

EFFECT OF BACTERIAL VOLATILE COMPOUNDS ON PAKCOY (*Brassica rapa L.*) GROWTH PROMOTION

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ABSTRACT

This research was aimed to test of the ability of MVOC-producing bacteria to increase plant growth of Pakcoy (*Brassica rapa L.*). The methodology including testing the ability of MVOC-producing rhizobacteria in Pakcoy plant growth enhancement, MVOC extraction and analysis of compounds in MVOC Extracts using Gas Chromatography. Different bacterial species produce different MVOC. *S. maltophilia* Sg3 emitted 20 MVOC compounds and MVOC that contribute to increasing plant growth, namely oxalic acid, cyclohexyl undecyl ester, 2-Furancarboxaldehyde, 5-methyl-, 1,2 butanediol, and Piperazine. *E. asburiae* MjSg48 emitted 12 MVOC compounds and those that contributed to increasing plant growth were oxalic acid, cyclohexyl dodecyl ester and 4-methyl oxazole. *E. asburiae* TK24 emitted 27 MVOC compounds and those that contributed to increasing plant growth were oxalic acid, isohexyl neopentyl ester, thiazole, Oxalic acid, and cyclohexyl decyl ester. Meanwhile *P. rettgeri* A12TT emitted 13 MVOC compounds and those that contributed to increasing plant growth were oxalic acid, diisohexyl ester, and Pyridine, 2,3,4,5-tetrahydro.

Keywords: Microbial volatile organic compound (MVOC), growth, pakcoy

INTRODUCTION

Rhizobacteria is bacteria that utilize plant root exudate to live as a colony in rhizosphere area. The metabolite products that released by this rhizobacteria is a chemical compound which easily evaporate or frequently categorized as an organic compound with a *volatile* property that produced by microbes or *Microbial volatile organic compound* (MVOC) (Kanchiswamy,

et al., 2015, Cappellari, et al., 2020). The microbes other than rhizobacteria, such as yeast dan fungi also can produce MVOC. The research on the ability of microbes in producing organic compounds which easily evaporate has been published in many journal (Ramirez, et al., 2010, Schmidt, et al., 2015, Gupta, et al., 2017) . The fresh smells from the pure culture of microbes like yeast and citric or lactic acid bacteria, and the strong

smells from various fermented products are a simple proof the existence of compounds from organic acid group as citric acid, acetic acid, lactic acid, propionate, alcohol, ester, mercaptan, pentylfuran and others which are metabolic product of microbes. However, scientific evidence on MVOC activity as a growth inducer and plant resistance was only published in 2003 (Ryu et al. 2003; Ryu et al. 2004). Ryu et al. (2004) reported that *Bacillus* sp. strain GB03 which emitted 2,3 butanediol and acetoin compounds was able to induce the growth of *Arabidopsis thaliana* plants when compared to water control and treatment with *Escherichia coli* strain DH5 α . Since then, the role of the MVOC compounds group for plant growth inducers has received a lot of attention and has attracted the interest of other researchers to explore and study its potential development and applications.

The exploration and characterization of the biological function of MVOCs can reveal a variety of biological processes that are important for plant growth (Castro et al., 2009, Bohm, et al., 2017). Castro et al., (2009) stated that Acyl homoserine lactone (AHL) is a compound that is emitted by bacterial cells to regulate gene expression when the population has reached a sufficient level of cell density and intermediate chain AHL compounds (C8-C14) such as N-

