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Molecular Identification of 16S rDNA and Polyketide Synthase Genes of Antagonist Bacteria against *Xanthomonas oryzae* Pathovar *oryzae* from Rice Phyllosphere

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ABSTRACT

Biocontrol using antagonist agent is one of environment-friendly method of controlling bacterial leaf blight disease in rice field. Eight antagonistic bacteria against *Xanthomonas oryzae* pathovar *oryzae* as well as the causal bacterial leaf blight disease in rice, have been isolated from rice phyllosphere of Wonogiri and Sukoharjo Regency, Central Java, Indonesia, using dual plate method. The aims of this study were to identify molecularly of 16S rDNA and polyketide synthase (PKS) genes of antagonist bacteria. The PKS gene is recorded as one of antibiotic compounds class which encode the polyketide biosynthesis. The amplification of 16S rDNA gene was performed using 63f and 1387r primers, while PKS gene detection was performed using degKS2F.gc and degKSR5.gc primers. The nucleotide sequences of 16S rDNA and PKS genes was aligned using GenBank database and BLAST-N program from NCBI site was operated. The results showed that the eight isolates identity of SH2a, MO142, MO22g, MO34h, MO34i, MO34j, MO43a and MO63j were Pseudomonadaceae SH2a, *Pantoea* sp. MO142, *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j, *Pantoea* sp. MO43a and *Pantoea* sp. MO63j, respectively. Bacterial antagonists of PKS genes have similarities with the gene of nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) *Lysobacter lactamgenus*. This indicates that the antagonist mechanism of antagonist bacteria is antibiosis.

Key words: Antagonist, phyllosphere, PKS gene, 16S rDNA gene, *Xanthomonas oryzae* pv. *oryzae*

INTRODUCTION

Xanthomonas oryzae pathovar *oryzae* are bacteria that caused Bacterial Leaf Blight (BLB) in rice field and causing a significant economic losses in almost all country producing rice in Asia (Nayak *et al*., 2008). Biocontrol approach using antagonist agent has been considered as one of the environment-friendly method of BLB disease in rice plantation (Donghua *et al*., 2013).

The identification of antagonist agent needs to be done in order to facilitate its development as a biocontrol agent of *Xanthomonas oryzae*. The 16S rDNA gene which is believed encode 16S rRNA, is widely used as a genomics marker for the prokaryotic identification (Vinje *et al*., 2014) due to the

fact that these genes are universally spread among the bacteria. The advantages of using the 16S rDNA gene were have highly conserved regions to construct a universal primer and highly variable regions for species identification (Nossa *et al*., 2010). The gene has been stored in GenBank and therefore can be used to identify bacteria that cannot be cultured (Drancourt *et al*., 2000).

The antagonist mechanism which is conducted by antagonist bacteria to phytopathogen microbes include antibiosis, competition and production of lytic enzymes (Bouizgarne, 2013). The Induced Systemic Resistance induction (ISR) (Pal and Gardener, 2006), as well as Plant Growth Promoting Rhizobacteria (PGPR) (Shivalingaiah and Umesha, 2013). The antibiosis is recorded as a mechanism that plays an important role in the suppression of plant diseases by antagonist bacteria (Lo, 1998; Mishra *et al*., 2013). One of the genes which were involved in many antibiotics biosynthesis is polyketide synthase (PKS) gene, that involved in encoding the polyketide biosynthesis enzymes.

Eight isolates phyllosphere bacteria antagonistic against *Xanthomonas oryzae* have been sampled and isolated from Wonogiri and Sukoharjo Regency, Central Java Province, Indonesia. Usually searching of biocontrol agent toward *Xanthomonas oryzae* were mostly done from rhizosphere (Donghua *et al*., 2013; Shivalingaiah and Umesha, 2013) and only a small portion was conducted from phyllosphere (Gangwar, 2013). The aims of this study were first to identify molecularly of 16S rDNA genes that can be used to determine the identity of antagonist bacteria. Secondly to identify polyketide synthase (PKS) genes molecularly so that it can be used as an early detection of antagonist mechanism which were applied by antagonist bacteria toward *Xanthomonas oryzae*.

