to 32.3% increase comparing to parental strain.

consistent with the proportion of 2 isoforms of chlorine (^{35}Cl and ^{37}Cl), the key characteristics of SRE.

Enhancing the production level of SRE

PS120 strain was exposed to various doses of UV to create mutant strains with enhanced SRE production. Following initial screening, 334 mutants were selected that survived after exposure to UV light for 180 s. The SRE production capacity of these mutants was assessed from their ability to inhibit the growth of *G. candidum.* The mutants with increased SRE production were selected following the method described by DeVay and Gross (1977). Of the 334 mutants, five were found to produce significantly higher levels of SRE (20 to 32.2%) compared with the parental PS120 strain (Figure 5). *P. syringae* mutant (PS120-15) showed the highest SRE production (47.6 U/ml), that is 32.2% higher than the parental strain (36 U/ml). This result was further supported by a larger inhibitory zone to *G. candidum* induced by PS120-15 compared to PS120 (Figure 2). PS120-15 mutant was further studied to understand the molecular mechanism responsible for the increased SRE production.

Analysis of syrB1 and syrB2 genes and their expression

SyrB1 and *syrB2* genes from both wild type (PS120) and mutant (PS120-15) strains were PCR amplified using specific primers as described earlier. The PCR products were then cloned, sequenced and analyzed using Bioedit (Tom Hall, Ibis Biosciences). While no change in *syrB2* sequence was detected following UV treatment, an additional nucleotide fragment was found inserted in *syrB1* sequence in PS120-15 mutant that led to the insertion of an amino acid fragment in syrB1 protein (Figure 6). Further analysis revealed that the inserted fragment was located at the AMP binding site (between the position of 304-408 of syrB1), an important element in the biosynthesis of SRE (Kleinkauf and Von Dohren, 1996).

The expression of *syrB1* and *syrB2* in wildtype and mutant was then measured by real-time PCR assay. The data showed that expression level of syrB1 in mutant strain was six-fold higher than that in the wild type strain (Figure 7). On the other hand, the expression level of *syrB2* remained unaffected (data not shown). This suggests that the insertion of amino acid fragment in syrB1 was associated with increased expression of *syrB1* and that this may be responsible for increased production of SRE in the mutant strains.

DISCUSSION

Because of the SRE antimicrobial activities and due to the fact that it has no impacts on both human health and environment, SRE has been the focus of much research with ultimate aim to develop new generation of antimicrobials. SRE has been shown to be an effective fruit preservative agent against a range of fungal species including *G. candidum, Rhodotorula pilimance, Botrytis cinerea, Fusarium, Pythium ultimum, Rhizoctonia, Greeneria uvicola, Aspergillus japonicus, Mucor* species,