hexanoylhomoserine lactone, N-3-oxo-hexanoyl-homoserine lactone, N-octanoyl-homoserine lactone, N-decanoyl-homoserine lactone, N-dodecanone-homoserine lactone, and N-tetradecanoylhomoserine lactone can be recognized by plants and are able to change gene expression in plants roots and shoots so that they are able to modulate plant cell growth and medium chain AHL compounds can change root architecture, alter primary root growth, stimulate lateral root formation, and root hairs development in *A. thaliana* plants. MVOC emitted by *Alternaria alternata* can trigger an escalation in starch accumulation in potato leaves accompanied by upregulation of sucrose synthase, invertase inhibitors, and starch synthase classes III and IV, glucose-6-phosphate transporter, plastidial thioredoxin enzyme, starch breakdown enzymes, and proteins involved in the provision of internal amino acids. This phenomenon is called MVOC-ISAP or MVOC-induced starch accumulation process (Ezquer et al., 2010, Orzechowski, et al., 2021). The rhizobacteria *Serratia odorifera* emitted compounds 2-pentanone, 4-heptanone, 2-heptanol, 2-undecanone, 2tridecanone, 2-pentadecanone, sodorifen, bicyclic oligomethyl octadiene, 1-hexanol and indole (Kai, et al. 2010, Kanchiswamy, et al., 2015). Indole and

pentadecane compounds produced by *S. odorifera* can stimulate the growth of *A. thaliana* plants (Blometal, 2011, Park, et al., 2015).

This research is aimed to aimed to test of the ability of MVOC-producing bacteria to increase plant growth of Pakcoy (*Brassica*

rapa L.). Pakcoy is a type of leafy vegetable crops that are very important in Indonesia because it has a high economic value. Pakcoy much in demand as a vegetable because of high nutrient content and it tastes good (Gustiar, et al., 2022).

MATERIALS AND METHODS

The research was conducted in October until December 2021 in Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Udayana University and in the

greenhouse in the Experimental Garden of the Faculty of Agriculture, Udayana University, Bali Indonesia. Figure 1 shows the map of the research location.



Figure 1. The Map of the Research Location.

Testing the ability of MVOC-producing rhizobacteria in *Brassica rapa* L. Plant Growth enhancement.

The ability of MVOC-producing rhizobacteria to increase plant growth was carried out in the Greenhouse. MVOC producing rhizobacteria were grown for 24 hours in Nutrient Broth medium (10 g peptone, 10 g Beef Extract, 5 g Sodium Chloride). Furthermore, the rhizobacteria were cultured in MRVP broth medium (7 g peptone, 5 g dipotassium phosphate, 5 g dextrose) for 48 hours. The seeds of pakcoy were sterilized using 0.5% Clorox and then soaked in sterile water for 24 hours. The seeds were drained and then soaked with rhizobacteria suspension from MRVP broth culture media for 2 hours.

Furthermore, the seeds were planted in pots, where the bottom of the pot contained a suspension of rhizobacteria from MRVP broth culture media. The measurement of chlorophyll levels in the leaves was carried out using the Minolta-SPAD 502 Chlorophyllmeter. Leaf chlorophyll measurements were carried out at the age of 14 DAP, 21 DAP and 28 DAP on the 2nd and 3rd leaves from the shoots.

MVOC Extraction

MVOC was extracted using ethyl acetate, hexane, and methanol. A total of 50

ml of the culture filtrate was centrifuged at 1.610 x g for 30 minutes, the supernatant was separated from the bacterial precipitate, filtered with Millipore filter paper (0.45 mm).

Then it was partitioned with ethyl acetate and methanol three times. MVOC compounds can be extracted by partitioning the supernatant using ethyl acetate. The ethyl acetate fraction was then separated from the methanol fraction, then the three ethyl acetate fractions were combined into the ethyl acetate fraction. Furthermore, the ethyl acetate fraction was evaporated using a Vacuum Rotary Evaporator at a temperature of 40°C. The extract or ethyl acetate fraction was then dissolved with 5 ml of methanol and stored in a refrigerator with minus 20° C temperature.

