Application of *Streptomyces nigrogriseolus GanoSA1* as a Preventive Treatment of Basal Stem Rot Disease Caused by *Ganoderma boninense*

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**ABSTRACT**

Research on the usage of biological control agents (BCA) for the management of basal stem rot disease of oil palm or better known as *Ganoderma* disease offers a greener solution than synthetic chemical fungicides. The actinomycete is one of the interesting groups of microbes with huge potential to be developed as a biological control agent. Rhizosphere actinomycetes identified as *Streptomyces nigrogriseolus GanoSA1* (*Streptomyces GanoSA1*) showed competent biological control activity in reducing *Ganoderma* incidence in nursery study. In this article, we described the roles and application of *Streptomyces* spp, and investigation of *S. nigrogriseolus GanoSA1* as BCA against *Ganoderma* disease of oil palm.

**INTRODUCTION**

*Ganoderma* disease in oil palm without proper disease management will cause reductions in yield production and financial losses (**Figure 1**). The usage of non-chemical control was considered as a green technology as a way towards sustainable agriculture. It offers a safer management strategy and contributes to the enrichment of biodiversity in environmentally friendly manners. Studies by researchers worldwide showed that many species of beneficial microorganisms, including actinomycetes, bacteria and fungi, have the ability to suppress plant diseases effectively.

The use of actinomycetes as biological control agents (BCA) to manage soil-borne diseases is of interest due to its characteristic and vast potential as a prolific producer of secondary metabolites. Actinomycetes (**Figure 2**) are widely distributed in natural or man-made environments. They constitute a large part of the rhizosphere microbiota and being isolated from various types of samples such as plant, soil, rhizosphere, water and air. The actinomycetes, especially *Streptomyces*, are used as a BCA in controlling soil-borne diseases in plants (Dias et al., 2017; Mun et al., 2020; Wonglom et al., 2019). Disease suppression by this genera might be related to several mechanisms such as i) production of antibiotics (Wonglom et al., 2019), ii) secretion of hydrolytic enzymes such as chitinase and laminarase (Wonglom et al., 2019; Mun et al., 2020),
iii) production of siderophores or competition (Zeng et al., 2018) and iv) induced resistance which acts individually or in combination to fight off pathogens (Dias et al., 2017; Senthilraja, 2016; Tariq et al., 2020). Apart from potential BCA traits tested in vitro and in vivo, reliable production-cost efficient and stable production on a large scale are crucial factors for a successful BCA product development. In this article, we described the roles and application of Streptomyces spp and the development of S. nigrogriseolus strain GanoSA1 as BCA against Ganoderma boninense of oil palm.

Figure 2. Different morphologies of a) actinomycetes (black circled), bacteria (red arrow) and fungi (blue arrow) observed on isolation plates, b) pure Streptomyces nigrogriseolus GanoSA1 on Yeast Malt agar (YMA) after seven days of incubation at 28°C (Idris and Shariffah, 2021).

Sources of bioactive compound

Actinomycetes are the major source for all types of bioactive metabolites, lytic enzymes of medical and industrial values. They were also able to decompose organic matter, especially biopolymers such as lignocelluloses, starch, and chitin in soil (Doumbou et al., 2002; Solecka et al., 2012). Production of chitinase and glucanase was also detected in some of the Streptomyces sp. isolates. Many species of actinomycetes, especially those belonging to the group Streptomyces are well known as biocontrol agents that inhibit or lyse several soil-borne and airborne plant pathogenic fungi (Jog et al., 2012; Mun et al., 2020; Wonglom et al., 2019) and continue to be a major source of beneficial secondary metabolite (Matsumoto and Takahashi, 2017). In vitro lysis of fungal cell walls either by microbial chitinase, glucanases alone or by a combination of both enzymes has been demonstrated to affect the mycelia structure of phytopathogen. Chitin has been known as the main component in the fungal cell wall. Thus, the production of lytic enzymes will increase the antagonistic effect towards phytopathogenic fungi.

Biological control agent

Extensive research on the use of actinomycetes as a BCA and bio-fertiliser in various crops have been established for a greener and sustainable crop protection (El-Tarabily et al., 2009; Gopalakrishnan et al., 2013; Goudjal et al., 2014; Yuan and Crawford, 1995) and some have been registered for commercial usage. Streptomyces griseoviridis, S. violaceusniger YCED9, S. nigrogriseolus GanoSA1 are examples of actinomycetes that have been developed into serve as nutrient enhancers (Amaresan et al., 2018). Besides producing siderophores and solubilising phosphate, they produce various enzymes that make the complex nutrients into simple mineral forms. This study did not find any adverse effects when the potential Streptomyces sp. was applied to the oil palm seedlings. All the treated seedlings were healthy. Observation on the vegetative growth indicated that the Streptomyces sp. significantly increased the plant height, stem diameter and relative leaf chlorophyll (Chl) content compared to those of the control seedlings. The PGP activities of Streptomyces sp. have also been reported widely (Nur Azura et al., 2016; Liotti et al., 2019). Dias et al. (2017) reported the use of Streptomyces spp. produced siderophores, ACC deaminase that can solubilise phosphate and produce volatile organic compounds related to tomato seedlings growth promotion. The use of Streptomyces griseoviridis R132 to control phytopathogen growth and promote the growth of pepper (Capsicum annum) was reported by Liotti et al. (2019).