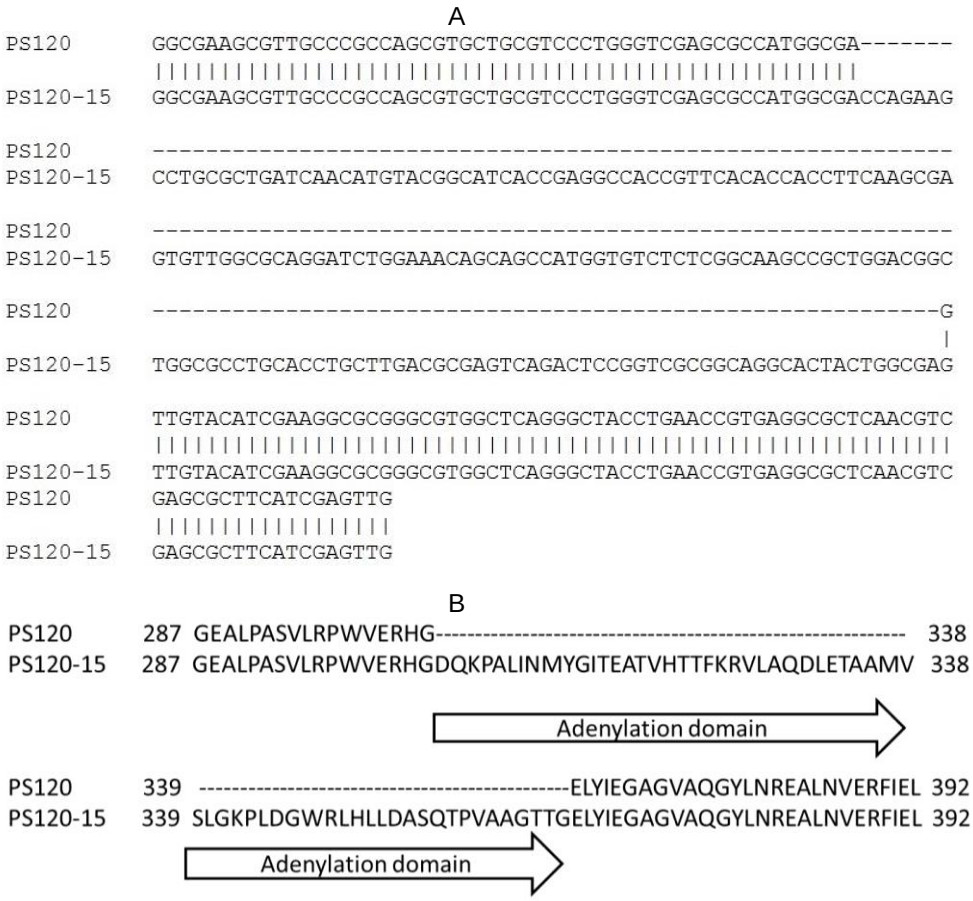

Figure 6. Comparison of nucleotide sequence (A) and amino acid sequence (B) of SyrB1 of the Ps120-15 with PS120. Sequence analysis using Bioedit showed an insertion in SyrB1 sequence in PS120-15 mutant strain leading to the insertion of an amino acid fragment located at the AMP binding domain of SyrB1 protein.

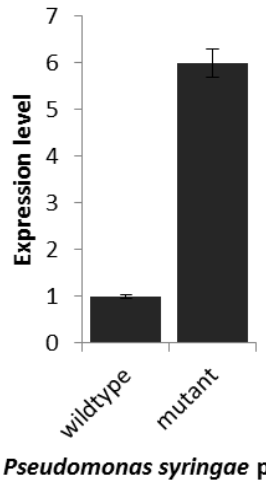

Figure 7. Comparing expression of SyrB1 in mutant and wildtype strains at transcriptional level. Expression level of SyrB1 in both strains was measured by realtime PCR using specific primers and normalized against 16S rRNA. Mutant strain expressed SyrB1 six times higher than that of parental strain.

Trichophyton species*, Curvularia brachyspora, Nigrospora sphaerica, Penicillium thomii* and *Penicillium sclerotiorum* (Breen et al., 2015; D'aes et al., 2010; Bull et al., 1998; Janisiewicz and Bors, 1995). In addition, it exhibits toxicity against Hela cells at 20-times higher concentrations than its fungicidal concentration that kills 95% of pathogenic fungi (De Lucca et al., 1999).

A number of studies have been conducted to isolate *P. syringae* pv. *Syringae* strains which produce SRE (Janisiewicz and Bors, 1995; Cirvilleri et al., 2005; Singh et al., 2007). In this study, a total of 260 *P. syringae pv syringae* strains was isolated and 27 strains produced antifungal metabolites characteristics of syringomycin was selected. Among these, PS120 showed the highest amount (36 U/ml). However, the production efficiency of SRE of PS120 is still low and has been the major barrier to its cost-effective production and industrial application. With the aim to create *P. syringae* pv. syringae mutants with enhanced ability to produce SRE, random mutagenesis approach was applied using UV light treatment. The effectiveness of UV light induced mutagenesis has

previously been reported (Ikehata and Ono, 2011), such as to increase production of clavulanic acid by *Streptomyces clavuligerus* (Lee et al., 2002) and bio-insecticides production in *Bacillus thuringenesis* (Ghribi et al., 2004). Interestingly, five of the 334 mutants, resulting from random mutagenesis induced by exposure of PS120 strain to UV light, produced significantly higher levels (20 to 32%) of SRE than the parental strain PS120. Out of the 334 mutants screened, a mutant strain (PS120-15) producing the highest level of SRE was selected that synthesized SRE up to 30% higher than its parental strain. These findings are consistent with the results of earlier studies that used UV light-induced mutagenesis to enhance production of bioinsecticides by *B. thuringiensis* (Ghribi et al., 2004) and clavulanic acid by *S. clavuligerus* (Muedi et al., 2011).