MATERIALS AND METHODS

Media and culture: *Xanthomonas oryzae* was stored in its agar (which is consisted of calcium carbonate ($CaCO_3$) 30 g L^{-1} (Merck), glucose 10 g L^{-1} (Himedia), yeast extract 5 g L^{-1} (Conda) and agar 15 g L^{-1}) in the form of stab and slant agar. Antagonist bacteria isolates (SH2a, MO142, MO22g, MO34h, MO34i, MO34j, MO43a and MO63j) were stored in nutrient agar (Himedia). Antagonistic ability of phyllosphere bacteria against *Xanthomonas oryzae* was tested using a dual plate method (Velusamy *et al*., 2013) on nutrient agar. Nutrient broth (Merck) was used for culturing antagonist bacteria in order to extract their genomic DNA.

Amplification of 16S rDNA gene: Genomic DNA extraction of antagonist bacteria was performed using Presto™ Mini gDNA Bacteria kit (Geneaid) according to the manufacturer's recommendation. Biophotometer was used to look at concentration and purity of genomic DNA. The 1,300 bp DNA fragment generated from genomic DNA was amplified using 63f forward primer (5'-CAGGCCTAACACATGCAAGTC-3') and 1387r reverse primer (5'-GGGCGGWGTGTACAAGGC-3') (Marchesi *et al*., 1998). The PCR mixture was consisted of 1.25 μL for each primer 63f and 1387r (10 pmol); 2 μL DNA template <25 ng μL^{-1}; 8 μL ddH_2O and 12.5 μL kit KAPA2G™ Fast Ready Mix (Kapa Biosystems). The PCR conditions were 95°C for 3 min followed by 30 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 30 sec and finally 72°C for 2 min. Amplicon separation was done by horizontal electrophoresis using 1% agarose on 90 V and 400 mA for 60 min. The agarose gel was then stained with ethidium bromide. The PCR product was then sequenced (At 1st Base Singapore).

Detection of polyketide synthase (PKS) gene: The PKS gene detection was done by amplifying the genomic DNA of antagonist bacteria using forward primer degKS2F.gc (5'-GCSATGGAYCCSCARCARCGSVT) and reverse primer degKSR5.gc (5'-GTSCCSGTSCCRT GSSCYTCSAC) (Schirmer *et al*., 2005). The PCR mixture was done by mixing of 1.25 µL for each primer degKS2F.gc and degKSR5.gc (10 pmol); 2 µL DNA template; 8 µL ddH_2O and 12.5 µL kit KAPA2G™ Fast Ready Mix. The PCR was performed by 30 cycles of 94°C for 40 sec, 55°C for 40 sec and 72°C for 75 sec. Amplicon separation was done by using 1% agarose. After running electrophoresis at 75 constant current for 90 min, the gel was then stained using ethidium bromide. The PCR product was then sequenced (At 1st Base Singapore).

Data analysis: Nucleotide sequences of 16S rDNA and PKS genes was aligned with the GenBank database using BLAST-N program (basic local alignment search tool-nucleotides) from NCBI (National Center for Biotechnology Information) site (www.ncbi.nlm.nih.gov). Percentage of similarities have been obtained from 16S rDNA gene alignment were used to determine antagonist bacteria identity, whereas PKS gene alignment was used to determine the similarities of PKS gene based on the GenBank database. Phylogenetic analysis of KS domain from PKS gene of antagonist bacteria with some other bacteria was conducted with phylogenetic tree construction using Molecular Evolutionary Genetics Analysis (MEGA) 6 software (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

Identity of antagonist bacteria: Rice phyllosphere bacteria that consistently antagonistic against *Xanthomonas oryzae* were SH2a isolate was collected from Wonogiri Regency and MO142, MO22g, MO34h, MO34i, MO34j, MO43a, as well MO63j isolates were collected from Sukoharjo Regency. All have capability with inhibition zone ranging 12-20 mm.

