The growth inducing endophyte: optimizing the non-host endophyte as growth promoter

by Sugiyarto Sugiyarto

IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

The growth inducing endophyte: optimizing the non-host endophyte as growth promoter

To cite this article: Sugiyarto *et al* 2022 *IOP Conf. Ser.: Earth Environ. Sci.* **980** 012011

IOP Conf. Series: Earth and Environmental Science **980** (2022) 012011 doi:10.1088/1755-1315/980/1/012011

The growth inducing endophyte: optimizing the non-host endophyte as growth promoter

Sugiyarto[1], C Trwidiarto[1], Supriyadi[1], R Firgiyanto[1*], H S Addy[2,3], A Wafa[2,3]

[1] Department of Agricultural Production, State Polytechnic of Jember, Jl. Mastrip, Jember, Indonesia
[2] Plant Protection Study Program Department of Plant Protection Faculty of Agriculture University of Jember, Indonesia, Jl. Kalimantan No 37 Jember, Indonesia
[3] Center of Development Center for Development of Advanced Science and Technology (CDAST), University of Jember, Indonesia

refa_firgiyanto@polije.ac.id

Abstract. The plants present a complex, spatially and temporally diverse ecological habitat. As living support of human, microorganism, microorganism and fungal. They are life in the host plant as endo, ecto, semi endo or other kind living mechanism. The symbiotic associations between microorganisms and plants are ancient and fundamental. Most of plant protection scientist develop the broad range of host in endphopytic microbes for the future purposes, such as biological control agents, growth inducer, the plant resistance inducer and soon. However, the development non true host of endophytic microbes are challenging. The research was done by isolating potential endophyte from three toga plant and then tested to spinach. The growing and plant pathogenic possibility test was done to eliminating the potential plant pathogen microbes. From 40 isolate, was detected more than 20 isolate have possibility to be a pathogen of Spinach, with different symptoms appearance level. The level of growth induced result also have different result among the potential endophyte. They appear on the growing time, the long of root and shoot number of spinach. The optimizing of growth promoter endophyte especially for non-host endophyte, need the extra path for convinced the isolated endophyte not to be potential plant pathogen.

1. Introduction

The plants present a complex, spatially and temporally diverse ecological habitat. The root and other part of plant became as living support system for macroscopic organisms, microbial communities and fungal. The symbiotic associations between microorganisms and plants are ancient and fundamental. Endophytic microbes are an intriguing group of organisms associated with various tissues and organs of terrestrial and some aquatic plants, and are the subject of increasing interest to mycologists, ecologists, and plant pathologists.

In general, "endophytic" infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal, either through histological means, by isolation from strongly surface disinfected tissues or, most recently, through direct amplification of fungal nuclear DNA from colonized plant tissue. Infections of land plants by endophytes are ubiquitous, having been found throughout a broad range of host orders, families, and genera worldwide, and representing a diverse array of terrestrial and aquatic habitats [1].

Most of scientist develop the broad range of host in endphopytic microbes for the future purposes, such as biological control agents, growth inducer, the plant resistance inducer and soon. It's isolated from a sample plant and optimizing for other plant, not only in same genus but also for some long distance relationship plant. For axample, the fungal endophytic from aquatic legumes, *Rhizobium leguminosarum* bv. Trifolii from their aquatic legumes, was use on aplicate them into rice. As the result the inducer reaction was appear on the infected host [2].

The same pathways are used in this project. Fungi and bacterial endophite was isolate from three different family medicinal plants or in Indonesia called Toga. There was Gotu Kola or Pegagan (*Centella asiatica*), the cat whiskers plant or kumis kucing (*Orthosiphon aristatus*) and Drumstick tree or Kelor (*Moringa oleifera*). The fungal and bacterial endophyte from thet plant were tested on spinach (*Spinacia oleracea*).