Analysis of Compounds in MVOC Extracts using Gas Chromatography – Mass Spectroscopy (GC-MS)

The Analysis of compounds that contained in the rhizobacterial filtrate was carried out using Gas Chromatography – Mass Spectroscopy (GC-MS QP2010 Ultra Shimadzu). The eluent used was liquid nitrogen, Wakosil ODS/5C18-200 column, size 4.6 x 200 mm, eluent flow rate of 1 ml/minute, temperature 250°C and detected using UV light at 254 nm. The results of the detection were carried out by matching the

molecular weights and fragmentation patterns of the isolated compounds with the compounds in the GC-MS library. By using

RESULTS AND DISCUSSIONS

Results

The results of the test of the ability of MVOC-producing bacteria to increase plant growth showed that the MVOC emitted by *S. maltophilia* Sg3, *E. asburiae* TK24, *E. asburiae* MjSg48, and *P. rettgeri* Al2TT bacteria was able to increase the growth of pakcoy plants. Growth parameter values such

GC-MS, the molecular weight and molecular structure of contained compounds in the bacterial filtrate can be known and identified.

as plant height, root fresh and dry weight, leaf area, leaf chlorophyll content, fresh and dry weight of plants treated with MVOC emitted by bacteria were higher than growth parameter values in control plants. Figure 2. shows performance of pakcoy plants treated with MVOC. KT: plants without MVOC (*S. maltophilia* Sg3 (Sg3); *E. asburiae* TK24 (TK24); *P. rettgeri* Al2TT (AL2T); *E. asburiae* MjSg48 (MjSg)).



Figure 2. Performance of Pakcoy plants treated with MVOC. **KT**: plants without MVOC (*S. maltophilia* Sg3 (**Sg3**); *E. asburiae* TK24 (**TK24**); *P. rettgeri* Al2TT (**AL2T**); *E. asburiae* MjSg48 (**MjSg**)).

The MVOC treatment in plants was able to increase plant height ranging from 21.44% to 33.97%. MVOC bacteria *P. rettgeri* Al2TT increased plant height by 21.44%, *S. maltophilia* Sg3 by 26.84%, *E. asburiae* TK24 by 26.98%, and *E. asburiae* MjSg48 by 33.97% when compared to the

control (Table 4.1). The MVOC treatment in plants was able to increase the fresh weight of roots ranging from 15.34% to 63.64%. MVOC bacteria *P. rettgeri* Al2TT increased root fresh weight by 15.34%, *S. maltophilia* Sg3 by 63.64%, *E. asburiae* TK24 by 18.18%, and *E. asburiae* MjSg48 by 59.09%

when compared to the control. MVOC treatment in plants was able to increase root dry weight ranging from 41.67% to 91.67%. MVOC of *P. rettgeri* Al2TT bacteria increased root dry weight by 41.67%, *S. maltophilia* Sg3 by 91.67%, *E. asburiae*

TK24 by 58.33%, and *E. asburiae* MjSg48 by 83.33% when compared to controls. Table 1. shows the effect of MVOC on plant height, root fresh weight, and root dry weight of pakcoy plants.

Table 1. The effect of MVOC on Plant Height, Root Fresh Weight, and Root Dry Weight of Pakcoy Plants.

Treatments	Plant Height (cm)	Root Fresh Weight (gr)	Root Dry weight (gr)
Control	20,75 a	17,6 a	1,2 a
<i>E. asburiae</i> MjSg48	27,8 d (33.97%)	28.0 c (59.09%)	2,2 c (83.33%)
<i>S. maltophilia</i> Sg3	26,32 c (26.84%)	28,8 c (63.64%)	2,3 c (91.67%)
<i>E. asburiae</i> TK24	26,35 c (26.98%)	20,8 b (18.18%)	1,9 b (58.33%)
<i>P. rettgeri</i> Al2TT	25,2 b (21.44%)	20,3 b (15.34%)	1,7 b (41.67%)