Based electrophoregram on Fig. 1, it was noted that the 16S rDNA gene amplicons of all eighth antagonist bacteria showed single band at ±1,300 bp. The similarities were obtained from the alignment using BLAST-N program, which can be used to determine the identity of antagonist bacteria. Determining identity of antagonist bacteria was carried out according

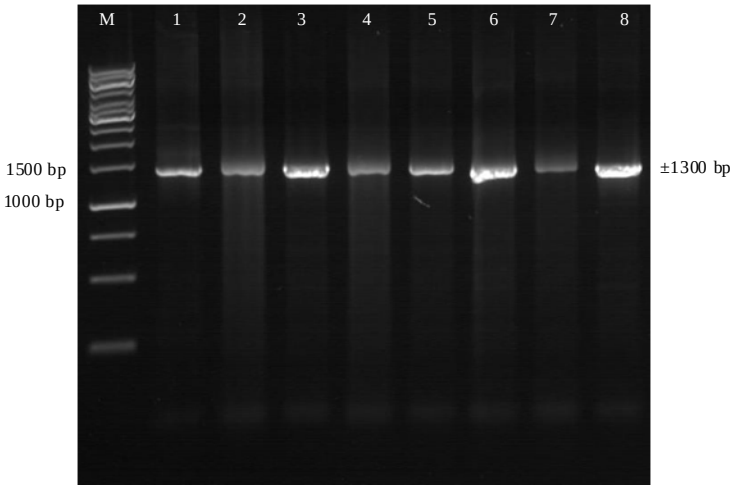

Fig. 1: Electrophoregram of 16S rDNA gene amplicons of the eight isolates tested, 1: SH2a, 2: MO142, 3: MO22g, 4: MO34h, 5: MO34i, 6: MO34j, 7: MO43a and 8: MO63j, M: 1 kb marker

Table 1: Percentage similarity of partial sequence 16S rDNA gene of eight antagonist bacteria to the GenBank database

Bacteria isolates	Related species	Accession	Identity (%)	Antagonist bacteria identity
SH2a	*Pseudomonas fulva* strain 1Y1103	JQ229796.1	93	Pseudomonadaceae SH2a
MO142	*Pantoea* sp. A1059	KC236721.1	98	*Pantoea* sp. MO142
MO22g	*Pantoea* sp. Ca04	KJ427829.1	96	*Pantoea* sp. MO22g
MO34h	*Erwinia* sp. ICB408	HM748066.1	95	*Erwinia* sp. MO34h
MO34i	*Pantoea* sp. Ca04	KJ427829.1	97	*Pantoea* sp. MO34i
MO34j	*Pantoea* sp. Ca04	KJ427829.1	96	*Pantoea* sp. MO34j
MO43a	*Pantoea* sp. Ca04	KJ427829.1	95	*Pantoea* sp. MO43a
MO63j	*Pantoea* sp. CT2	EF585309.1	98	*Pantoea* sp. MO63j

Bosshard *et al*. (2003), in which the ≥99% similarity was recorded as the same species, while the similarity in between 95 and 99% was grouped as the same genus and the similarity index bellow <95% included in the same family. Based on these criteria, it was known that antagonist bacteria SH2a isolate was a bacterium from Pseudomonadaceae family. The isolates of MO142, MO22g, MO34i, MO34j, MO43a and MO63j belongs to the *Pantoea* genus, while MO34h isolate was included to the *Erwinia* genus (Table 1).

Antagonist bacteria which were isolated from rice phyllosphere dominated by bacteria from *Pantoea* genus. According to Bulgarelli *et al*. (2013) that genera of bacteria commonly found in phyllosphere were *Pseudomonas*, *Sphingomonas*, *Methylobacterium*, *Bacillus*, *Massilia*, *Arthrobacter* and *Pantoea*. *Pseudomonas*, *Pantoea* and *Erwinia* are genus that commonly used as an antagonist bacteria. Based on this research, there were known that the genus has wide inhibitory spectrum against phytopathogen microbes, started by *Pseudomonas*, *Pantoea* and *Erwinia* genera, respectively.