ROLES AND APPLICATION OF Streptomyces spp.

Plant growth promotion (PGP)

The actinomycetes influence soil fertility through the involvement of many mechanisms and
commercial biological control agents (Shariffah-Muzaimah et al., 2021, Idris and Shariffah-Muzaimah, 2021). Numerous studies have reported on the parasitism effect of fungal pathogens by Streptomyces spp. and other actinomycetes. The actinomycetes have also been reported to induce systemic resistance in apple fruit (Zhang et al., 2016), rice (Senthilraja, 2016; Shao et al., 2018), tomato (Dias et al., 2017; Singh and Gaur, 2017) and eucalyptus (Salla et al., 2016). We observed quantitative changes in the activities of oil palm defence related enzymes. Activities of Peroxidase (POX), Polyphenol oxidase (PPO), Phenylalanine lyase (PAL), chitinase and β-1,3-glucanase in both oil palm leaves and roots treated with our potential isolates were enhanced to some extent in comparison with the untreated seedlings indicating possible induction of systemic resistance in oil palm seedlings.

RESEARCH ON ACTINOMYCETES AS BIOLOGICAL CONTROL OF Ganoderma DISEASE

The selection of potential strain is a critical step in the development of BCA. A two-component screening, as exclusively related to interaction studies and potential antagonists were typically ranked according to their ability to inhibit the growth of the pathogen expressed by the inhibition zone. Strain with potential BCA activity against pathogenic Ganoderma was selected based on inhibition percentage of radial growth (PIRG) observed on petri plates. Many studies have reported the ability of Streptomyces spp and some non-Streptomyces actinomycetes in the whole cell and also its metabolite to cause inhibition and lesion on Ganoderma mycelia (Idris and Shariffah-Muzaimah, 2021; Lim et al., 2018; Nur Azura et al., 2016; Pithakkit et al., 2015; Queendy and Roza, 2019; Shariffah-Muzaimah et al., 2015, 2020; Sujarit et al., 2020; Tan et al., 2002; Ting et al., 2014). In our experiment, four isolates (19.95%) of rhizospheric actinomycetes exhibited significant activity in inhibiting the growth of G. boninense in vitro (Figure 3).

The two-compartment screening or the primary in vitro test is a good method to distinguish potential BCA. However, it is just an initial from experiments required to identify novel biocontrol agents. Microbial interaction in soil under natural ecosystems may alter their competitive ability and potential to produce antimicrobial compounds. Therefore, an in planta experiment at a greenhouse and field is necessary to ensure the isolates potential in plant protection. As far as our concern, there is not much research reported in greenhouse experiments or field trials to study Streptomyces spp. in oil palm growth promotion and protection against Ganoderma. Nur Azura et al. (2016) reported that the application of S. sanglieri strain AUM 00500 at 10⁹ CFU ml⁻¹ showed significance in oil palm growth promotion compared to the control. Meanwhile, Shariffah Muzaimah et al. (2018) the main causal agent of oil palm (Elaeis guineensis) reported a significant reduction of disease incidence and oil palm growth when oil palm seedlings were treated with Streptomyces spp. under a shaded nursery experiment (Figure 6).

Research and development (R&D) in mass production of Streptomyces nigrogriseolus GanoSA1 and utilisation of the Streptomyces powder

Mass production of a stable and inexpensive formulation is one of the most critical steps in developing an effective biological control product. Further R&D in the downstream process to develop BCA products on a large scale was established based on the laboratory scale data. The downstream process related to the inoculum production in large amounts includes the choices of culture medium and equipment to be used, the method for harvesting and storage of stable inoculum and its product. Scaling up from laboratory to large scale is one of the crucial factors in developing BCA products. It requires optimising parameters involved during flask to bioreactor process, which is tailored specifically to a BCA used. Overall, this process must be focused on obtaining the optimal

Figure 3. In vitro assay plates of actinomycetes isolates against G. boninense; a) control plate of G. boninense, b) Positive inhibition observed between actinomycetes during in vitro assay and, c) Inhibition of G. boninense by Streptomyces nigrogriseolus GanoSA1 (Shariffah Muzaimah et al., 2020).
BCA with effective results and possibly low costs. The final developed BCA product must be stable with a long shelf-life, and providing the required control results when applied in the field with an appropriate delivery system at the lowest effective dose (Ravensberg, 2010; Shaikh and Sayyed, 2015; Shen et al., 2016). The mass production of *S. nigrogriseolus* GanoSA1 (Figure 5) was achieved through research collaboration and continuous R&D.