The MVOC treatment in plants was able to increase leaf chlorophyll levels ranging from 25.21% to 40.47%. MVOC bacteria *P. rettgeri* Al2TT increased leaf chlorophyll content by 25.21%, *S. maltophilia* Sg3 by 40.47%, *E. asburiae* TK24 by 28.13%, and *E. asburiae* MjSg48 by 30.41% when compared to the control. (Table 4.2). The MVOC treatment in plants was able to increase the fresh weight of plants ranging from 75.43% to 94.48%. MVOC of *P. rettgeri* Al2TT bacteria increased plant fresh weight by 79.63%, *S. maltophilia* Sg3 by 94.48%, *E. asburiae* TK24 by 75.43%, and *E. asburiae* MjSg48 by 87.25% when compared

to controls. Table 2 show the effect of MVOC on leaf chlorophyll content, plant fresh weight, and plant dry weight pakcoy plants.

The MVOC treatment in plants was able to increase the dry weight of the plants ranging from 14.67% to 48.00%. MVOC of *P. rettgeri* Al2TT bacteria increased plant dry weight by 14.67%, *S. maltophilia* Sg3 by 48.00%, *E. asburiae* TK24 by 34.67%, and *E. asburiae* MjSg48 by 41.33% when compared to controls. The results of MVOC compounds analysis in *S. maltophilia* Sg3 using GC-MS showed that there were 20 compounds detected shows in Figure 3.

Table 2. The effect of MVOC on Leaf Chlorophyll Content, Plant Fresh Weight, and Plant Dry Weight of Pakcoy Plant.

Treatments	Leaf Chlorophyll Content (SPAD unit)	Plant Fresh Weight (gr)	Plant Dry Weight (gr)
Control	41,16 a	152,2 a	7,5 a
<i>E.asburiae</i> MjSg48	53,68 c (30.41%)	285,0 d (87.25%)	10,6 c (41.33%)
<i>S. maltophilia</i> Sg3	57,82 d (40.47%)	296,0 e (94.48%)	11,1 d (48.00%)
<i>E. asburiae</i> TK24	52,74 b (28.13%)	267,0 b (75.43%)	10,1 c (34.67%)
<i>P. rettgeri</i> Al2TT	51,54 b (25.21%)	273,4 c (79.63%)	8,6 b (14.67%)

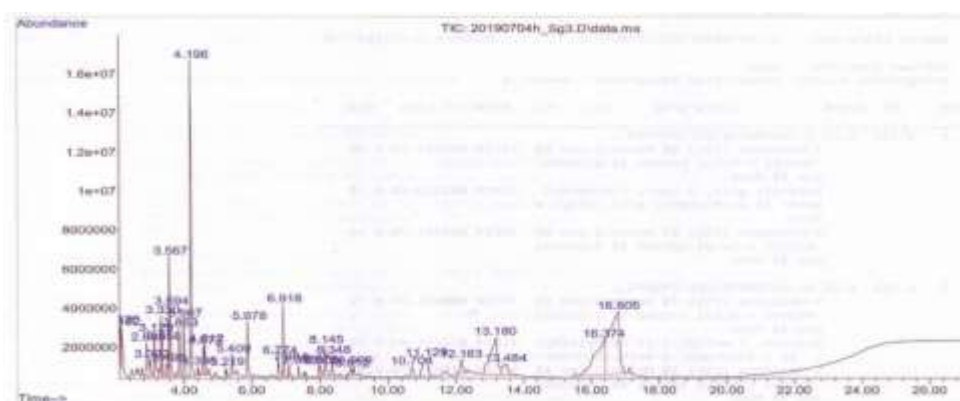


Figure 3. Chromatogram of mVOC in *S. maltophilia* Sg3

The MVOC compounds in *S. maltophilia* Sg3 are Cyclobutanol, 1-(trans-2-Phenylcyclopropyl)-2-methylpropan-1-ol, 1-Pentyl-3,3-D2 Acetate, Cyclopropane carboxylic acid, 2(3H)-Furanone, dihydro-, Oxalic acid, cyclohexyl undecyl ester, 7-oxabicyclo[4.1.0] heptane, 1-methyl-, 2-Furancarboxaldehyde, 5-methyl-, 1,3,5-Triazine-2,4,6-triamine, 1,4-Dioxan-2-one, 2-hydroxy-butanediol, N-(methyl-d3) pyrrole, Piperazine, 2-Furancarboxaldehyde,5-(hydroxymethyl)-, 2,3-Propanetriol, monoacetate, N,N' -

Diethyl oxamide, n-Pentanal, Tetrahydro-4H-Pyran-4-ol, 1-Pentanamine, N-methyl-N-(1-methylethyl)-, 1,3-Dioxan-5-ol.