It was recorded that species within the genus *Pseudomonas* has great potential to be developed into biocontrol agents for various phytopathogen microbes. Several examples that support this statement were the ability of *Pseudomonas fluorescens* against *Xanthomonas oryzae* (Shivalingaiah and Umesha, 2013), *P. fluorescens* 1100-6 against *Rhizobium vitis* causes tumors in wine (Eastwell *et al*., 2006), *P. fluorescens* and *P. putida* against *P. solanacearum* causes wilt disease in mulberry (Nuraeni and Fattah, 2007); *P. rhizosphaerae* JAN against *Erwinia amylovora* causes fire blight disease (Paternoster *et al*., 2010) and *P. brassicacearum* J12 against *Ralstonia solanacearum* causes bacterial wilt in tomatoes (Zhou *et al*., 2012).

Related research in using *Pantoea* as antagonist bacteria against phytopathogen bacteria has been conducted by several researchers including Sammer *et al*. (2009) using *Pantoea agglomerans* against *Erwinia amylovora* and *Pseudomonas syringae* pv. glycinea (Sammer *et al*., 2012) and *P. ananatis* BRT175 against *E. amylovora* causes fire blight disease on apples and pears (Walterson *et al*., 2014). Accordingly *Pseudomonas* spp., *Pantoea* spp. and *Erwinia* spp. have capability in preventing ability against some phytopathogen microbes, such as *Erwinia persicinus* that can inhibit *Bemisia tabaci* (Ateyyat *et al*., 2009) and *E. chrysanthemi* RK-67 in inhibit *Aspergillus flavus*, causing post harvest diseases on lemon cultivars Meyer and Interdonato (Kotan *et al*., 2009).

Detection of polyketide synthase (PKS) gene: The detection of PKS genes was the first step should be performed in order to detect the antagonist mechanism were applied antagonist bacteria to the *Xanthomonas oryzae*. This gene was believed involved in polyketide biosynthesis. Polyketide is a class of antibiotic compounds that have various biological activities (Wang *et al*., 2014). This antibiotic was synthesized by the PKS genes including erythromycin (Faizal *et al*., 2008), diacetylphloroglucinol (Zha *et al*., 2006) and pyoluteorin (De Souza and Raaijmakers, 2003).

The detection of PKS gene was done by amplifying ketosynthase (KS) domain from genomic DNA of antagonist bacteria. Schmitt and Lumbsch (2009) recorded that the regions were very

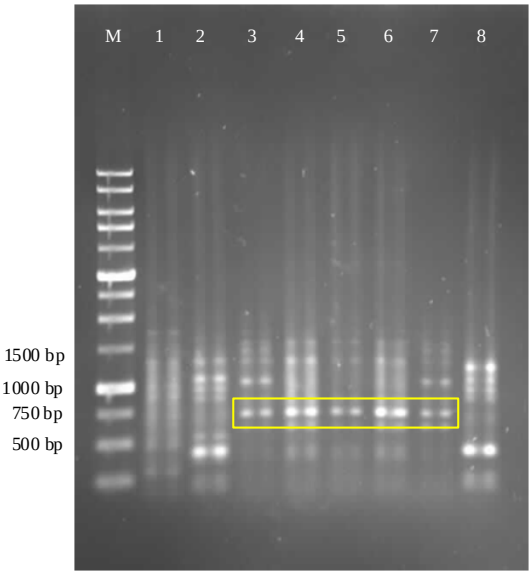

Fig. 2: Electrophoregram of polyketide synthase (PKS) gene amplicons, M: (1) kb marker, 1: Pseudomonadaceae SH2a, 2: *Pantoea* sp. MO142, 3: *Pantoea* sp. MO22g, 4: *Erwinia* sp. MO34h, 5: *Pantoea* sp. MO34i, 6: *Pantoea* sp. MO34j, 7: *Pantoea* sp. MO43a and 8: *Pantoea* sp. MO63j, bands in the yellow box is the targeted bands

highly conserved in type I PKS, namely a KS and acyltransferase (AT) domains. These two domains which is often used to predict the evolution of PKS gene. The primers used for amplification of KS domain produced approximately 700 bp band (Schirmer *et al*., 2005).