2. Material and Methods

2.1. Microbial Endophytic Isolation

The isolation methodology of endophytic fungi from three different described plant performed by methods described by Braun *et al*. [3]. Hyphaltips were taken from initial isolates and transferred onto plates of potato dextrose agar (PDA, Hi Media India), Malt Ekstract Agar (MEA, Diftco Franklin Lakes, N.J.) and Tryptic Soy Agar (TSA, Merk), maintained at 18 8C or room temperature, and transferred every few months. Each isolates were selected for further morphological characterization based upon differences in gross colony morphology. The bacterial endophytic was isolate by maceration technique.

2.2. Endophytic Screening

For fungal morphological examination, isolates were cultured on PDA, and water agar. Cultures were incubated the 14 h light (dark) at 22-28^{o}C. Following 2 weeks incubation, isolates were examined for colony morphology and morphological characteristics of the conidia and conidiophores. Conidia and conidiophores were taken approximately 2 mm inside the colony margin, mounted in water on a microscope slide, and observed with an Olympus BX61 microscope at 40x magnification.

The bacterial endhophyte was examine by bacterial colony examination in TS Agar medium. Than a microscope slide, and observed the colony shape and other morphological type. The first screening of microbial endophytic was done by seed pathogenicity test. By follow [4] methodology for seed health. The *S. oleracea* seed were tested individually on each isolated endophyte. The necrotic reaction in the test indicated the microbial endophytic have a possibility as plant pathogenic in spinach seedling. And for that microbes will not use for more purpose.

2.2. Growing test

The possibility of endophytic growth inducer on the Spinach was done by [5] modification methodology. The modification was done by adding the medium test for each endophytic microbes type. The bacterial endophytic was growth in LB medium for 48 hour and shake in 150 rpm at 28^{o}C and incubated for 2 weeks. The surface sterilized seed adding before the shaker turn on. The seed planted on sandy soil on the tray. Each bacteria replicated three times with five plant unit per replication. The fungi was test by water agar seedling test. Each fungi test was replicated three times with five plant unit per replication at 28^{o}C and incubated for 2 weeks 14 h light.

3. Result and Discussion

The plant endophytic life as an endosymbiont. It mostly from bacterium and fungus. That lives within a host on specific host part or cell, without causing apparent disease. According to Ahmad *et al* [6] It live asymptomatically inside plant tissue and found the most plant kingdom. The symbiont of endophyte and plant are mostly mutualism, where each species has a net benefit. For example fungi Endophytic fungi receive shelter and nutrition from host plants and in return provide great advantages to the host.

However, according to the [7], the evolution of endophytic microbes was start as normal plant pathogen, attacks their host and absorbed the nutrition from their host just like other plant pathogens.

Moreover, [8] informed that endophytic-host plant associations are generally treated separately from parasitic, pathogenic and saprophytic interactions and are viewed as mutualistic associations. However, endophyte–host interactions are based on mutual exploitation. Benefits to the partners are rarely symmetric and conflicting selection forces are likely to destabilize them.

The mutualistic associations of endophytic and host plant appear not only at one time. They develop their symbiotic interaction for long time, from geneteration to generation, to specific plant to specific microbe's species. To specific microbes to specific plant part. Some of them can more specifically to the strain, race, or pathovar. It fact make the development of non-true host endophytic microbes are challenging. Need to extra purposes for development of endophytic microbes from other plant host to other plant genus or species.

The same result and theory was showed in this project. According to research, the necrotic reaction (Table 1) and seedling symptom disease (Figure 1) was appear on the spinach body part. The appearance of necrotic in the spinach part indicated the interaction e.g infection process was started. According to [9] the fungal endophytic behaviour changed due to abiotic and biotic factor. By that factor the effector and elicitor pathways on plant was started. Moreover, [10] reported that the abiotic factor like high light triggers endophyte pathogenicity, while low light supports endosymbiotic development. The pathogenicity under high light resulted from light-induced production of H_2O_2 by the fungus, triggering hypersensitivity, cell death, and tissue necrosis.