The results showed that the MVOC which emitted by *S. maltophilia* Sg3 and contributed to increasing plant growth were oxalic acid, cyclohexyl undecyl ester, 2-Furancarboxaldehyde, 5-methyl-, 1,2 butanediol, and Piperazine. The results of the analysis of MVOC compounds in *E. asburiae* MjSg48 using GC-MS showed that there were 12 compounds detected. Figure 4.

shows chromatogram of MVOC in *E. asburiae* MjSg48.

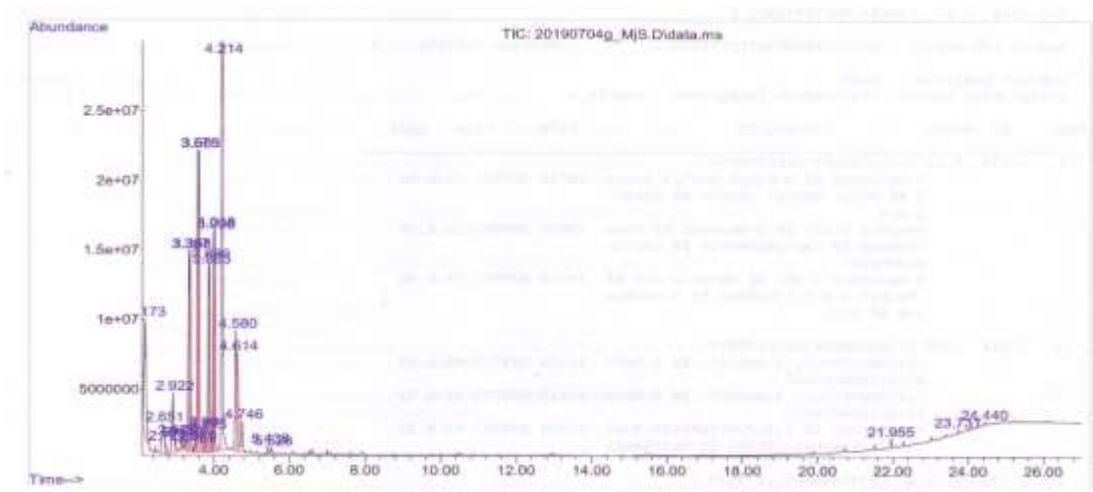


Figure 4. Chromatogram of MVOC in *E. asburiae* MjSg48

The MVOC compounds detected in *E. asburiae* MjSg48 were Propanoic acid, 2-hydroxy-, Benzene, 1,4-dimethyl-, Acetic acid, anhydride, Propanal, dimethylhydrazone, 1-Pentanol, 2,2-dimethyl-, Sulfurous acid, isohexyl 2-propyl ester, Oxalic acid, cyclohexyl dodecyl ester, 4-methyl oxazole, Oxalic acid, cyclohexyl tetradecyl ester, Oxalic acid, cyclohexyl pentyl ester, Acetic acid, sodium salt, and 1H-Indole-3-ethanamine, 6-fluoro -beta.-methyl-.

The results showed that the MVOC emitted by *E. asburiae* MjSg48 and contributed to increasing plant growth were

oxalic acid, cyclohexyl dodecyl ester and 4-methyl oxazole. The analysis result of MVOC compounds in *E. asburiae* TK24 using GC-MS showed that there were 27 compounds detected. Figure 5. shows the MVOC chromatogram of *E. asburiae* TK24.

