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Characterization of rhizobacteria secondary metabolites on maize (Zea mays) in marginal land

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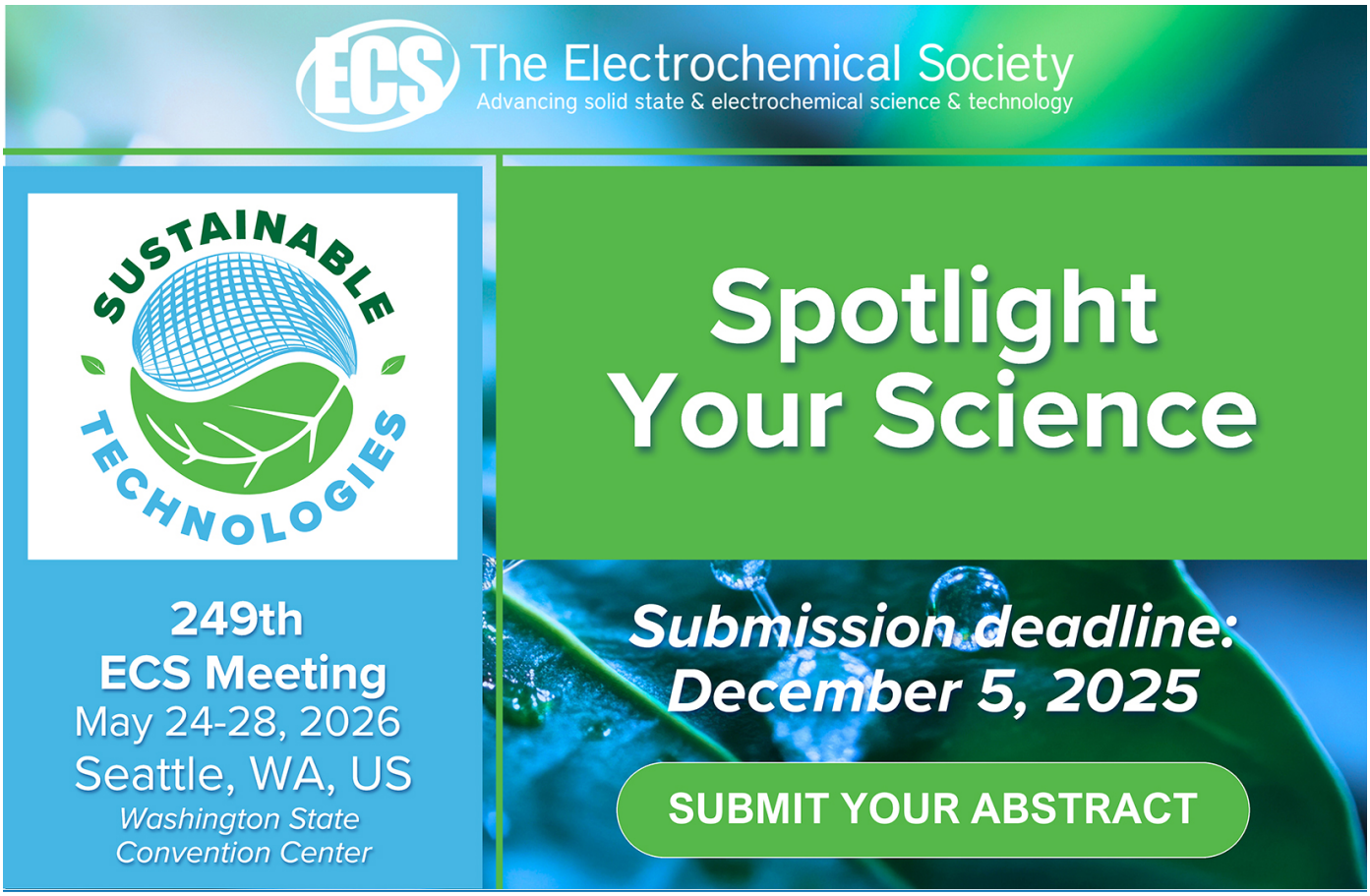

Characterization of rhizobacteria secondary metabolites on maize (*Zea mays*) in marginal land