The electrophoregram of PKS gene amplicons showed that the Pseudomonadaceae SH2a did not produce specific band, accordingly. *Pantoea* sp. MO142 and *Pantoea* sp. MO63j generate unspecific bands and the thickest band showed a smaller size than the targeted size, therefore sequencing DNA was not to do. Smaller amplicon size than the targeted size presumably because of the used primers which was constructed from degenerated primer and this could produce less specific that causes primers anneal on more than one place. *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j and *Pantoea* sp. MO43a produce unspecific bands but the thickest band showed close to targeted size (Fig. 2). The alignment results of fifth antagonist bacteria showed low similarities with nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene from *Lysobacter lactamgenus*. This occurrence was because there is a lot of bases at the beginning, middle and end of the sequence were different. By contrast conversely, the fifth nucleotide sequence of antagonist bacteria has a specific features and this has been proved in their base similarities, not only on of the beginning nucleotide, but also to middle and end of the sequence (Fig. 3).

The alignment results of query cover showed a low percentage, (12-33%), while the lowest E value was recorded 8×10^{-7} and the similarities was 78-80%. This was realized because the size of the amplicons were only about 700 bp compared to the size of the nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus* that have 15,150 bp on size (Table 2).

Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene from *Lysobacter lactamgenus* contains three modules nonribosomal peptide synthetase (NRPS), namely module 1 (M1), module 2 (M2), as well as module 3 (M3). The type I PKS consists of ketosynthase

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cpbI gene          GAGTTCTTCTGGCCCCTGCTGCACGGCGCCACCCTGGCGCTGGCGCCGCCGGACGCGCAC 60
Pantoea sp. MO22g  .GA.N--.....G.TGAGACNGG.....AG.--..CT.A.............T.....T 60
Erwinia sp. MO34h  .GA..--.....G.TATGAC.GG.....AG.--..CT.A.............T.....T 60
Pantoea sp. MO34i  .GA.........G.TATGAC.GG.....AG.--..CT.A.............T.....T 60
Pantoea sp. MO34j  .GA..--.....G.TATGAC.GG.....AG.--..CT.A.............T.....T 60
Pantoea sp. MO43a  .GA.........G.TGATGA.GG.....AG.--..CT.A.............T.....T 60

cpbI gene          AAGGACCCGGCCGCGCTGATCGAGCTGATCGTGCGCCACCGCGTCACCACCGTGCACTTC 120
Pantoea sp. MO22g  CGC..T....A...C....CGCGCT.A...AATGAGT.............GC........ 120
Erwinia sp. MO34h  CGC..T....A...C....CGCGCT.A...AATGAGT.............GC........ 120
Pantoea sp. MO34i  CGC..T....A...C....CGCGCT.A...AATGAGT.............GC........ 120
Pantoea sp. MO34j  CGC..T....A...C....CGCGCT.A...AATGAGT.............GC........ 120
Pantoea sp. MO43a  CGC..T....A...C....CGCGCT.A...AATGAGT.............GC........ 120

cpbI gene          GTGCCGTCGATGCTGGCGGTGT------TCCTGCAGGCCGACGGGGTCGA-ACGCTGCGC 180
Pantoea sp. MO22g  ........C........A.CC.GGATCGC.AC..T..A.AG...A.CT..CG..GA.-A. 180
Erwinia sp. MO34h  ........C........A.CC.GGATCGC.AC..T..A.AG...A.CT..CG...C..A. 180
Pantoea sp. MO34i  ........C........A.CC.GGATCGC.AC..T..A.AG...A.CT..CG...C..A. 180
Pantoea sp. MO34j  ........C........A.CC.GGATCGC.AC..T..A.AG...A.CT..CG...C..A. 180
Pantoea sp. MO43a  ........C........A.CC.GGATCGC.AC..T..A.AG...A.CT..CG...C..A. 180