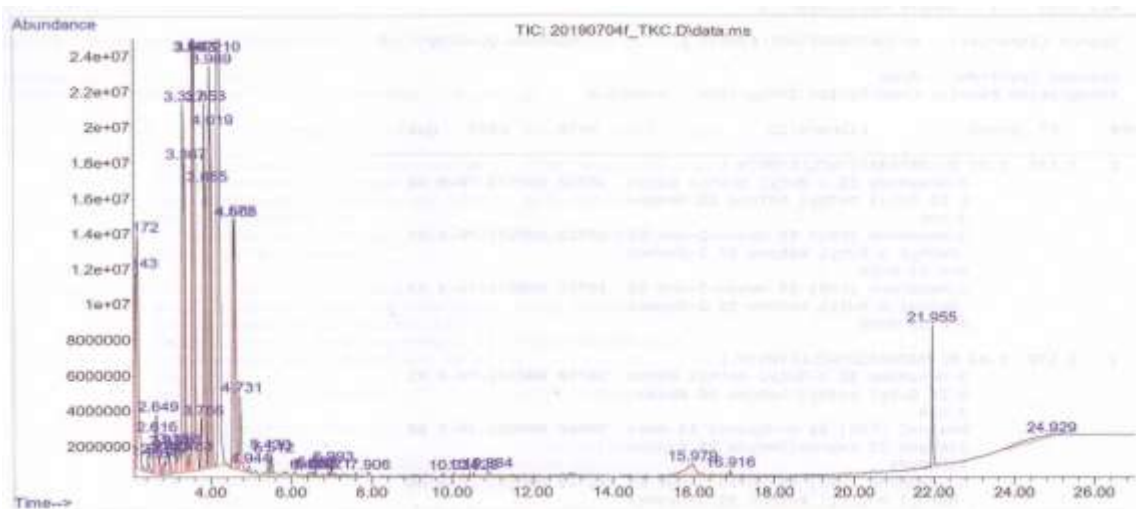


Figure 5. The MVOC Chromatogram of *E. asburiae* TK24

The MVOC compounds detected in *E. asburiae* TK24 were Propanal, 2,3-dihydroxy-, Benzene, ethyl-, XYLENE, 4-Amino-furazan-3-carboxylic acid (2-acetylamino-ethyl)-amide, 1,2- Xylene, Butanoic acid, 2-ethyl-2-methyl-, Pentane, 3-ethyl-3-methyl-, Oxalic acid, isohexyl neopentyl ester, 2,4-Octanedione, Thiazole, 1-Pentanol, 2,2-dimethyl-, Oxalic acid, cyclohexyl decyl ester, Oxalic acid, cyclohexyl pentyl ester, Oxalic acid, cyclohexyl hexyl ester, N1-METHYL-1,4-BUTANEDIAMINE, 5-Hexen-2-one, 2,3-Anhydro-d-galactosan, 1,3-Propane diamine, N-methyl-, Benzene, 1,2,3,4-tetramethyl-, 4-

Heptanone, Heptanamine, 5-methyl-, Piperidine, 3,3-dimethyl-, Octadecane, Pentanal, Dibutyl phthalate, and 1,2-Benzenedicarboxylic acid, mono (2-ethyl) ester.