Table 1. Plant Pathogenic Posibility of Isolated Endophytic

Isolate Code	Necrotic Reaction on Spinach	Necrotic Clearance Level	Appearance Level
B.C.R1	-	-	-
B.C.R2	-	-	-
B.C.R3	-	-	-
B.C.R4	-	-	-
B.C.R5	-	-	-
B.C.R6	-	-	-
B.C.R7	-	-	-
B.C.R8	-	-	-
B.C.S1	+	5	Clear Spot
B.C.S2	+	1	Ambiguous
B.C.S3	-	-	-
B.C.L1	+	1	Ambiguous
F.C.L1	+	3	Mostly Clear
F.C.R1	-	-	-
F.C.R1	-	-	-
F.C.S1	-	-	-
F.C.L1	-	-	-
F.C.L2	-	-	-
B.K.R1	-	-	-

B.K.R2	-	-	-
B.K.R3	-	-	-
B.K.R4	+	1	Ambiguous
B.K.R5	-	-	-
B.K.R6	-	-	-
B.K.R7	-	-	-
B.K.R8	-	-	-
B.K.S1	-	-	-
B.K.L1	-	-	-
B.K.L2	-	-	-
F.K.R1			
F.K.R2	+	1	Ambiguous
F.K.R3	-	-	-
F.K.L1	-	-	-
B.M.R1	-	-	-
B.M.R2	+	1	Ambiguous
B.M.R3	-	-	-
B.M.R4	-	-	-
B.M.R5	-	-	-
B.M.L1	+	1	Dim
B.M.L2	+	1	Dim
F.M.R1	-	-	-
F.M.R2	-	-	-
F.M.L1	+	5	Mostly Clear
F.M.L2	+	3	Dim
F.M.L4	+	4	Mostly Clear
F.M.L3	+	3	Mostly Clear

The clearance level of necrosis in the spinach indicated two think. The microbial endophyte was triggers a hypersensitive response visible as local necrotic lesions of the host. And the second indication was the necrosis on the development non-host endophyte should be treated as independent features in the evaluation system. The clearance level of necrosis on the spinach indicate the conflicting selection forces by the plant host was undergo. According to the [11], most plant pathogens carry genes encoding plant cell wall-degrading enzymes. However, nonphytopathogens may possess glycoside hydrolase other than cellulase/hemi-cellulase (or cell wall degradation hydrolases). Presence of this enzyme in numerous endophytes is consistent with its possible role in the diversity of sugar utilization that might be a useful component of a competent endophyte.

The growth promoter is the one of several symbiotic reactions of the microbial endophyte and the true-new host. According to Gomez *et al* [12], plant growth-promoting endophyte may be used to reduce the need for chemical fertilizers, but their efficiently be affected by several factor. The utilization of endophytes can be protected the plant host from the rhizospheric competitors and extreme environmental conditions, could overcome those problems and successfully promote the crops under field conditions.

The plant host species, the microbe type, species, race and mechanism are the most factors to influence the growth of plant. Not all endophytic microbes have mechanism to boost the growth of the host. The growth promoter is just one of symbiotic mechanism among plant host and endophytes. However, the deep screening is the fist pathway to the develop the endophytic. Its also the most important path on the development non host endophytic microbes. While, the visible necrotic symptom is the most part on evaluation system during the endophytic sreening.