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Abstract. Maize is one of the strategic food crops and has economic value and could be developed because of its position as the main carbohydrate and protein source after rice. This could be a reference for us to increase maize cultivation, seeing the role of maize is quite large, and the availability of maize is less stable every year. maize can grow optimally on productive land and marginal land, but at this time more productive land is used for rice production. One alternative for maize production is on marginal land especially dryland. The main problem in dryland is limited water. One way to overcome this is by inoculating Rhizobacteria which can increase the growth and resistance of maize to drought. Rhizobacteria possess tremendous potential for modulating the physiological response to water deprivation, thus ensuring plant survival under such stressful conditions. The research aimed to find rhizobacteria have secondary metabolite for resistance to drought. Rhizobacteria exploration and secondary metabolite isolation was conducted at University of Muhammadiyah Malang. The research results have obtained 10 isolates capable of synthesizing extracellular secondary metabolites: osmoprotectants, phytohormones, and organopesticides that can be used by plants. The results of the analysis by GS-MS (Gas Chromatography-Mass Spectrometry) rhizobacteria showed that three compounds were synthesized. The osmoprotectants compound synthesize ware found namely: (a) (2E)-3,7-dimethylocta-2,6-dienal 16.66%;(b) (E)-hept-2-enal12.55%;(c)2-methylsulfanylethanamine 5.34%; (d) 1,3,5-trimethoxybenzene 4.42%. It is also capable of synthesizing phytohormones: (a) cinnolino [4,3-b] quinoxaline 6.00%; (b) 1-methyl-2-phenylindole 5.90%; (c) 1H-Indole 3.64%; (d) N-2-Fluorenylperfluorpropionamid 3.02%. These rhizobacteria were also able to synthesize: (a) 2-chloro-4-methoxy phenol 11.32%; (b) (E)-non-2-enal 9.87%; (c) 4-chloro-2,6-dipyridin-2-ylpyridine 7.07%; (d) 2-(4-chlorophenyl) hexane nitrile 6.45%. The Osmprotectant compounds aim to protect cells from drought conditions, phytohormones to stimulate plant growth and organopesticides to overcome pests and diseases.
Keywords: maize, osmoprotectants, phytohormone, organopesticide

1. Introduction

Maize is one of the strategic food crops and has economic value and could be developed because of its position as the main carbohydrate and protein source after rice [1]. This aligns with the demand for national corn in 2018 of 20.33 million tons [2]. This could be a reference for us to increase corn plants' cultivation, seeing the role of corn plants is quite large, and the availability of corn plants is less stable every year. The need for corn in 2016 is projected at 13.8 million tons, where the need for corn is divided into two needs, namely for food which reaches 8.6 million tons and feed 5.2 million tons. The demand for corn in 2016 increased compared to 2015, which was only 13.1 million tons. While in 2018, the total

production of corn crops in Indonesia reached 30.1 million tons, an increase of 6.5 million tons over two years.

Corn plants can grow optimally on productive land and marginal land. But at this time more productive land is used for rice production. One alternative for corn production is only on marginal land. However, the main problem found in marginal land is the soil's poor physical, chemical, and biological properties. Soil physical properties included compacted soil, hard, dry, prone to erosion, and rocky (gravel, kraal, and large stones). Soil chemical properties such as low soil pH, low macro and micronutrient reserves, low cation/anion exchange capacity, contain toxic metal elements aluminium, iron, manganese. Soil biological properties, for example, are inferior in beneficial soil micro-organisms. Still, the abundance of micro-organisms detrimental to plant growth and decomposition of soil organic matter is very low [3]. Based on the explanation of these problems, marginal land is still possible to be used as agricultural land because there is input and technology to increase production, for example, by using rhizobacteria.

The use of rhizobacteria as bio stimulants and biofertilizers (biological fertilizers) to increase agricultural production is still minimal. However, various studies have shown that rhizobacteria have enormous potential in increasing agricultural production. Biofertilizer is a fertilizer that contains a group of live microorganisms that are useful for plants. These microorganisms were provided nutrients to increase soil fertility and plant quality through biological activities that interact with the physical and chemical properties of the soil [4]. These microorganisms contribute greatly to soil function because they contain several secondary metabolites that can maintain soil quality and increase plant growth and health. The characterization of rhizobacteria secondary metabolism has three potential function compounds consisting of osmoprotectant, phytohormone, and organopesticide. Based on research aims ware to find rhizobacteria living in dryland, it assumed some rhizobacteria living in dryland could be resistant to drought areas, and that has the potential compound to increase induced resistant plants to drought

2. Materials and Methods

The research was conducted at the Integrated Laboratory of the Faculty of Agriculture and Animal Husbandry, the University of Muhammadiyah Malang, from September 2020 till November 2020.

2.1. Sampling

Sampling was carried out at five points of collection by taking soil as deep as 10 cm. The soil taken is positioned close to the roots of the corn plant.