The results showed that the MVOC which emitted by *E. asburiae* TK24 and contributed to increasing plant growth were oxalic acid, isohexyl neopentyl ester, thiazole, oxalic acid, and cyclohexyl decyl ester. The results of MVOC compounds analysis in *P. rettgeri* A12TT using GC-MS showed that there were 13 compounds detected. Figure 6. shows the chromatogram of MVOC in *P. rettgeri* A12TT



Figure 6. The Chromatogram of MVOC in *P. rettgeri* Al2TT

The MVOC compounds which detected in *P. rettgeri* Al2TT were Propanal, 2,3-dihydroxy-, Benzene, 1,4-dimethyl-, Oxalic acid, diisohexyl ester, Octanal, 2,4-Pentanedione, Oxalic acid, cyclohexyl octyl ester, Oxalic acid, cyclohexyl tetradecyl ester, Pyridine, 2,3,4,5-tetrahydro-, Carbamic acid, (3,4,4-trimethyl-1, 2-dioxetan-3-yl) methyl ester, 1-Butanol, 2- amino-, delta. 2-tetrazaboroline, 5-ethyl-1,4-dimethyl-, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and 1,2-Benzenedicarboxylic acid. The results showed that the MVOC emitted by *P. rettgeri* Al2TT and contributed to increasing plant growth were oxalic acid, diisohexyl ester, Pyridine, 2,3,4,5-tetrahydro.

Discussions

A wide variety of microorganisms found in the rhizosphere are capable of

producing substances that regulate plant growth and development. Chemical signals from microbes have been found to play a role in plant morphogenetic processes, including N-acyl-L-homoserine lactones (AHL) and volatile organic compounds (VOCs). AHL is a bacterial quorum sensing signal from Gram-negative bacteria such as *Pseudomonas*. These compounds allow bacterial cells to regulate gene expression depending on population density. Recently, it was found that AHL can be recognized by plants, alter gene expression in roots and shoots also modulate cell growth defense and response. In the same way, bacterial volatiles such as acetoin and 2,3-butanediol produced by certain rhizobacteria can be used for bacterial and plant communication, as well as plant growth triggers. Acetoin VOC-producing bacteria were able to increase the

root growth of *Lactuca sativa* plants (Fincheira et al., 2016).

Paracoccus halophilus G062 emits 1,2-Dimethoxy-4-(2propenyl) benzene or Methyl eugenol (ME) compounds which can increase potato plant growth. *B. subtilis* GB03 which emits compounds 3-hydroxy-2-butanone, 2,3-butanediol, decanal, dean, tetramethyl pyrazine, and undecane which can increase the growth of *A. thaliana* (Ryu, 2004, Akhdiya, 2014, Ditengou, et al., 2015). The results of VOC trapping of *B. subtilis* G8 which was carried out using SPME fibers and followed by identification using GC-MS

CONCLUSION

Different bacterial species produce different MVOC. *S. maltophilia* Sg3 emitted 20 MVOC compounds and MVOC that contribute to increasing plant growth, namely oxalic acid, cyclohexyl undecyl ester, 2-Furancarboxaldehyde, 5-methyl-, 1,2 butanediol, and Piperazine. *E. asburiae* MjSg48 emitted 12 MVOC compounds and those that contributed to increasing plant

obtained 30 types of compounds consisting of alkyl groups, alcohols, esters, ketones, acids, amines, oxime, phenol, and heterocyclic compounds. Ectomycorrhiza produce VOC sesquiterpene which able to increase the roots growth of *A. thaliana*. reported that *Phoma* sp. GS8-3 emits VOCs 2-Methyl-propanol, 3-Methyl-butanol, Phenyl ethyl alcohol, 3-Hydroxy-2-butanone, 2,3-Butanediol, 1-Octen-3-ole, Methacrylic acid, Isobutyl acetate, Acetic acid, Tiglic acid can increase the growth of tobacco plants (Liu et al., 2008, Naznin et al., 2013)

growth were oxalic acid, cyclohexyl dodecyl ester and 4-methyl oxazole. *E. asburiae* TK24 emitted 27 MVOC compounds and those that contributed to increasing plant growth were oxalic acid, isohexyl neopentyl ester, thiazole, Oxalic acid, and cyclohexyl decyl ester. Meanwhile *P. rettgeri* A12TT emitted 13 MVOC compounds and those that contributed to increasing plant growth were oxalic acid, diisohexyl ester, and Pyridine, 2,3,4,5-tetrahydro.

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