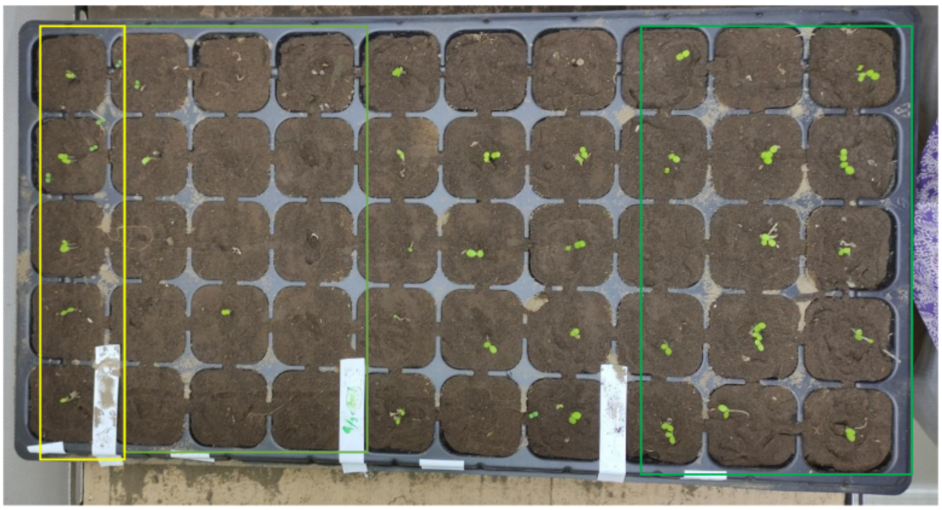

Figure 1. The growth promoter reaction compared to control (Green Box) and Seedling Symptoms (Red box) in Seedling Test compared to control (yellow box)

4. Conclusion
The growing and plant pathogenic possibility test was done to eliminating the potential plant pathogen microbes. From 40 isolate, was detected more than 20 isolate have possibility to be a pathogen of Spinach, with different symptoms appearance level. The level of growth induced result also have different result among the potential endophyte. They appear on the growing time, the long of root and shoot number of spinach. The optimizing of growth promoter endophyte especially for non-host endophyte, need the extra path for convinced the isolated endophyte not to be potential plant pathogen.

Acknowledgment
To the Research and Social Service Center of State Polytechnic of Jember by The Applied Research (Penelitian Terapan) Grand on 2021 with The contrack number: 23/PL17.4/PG/2021

References
[1] Stone, Jeffrey K, Charles W B, and James F White Jr 2000 Microbial Endophytes 17–44
[2] Yanni, Youssef G, Rizk R Y, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, De Bruijn F, Stoltzfus J, and Buckley D 1997. "Natural Endophytic Association between Rhizobium Leguminosarum Bv. Trifolii and Rice Roots and Assessment of Its Potential to Promote Rice Growth." Pp. 99–114 in Opportunities for biological nitrogen fixation in rice and

other non-legumes. Springer
[3] Braun, Karen, Romero J, Liddell C, and Creamer R 2003 Mycological Research 107 980–88
[4] Dube, Praxedis, Struik P C, and Ngadze E 2018 African Journal of Agricultural Research 13 753–70
[5] Zou, Tengyue, Huang C, Wu P, Ge L, and Xu Y 2020 Plants 9 490
[6] Ahmad R Z, Ameen F, Khalid R, Alghuthaymi M A, Alsalmi R, and Li C 2019 Sustainable Agriculture Research 8 66-72
[7] Wilson D 1995 Endophyte: the evolution of a term, and clarification of its use and definition Oikos 274-276
[8] Saikkonen K, Wäli P, Helander M, and Faeth S H 2004 Trends in plant science 9 275-280
[9] Mengistu A A 2020 Endophytes: Colonization, behaviour, and their role in defense mechanism. International Journal of Microbiology https://doi.org/10.1155/2020/6927219
[10] Alvarez-Loayza, J. F. White Jr., M. S. Torres et al., 2011. PLoS One, vol. 6, 2011
[11] Ali S, Duan J, Charles T C, and Glick B R. 2014. Journal of6eoretical Biology 343 193–198
[12] Jiménez-Gómez A, Saati-Santamaría Z, Kostovcik M, Rivas R, Velázquez E., Mateos P F, Menendez E, and García-Fraile P 2020 Agronomy 10 1788 https://doi.org/10.3390/agronomy10111788

The growth inducing endophyte: optimizing the non-host endophyte as growth promoter

ORIGINALITY REPORT

22%	23%	23%	10%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.nrcresearchpress.com Internet Source	5%
2	Submitted to Universitas Airlangga Student Paper	4%
3	www.researchgate.net Internet Source	4%
4	www.powerlogic.com Internet Source	3%
5	repository.ias.ac.in Internet Source	2%
6	Submitted to University of Science and Technology Student Paper	2%
7	mie-u.repo.nii.ac.jp Internet Source	2%

Exclude quotes Off

Exclude matches < 2%

Exclude bibliography On