cpbI gene          CGGCCTGCGCCGGGTGA-----TCTGCAGCGGCGAAGCCCTGCCCGGCGCCAGCGTGCGG 240
Pantoea sp. MO22g  T.C.GCA.....AACA.AGATC.TCTTT..N...-G..G..TA.AT.A.ATC-...CT.A 240
Erwinia sp. MO34h  T.C.GCA...T.C.CCGGGTCT.............G..G..CT..T...ATC-T.GCT.A 240
Pantoea sp. MO34i  T.C.GCA...T.C.CCGGGTCT.............G..G..CT..T...ATC-T.GCT.A 240
Pantoea sp. MO34j  T.C.GCA...T.C.CCGGGTCT.............G..G..CT..T...ATC-T.GCT.A 240
Pantoea sp. MO43a  T.C.GCA...T.C.CCGGATCT.............G..G..CT..T...ATC-T.GCT.A 240

cpbI gene          CTGCTGCACAAGCGCCTGCCGCAGACCGCGATCCACAACCTGTACGGCCCGACCGAAGCC 300
Pantoea sp. MO22g  ACA..CT-...T.-.AC...T.C.-...TTC..A...-T..A..NT-TAT...------- 300
Erwinia sp. MO34h  AGAT.AT-..GT...GG.TT..C.-..C..C.G.......................... 300
Pantoea sp. MO34i  AGAT.AT-..GT...GG.TT..C.-..C..C.G.......................... 300
Pantoea sp. MO34j  AGAT.AT-..GT...GG.TT..C.-..C..C.G.......................... 300
Pantoea sp. MO43a  GGAT.AT-..GT...GG.TT..C.-..C..C.G.......................... 300

cpbI gene          GCGATCGAGGCGACCGCCTGGACCTGTCCGCGCGAT-----------TTCGCCGGCGAC 360
Pantoea sp. MO22g  ------------------------------------------------------------ 360
Erwinia sp. MO34h  ..CG.T..C.T....TGG.ATC.GGC.T.CG.....GCGCTGGCCGCC.GTCAG..T.C. 360
Pantoea sp. MO34i  ..CG.T..C.T....TGG.ATC.GGC.T.CG.....GCGCTGGCCGCC.GTCAG..T.C. 360
Pantoea sp. MO34j  ..CG.T..C.T....TGG.ATC.GGC.T.CG.....GCGCTGGCCGCC.GTCAG..T.C. 360
Pantoea sp. MO43a  ..CG.T..C.T....TGG.ATC.GGC.T.CG.....GCGCTGGCCGCC.GTCAG..A.C. 360

cpbI gene          ACCGTGCCGATCGGCCGCCCGATCGCCAACGCCCGCATCTATCTGCTCGACCCGCGGCGC 420
Pantoea sp. MO22g  ------------------------------------------------------------ 420
Erwinia sp. MO34h  GGA..C..TG.T...TAT...G..TGG...A....AC.GCGCA.T..G..TA.C.T..TG 420
Pantoea sp. MO34i  GGA..C..TG.T...TAT...G..TGG...A....AC.GCGCA.T..G..TA.C.T..TG 420
Pantoea sp. MO34j  GGA..C..TG.T...TAT...G..TGG...A....AC.GCGCA.T..G..TA.C.T..TG 420
Pantoea sp. MO43a  GGA..C..TG.T...TAT...G..TGG...A....AC.GCGCA.T..G..TA.C.T..TG 420