2.2. Media Preparation

The media needed in this research is M63 media. The composition of the media is M63 KH_2PO_4 100 mM, KOH 75 mM, $(NH_4)_2SO_4$ 15 mM $MgSO_4$ 0.16 mM, $FeSO_4$ 4 mM, Glucose 10 mM, making 1-liter stock with a note that glucose is not mixed because it can mushroom more quickly. All ingredients are dissolved in 1 litre of distilled water using a stirrer. Next, take stock of NaCl by weighing 5.85 g/L material dissolved in 1 litre of distilled water. If all the ingredients are ready to make the media, it takes 100 mM M63, 100 mM NaCl and 10 mM tryptophan dissolved in 1 litre of distilled water, then sterilized using an autoclave 121 °C for 15 minutes.

2.3. Rhizobacteria Isolation

Soil samples that have been prepared are separated between the roots and the soil powder. Then the dilution was carried out with a series of 101-109, and the droplet method was carried out, namely the dilution of 10^6,10^7,10^8, 10^9 then planted on NA media (NB 18g+ Agar 15g per 1 litre of aquadesh) with the droplet method using glass L. Incubated for 1×24 hours for bacteria to grow [5].

2.4. Purification

Purification is carried out after isolation. Purification aims to obtain single colonies of corn plant root bacteria. Purification was carried out by the steak method by taking one ose of homogeneous bacterial isolate. The scratch method was carried out to purify and separate the isolates of each bacterium. Colony purification was separated based on differences in colour, texture, and colony shape (Pamungkas 2014). Incubation was carried out to see the growth of bacteria for 1×24 hours. After obtaining pure ten bacterial isolates potential, they were stored on media so that they were tilted.

2.5. Osmoprotectant and Growth Hormone Metabolite Test

The sterile media was then transferred to 8 ml test tubes, and then the selected isolate was taken and added to 8 ml of M63+Tryptophan media. The stages of media transfer and bacterial isolation were carried out in a sterile LAF, and then the isolates were incubated for 3 days using a shaker. After 3 days, the isolate was centrifuged at 2000 rpm for 5 minutes. After the isolates were centrifuged, the supernatant. Taken, then added absolute methanol in a ratio of 1:1 with the number of isolates is 5 ml.

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3. Results and Discussion

3.1. Result

3.1.1 Metabolites belonging to the osmoprotectant group

The results of the GCMS analysis of the 10 tested isolates found that metabolites that lead to the osmoprotectant group can be seen in Table 1. Based on table 1. Classification of secondary metabolites in 10 isolates of compounds classified as osmoprotectants. This osmoprotectant compound is produced naturally by organisms or synthetically, which aims to protect cells from conditions of low air activity.

Table 1. Metabolites classified as osmoprotectant

No.	Retention time	Compound	Molecular formula	Area (%)
1.	16.076	2,3,4,5,6-pentaacetyloxyhexyl acetate	$C_{18}H_{26}O_{12}$	1.53
2.	20.394	Hexadecamethyl octasiloxane	$C_{16}H_{48}O_7Si_8$	3.30
3.	20.745	1,3,5-trimethoxybenzene	$C_9H_{12}O_3$	4.42
4.	23.750	benzyl N-phenylmethoxycarbonyliminocarbamate	$C_{16}H_{14}N_2O_4$	3.56
5.	2.053	2-methylsulfanylethanamine	C_3H_9NS	5.34
6.	14.701	oxepane-2,7-dione	$C_6H_8O_3$	0.50
7.	17.236	bis[[bis(dimethylsilyloxy)-methylsilyl]oxy]-methyl-prop 2-enylsilane	$C_{14}H_{42}O_6Si_7$	2.43
8.	3.534	1,3-thiazolidine	C_3H_7NS	2.70
9.	15.360	cyclohex-4-ene-1,2-dicarboxylic acid	$C_8H_{10}O_4$	4.42
10.	18.497	N-benzyl-N-(2-pyrrolidin-1-ylethyl)aniline	$C_{19}H_{24}N_2$	4.29
11.	23.759	(E)-hept-2-enal	$C_7H_{12}O$	12.55
12.	1.474	(2E)-3,7-dimethylocta-2,6-dienal	$C_{10}H_{16}O$	15.66
13.	20.558	octadecan-1-ol	$C_{18}H_{38}O$	2.02

3.1.2 Metabolites belonging to the phytohormone group

The GCMS analysis of the 10 tested isolates showed that there were 10 metabolites in the phytohormone group. Phytohormone compounds are thought to have the potential as biofertilizers for plants. It can be seen in Table 2. Based on table 2. The compounds found are included in the phytohormone group, which functions to stimulate plant growth.