Although significant efforts have been made toward understanding the molecular mechanisms that trigger and control the biosynthesis of SRE, much is still unknown about this process (Roongsawang et al., 2011). Transposon mutagenesis performed by Xu and Gross (1988) and Zhang and Gross (1995) has proven that a chromosomal region larger than 25 kb is involved in the biosynthesis of SRE. Within this region, four genes, namely, *syrB, syrC, syrD* and *syrP* have been sequenced and partially characterized (Martinie et al., 2015; Singh et al., 2007; Gross, 1991; Zhang and Gross, 1997; Quigley et al., 1993). *SyrD* has similar sequence to the ATP binding cassette transporter superfamily, thus, it is hypothesized that *syrD* product is involved in the transportation of SRE across the cytoplasmic membrane (Quigley et al., 1993). Further sequencing investigation by Guenzi et al. (1998) showed that *syrB* is actually organized in two ORFs, *syrB1* and *syrB2* encoding a 67 kD protein and a 36 kD protein, respectively. The 67 kD protein encoded by *syrB1* carries all known conserved regions of the amino acid binding modules of peptide synthetases.

It is well documented that *syrB1* and *syrB2* are involved in the biosynthesis of SRE of *P. syringae* pv *syringae*. It was hypothesized that UV radiation is likely to induce mutations and/or enhance the expression of these two enzymes to influence the production of SRE directly or indirectly. To test this hypothesis, *syrB1* and *syrB2* genes was cloned and sequenced. Previous studies have shown while *syrB1* carries all consensus sequences of amino acid binding module of peptide synthetases, *syrB2* sequence shows little to no consensus to other known peptide synthetase (Guenzi et al., 1998). In this study, the sequence analysis showed that an insertion occurred at AMP binding site in *syrB1* gene at nucleotide position 304 to 408, which belongs to adenylation domain responsible for recognizing and activating a specific amino acid (Kleinkauf and Von Dohren, 1996; Marahiel et al., 1997). Previous study has indicated that SRE is synthesized by nonribosomal mechanism (Singh et al., 2007) with the participation of *syrB1, syrB2, syrC* and *syrE*. Nonribosomal peptide synthetases, such as *syrB1* and *syrB2*, are multimodular enzymes that produce peptide via thiotemplate mechanism independent of ribosomes. Well-known products of nonribosomal peptide synthetases are antibiotics (such as penicillin, erythromycin, vancomycin), immune suppressants (such as cyclosporine and rapamycin) and antitumor agents (Lee et al., 2005). Minimal nonribosomal peptide synthetase module consists of an AMP-binding adenylation domain (A) for substrate recognition and activation; and a thiolation (T, also called peptidyl carrier protein) domain, downstream of A domain, functioning as cofactor binding site (Schwarzer and Marahiel, 2001). This A domain is highly conserved; therefore, mutations in AMP-binding site might lead to the improvements in SRE production ability of the mutant strain.

To test whether the UV treatment induced the expression of syrB1, a realtime PCR assay was conducted. The real-time PCR result showed that expression level of *syrB1* in mutant strain was six-times higher compared with the wildtype strain. The increased expression level of *syrB1* might be due to some acquired mutations in its promoter region and responsible for the increased biosynthesis of SRE. It is likely that insertion of new fragment of amino acid into the adenylation domain of SyrB1 increases its amino acid activation rate, indirectly boosting SRE biosynthesis. At present, the molecular mechanism(s) underlying the higher SRE production ability of the mutant strain was not fully understood. This study showed a successful enhancement of SRE production in *P. syringae pv syringae* using UV induced mutagenesis, proving involvement of at least one gene, *syrB1*. These results shed more lights into the biosynthesis pathway of SRE in *P. syringae*, giving directions for further characterization of the pathway in the future.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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