cpbI gene          CAGCCGGTGCCGCTCGGCGCGGTCGGCGAGCTCTACATCGGCGGCGTCGGCGTCGCCCGC 480
Pantoea sp. MO22g  ------------------------------------------------------------ 480
Erwinia sp. MO34h  .GC...........G..T.TA.CG.....T.....TC.GAC......ACAGC.G..AGA. 480
Pantoea sp. MO34i  .GC...........G..T.TA.CG.....T.....TC.GAC......ACAGC.G..AGA. 480
Pantoea sp. MO34j  .GC...........G..T.TA.CG.....T.....TC.GAC......ACAGC.G..AGA. 480
Pantoea sp. MO43a  .GC...........G..T.TA.CG.....T.....TC.GAC......GCAGC.G..AGA. 480

cpbI gene          GGCTATCTGAACCGTCCCGAGCTCACCGACGAACGCTTCCTGCCCGACCCGTTCGCCGCC 540
Pantoea sp. MO22g  ------------------------------------------------------------ 540
Erwinia sp. MO34h  .........CG...C.....C..G....C.C.G.....TG.CG....T............ 540
Pantoea sp. MO34i  .........CG...C.....C..G....C.C.G.....TG.CG....T............ 540
Pantoea sp. MO34j  .........CG...C.....C..G....C.C.G.....TG.CG....T............ 540
Pantoea sp. MO43a  .........CG...C.....C..G....C.C.G.....TG.CG....T............ 540

cpbI gene          GATCCCGACGCGCGCATGTACCGCAGCGGCGATCTGGCGCGCCATCTCGCCGGCGGCGAC 600
Pantoea sp. MO22g  ------------------------------------------------------------ 600
Erwinia sp. MO34h  .GG------.AA.............C.......A.C.TC...TGG..G.A.AA.....CG 600
Pantoea sp. MO34i  .GG------.AA.............C.......A.CATC...TGG..G.A.AA.....CG 600
Pantoea sp. MO34j  .GG------.AA.............C.......A.C.TC...TGG..G.A.AA.....CG 600
Pantoea sp. MO43a  .GG------.AA.............C.......A.C..C...TGG..G.A.AA.....CG 600

cpbI gene          ATCGAATTCCTCGGCCGCAACGACCATCAGGTCAAGCTGCGCGGCTTCCGCATCGAACTC 660
Pantoea sp. MO22g  ------------------------------------------------------------ 660
Erwinia sp. MO34h  G.G....A...G.......GT...G.....C.G..AA.T.....TCAG.....T..G..G 660
Pantoea sp. MO34i  G.G....A...G........T...T.C...C.G..AA.T.A...TCAT.....T..T..T 660
Pantoea sp. MO34j  G.G....A...G.......GT...G.....C.G..AA.T.....TCAG.....T..G..G 660
Pantoea sp. MO43a  G.G....A...T.......GT...G.....C.G..AA.T.....TCAG.....T..G..G 660

cpbI gene          GGCGAAATCGAGACCCGGCTCGCCGCCCATGCCGAGGTC 699
Pantoea sp. MO22g  ----------------------------------- 699
Erwinia sp. MO34h  ..T.........CANGC...GCTGCGGATCCAT.GCACG 699
Pantoea sp. MO34i  TCGTG..ATCGAG.ATAA.ATTATTG.GGAT..ATTCG. 699
Pantoea sp. MO34j  ..T.........CATGC...GCTGCGGATCCAT.GCAAG 699
Pantoea sp. MO43a  ..T.........CATGC...GCTG.GATTCC.ATG.CAA 699
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Fig. 3: Comparison of sequence nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus* with partial sequences polyketide synthase (PKS) genes antagonist bacteria *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j and *Pantoea* sp. MO43a, A point (.) indicates the similarity between sequences of nucleotide bases, a dash (-) indicates a gap