Table 2. Metabolites belonging to the phytohormone group

No.	Retention time	Compound	Molecular formula	Area (%)
1.	23.751	N-2-Fluorenylperfluorpropionamid	$C_{16}H_{10}F_5NO$	3.02
2.	18.497	cinnolino[4,3-b]quinoxaline	$C_{14}H_8N_4$	6.00
3.	3.430	1-methyl-2-phenylindole	$C_{15}H_{13}N$	5.90
4.	11.008	1H-Indole	C_8H_7N	3.64
5.	11.066	4-(1H-benzimidazol-2-yl)benzonitrile	$C_{14}H_9N_3$	2.41
6.	17.245	N,N'-diphenylhexanediamide	$C_{18}H_{20}N_2O_2$	2.13
7.	20.305	2,5-bis(diethylaminomethyl)benzene-1,4-diol	$C_{16}H_{28}N_2O_2$	2.35
8.	2.055	1-methyl-2-phenylindole	$C_{15}H_{13}N$	2.81
9.	1.429	cyclohexanone	$C_6H_{10}O$	1.92

3.1.3 Metabolites belonging to the organopesticide group

The results of the GCMS analysis of the 10 tested isolates found that metabolites that lead to the organopesticide group is shown in Table 3. Based on table 3. The isolates were found to have the characteristics of metabolites classified as organopesticides. This group of compounds is a group of compounds that can be used as substances in controlling pests.

Table 3. Metabolites belonging to the organopesticide group

No.	Retention time	Compound	Molecular formula	Area (%)
1.	20.594	2-(4-chlorophenyl)hexanenitrile	$C_{12}H_{14}ClN$	6.43
2.	1.468	2-chloro-4-methoxyphenol	$C_7H_7ClO_2$	11.32
3.	23.145	2,6-dichloro-4-nitrophenol	$C_6H_3Cl_2NO_3$	3.84
4.	18.498	2,6-dichloro-4-nitrophenol	$C_6H_3Cl_2NO_3$	4.37
5.	19.535	4-chloro-2,6-dipyridin-2-ylpyridine	$C_{15}H_{10}ClN_3$	7.07
6.	1.428	3,4-dichlorotridecanoic acid	$C_{13}H_{24}Cl_2O_2$	1.57
7.	2.054	aminothiourea	CH_5N_3S	4.80
8.	1.430	ethyl 2-chloroacetate	$C_4H_7ClO_2$	2.65
9.	20.581	(E)-non-2-enal	$C_9H_{16}O$	9.87

3.2. Discussion

Secondary metabolites which act as osmoprotectant should not be inhibitory to cells. Compounds used as osmoprotectants are water-soluble, such as sugar derivatives, alcohols, and amino acids [6]. Osmoprotectant compound accumulation and stress tolerance acquisition. Changing the ratio between the carbohydrates provided and non-protein nitrogen during growth under osmotic constraint modulated osmoprotectant accumulation [7].

Plant hormones are produced by the individual in question ("endogenous"). Hormones from outside the individual's system can also be administered ("exogenously"). Exogenous administration may also involve non-natural chemicals (synthetic, not made from plant extractions) that produce stimuli similar to natural phytohormones. Therefore, to accommodate differences from animal hormones, the term plant growth regulator is also used. There are five main groups of plant hormones, namely auxins (AUX), cytokinins (CK), gibberellins (or gibberellic acid, GA), ethylene (ethene, ETH), and abscisic acid

(ABA). The first three groups tend to be positive for growth at physiological concentrations, ethylene can support or inhibit growth, and abscisic acid is primarily a growth inhibitor. The above compounds are compounds that makeup plant growth hormones [8].

Besides osmoprotectan and plant hormones, secondary metabolites can have a role and categorized as organopesticide. This group of compounds is a group of compounds that can be used as substances in controlling pests. Organopesticides is a biochemical pesticide composed of natural and non-toxic compounds used to control pests. Mathew [9] and Kumar [10] add that besides being non-toxic, organopesticides are natural pesticides that are also friendly or safe for the environment. According to Mishra et al. [11], the commonly used definition of a biopesticide is that from the US Environmental Protection Agency (USEPA). Organopesticides are defined as pesticides derived from nature composed of animals, plants, bacteria, and minerals. Organopesticides also include living organisms that can control agricultural pests.

4. Conclusion

This research concludes that the 10 isolates have three characteristics of secondary metabolites i.e., osmoprotectants, phytohormone, and organopesticide. The greatest value on osmoprotectans that is (2E)-3,7-dimethylocta-2,6-dienal 16.66%, on phytohormone that is cinnolino[4,3-b]quinoxaline 6.00%, on organopesticide that is 2-chloro-4-methoxyphenol 11.32%.

5. Acknowledgement

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