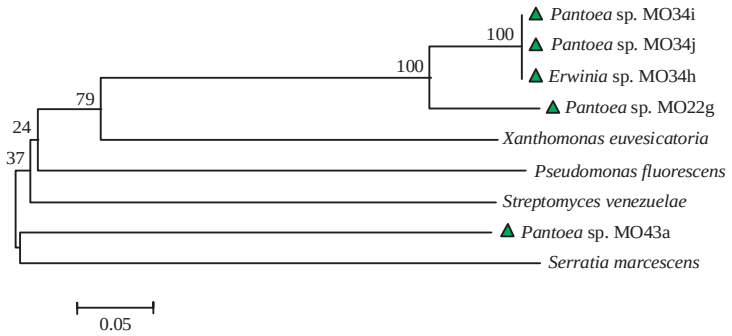

Fig. 4: Phylogenetic tree of KS domain antagonist bacteria with some other bacteria, *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j, as well as *Pantoea* sp. MO43a are the antagonist bacteria that were obtained from this study (marked with a triangle). The KS domain sequence of *Xanthomonas euvesicatoria* that was isolated from *Capsicum annuum* cv. Kurtovska kapija leaves from Macedonia was obtained from European Nucleotide Archive (ENA) with accession no. >ENA|KHL65014|KHL65014.1, *Pseudomonas fluorescens* (>ENA|ADQ55965|ADQ55965.1) that was isolated from sponge in Baikal lake Russia, *Streptomyces venezuelae* (>ENA|AB024980|AB024980.1) is one of the actinomycetes and *Serratia marcescens* (>ENA|AFX60305|AFX60305.1) belong to Enterobacteriaceae family

Table 2: Similarity of partial sequence polyketide synthase (PKS) genes of five antagonist bacteria to the GenBank database

Bacteria isolates	Similarity	Query cover (%)	E value	Identity (%)	Accession
Pantoea sp. MO22g	Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus*	33	5e-10	80	DQ278492.1
Erwinia sp. MO34h	Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus*	12	8e-08	80	DQ278492.1
Pantoea sp. MO34i	Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus*	13	7e-08	80	DQ278492.1
Pantoea sp. MO34j	Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus*	13	7e-08	80	DQ278492.1
Pantoea sp. MO43a	Nonribosomal peptide synthetase-polyketide synthase hybrid (*cpb*I) gene *Lysobacter lactamgenus*	21	9e-17	78	DQ278492.1

(KS), acyltransferase (AT), ketoreductase (KR), acyl carrier protein (ACP) and thioesterase (TE) domains. The NRPS gene is a gene that involved in nonribosomal peptide biosynthesis. Nonribosomal peptides are a group of compounds that have various biological activities such as antibacterial (vancomycin), anticancer (bleomycin), immunosuppressants (cyclosporine) (Tang *et al*., 2007), siderophores (pyoverdin), toxins (HC toxins) and surfactant (surfactin) (Caboche *et al*., 2008).

Type I PKS is a multi-domain enzymes that use modular strategy, with each module consisting of at least three domains, namely ketosynthase (KS), acyltransferase (AT) and acyl carrier protein (ACP) domain. This type I PKS module may contain additional domains such as ketoreductase (KR), dehydratase (DH) and enoylreductase (ER) (Ayuso-Sacido and Genilloud, 2005). Based on the detection results of the PKS gene in genomic DNA of *Pantoea* sp. MO22g, *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j and *Pantoea* sp. MO43a, this indicated that antagonist mechanism of antagonist bacteria with antibiotics production (antibiosis).

Genetic relationship of KS domain: Based on the phylogenetic tree in Fig. 4, showed that the KS domain of antagonist bacteria i.e., *Erwinia* sp. MO34h, *Pantoea* sp. MO34i, *Pantoea* sp. MO34j

and *Pantoea* sp. MO22g have a very closely genetic relationship and clustering in a group. These KS domain of antagonist bacteria have a closely genetic relationship with *Xanthomonas euvesicatoria* that alike as phyllosphere bacteria though in the different plants. Fourth of these KS domain have a far distant compare to the KS domain of *Streptomyces venezuelae* and *Pseudomonas fluorescens*. The KS domain of *Pantoea* sp. MO43a is in a group with KS domain of *Serratia marcescens* and separated from the other group.

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