In order to ensure the safety of our *Streptomyces* strain, a toxicology test on acute oral and acute dermal were performed in School of Pharmaceutical Sciences, University Sains Malaysia (USM), Penang, Malaysia by using Spraque-Dawley rats (Female, nulliparous and non-pregnant). The LD$_{50}$ of orally and dermal administration of the powder in female rats is higher than 2000 mg kg$^{-1}$. No toxic signs and symptoms were observed. Necropsy studies done two weeks after treatment also did not show any physical changes in rats’ organs. All of the dermally treated rats gained weight every week, similar to the control group. The product is non-polluting, non-pathogenic to humans and animals as compared to chemical fungicides.

In *plantae* assessment of the *S. nigrogriseolus* GanoSA1 powder was conducted in a period of sixth months under nursery conditions (Figures 4a and 4b). A series of repeated experiments was performed in order to obtain reliable data. Based on the observation, the disease development in seedlings treated with *S. nigrogriseolus* GanoSA1 was slower compared to the negative control. After sixth months of artificial inoculation with *G.,
Application of Streptomyces nigrogriseolus GanoSA1 as a Preventive Treatment of Basal Stem Rot Disease Caused by Ganoderma boninense

Boninense PER71, seedlings treated with soil isolates Streptomyces sp. GanoSA1 showed the significantly lowest percentage of DI and SFS (53.33% and 41.70%, respectively) compared to the untreated with 93.33% and 83.84%, respectively. Apart from disease assessment, nursery evaluation of S. nigrogriseolus GanoSA1 revealed the potential used as the strain as a plant growth promoter of oil palm. No adverse effect was observed on all of the seedlings treated with our Streptomyces strain. All of the seedlings were healthy. The assessment on vegetative growth indicated that our strain significantly increased the plant height, stem diameter, and relative leaf chlorophyll (Chl) content compared to those of the control seedlings.

The in plantae assessment was also conducted under a field condition through the seedling baiting technique (Figure 5). Based on the observation of dead seedlings due to Ganoderma infection at 36 months, 6.6% of palms treated with S. nigrogriseolus GanoSA1 showing BSR symptoms and dead compared to the control (75.0%). Disease reduction of 68.4% was calculated based on dead seedlings percentage after three years of planting (Idris and Shariffah, 2021). These trials highlight the potential of the Streptomyces GanoSA1 powder to reduce BSR disease in oil palm and promote oil palm growth.

The Streptomyces GanoSA1 powder can be used at the nursery, during replanting and in the field. It is recommended to apply the powder in the nursery (oil palm seedlings at 3, 6 and 9 months, total of 150 g/palm), at replanting area (250-300 g/hole), and oil palm cultivation area; immature palm (palm less than 5 years at 300 g/palm/year) and mature palm (palm more than 5 years at 600 g/palm/year) (Figure 7). This could prevent Ganoderma infection and reducing the risk of disease incidence in oil palm cultivation areas.

As part of the effort to reduce the incidence of Ganoderma disease in oil palm, 431 430 sachets

Figure 6. Field evaluation using seedling baiting trial in field, a) palm treated with Streptomyces nigrogriseolus GanoSA1 showing healthy and normal growth, b(i) palm untreated with Streptomyces GanoSA1 showing Ganoderma symptom (yellowing foliar symptoms) and b(ii) dead palm observed in untreated palm (Idris and Shariffah-Muzaimah, 2021).

Figure 7. Application of commercialised Streptomyces GanoSA1 powder in a) nursery, b) immature/mature palm of field planted oil palm and c) planting hole.
(129.43 tonnes) of Streptomyces GanoSA1 powder has been distributed to 2 915.07 ha of field planted oil palm in Malaysia (Table 1).

**CONCLUSION**

Research on BCA is very promising, with significant economic and greener alternatives to reduce environmental impacts. Safer management strategy contributes to the enrichment of biodiversity in eco-friendly manners were possibly gained through the adoption of the biological control agent. The success of plant disease control by using Streptomyces as BCA is related to several factors, including finding potential BCAs. As the major soil inhabitants and their prolific antimicrobial producer criteria, actinomycetes have a huge potential to be used as biological control agents for controlling plant disease. Apart from preliminary antagonistic activity, study on enzyme, siderophores, antimicrobial compound production, plant growth promotion traits or induce systemic ability is additional information that will highlight the potential of the mechanism involved. In plantae experiment in nursery and field by using plant treated by BCA and artificially inoculated by the pathogen is a sequel in order to look into the efficacy of each BCA under natural conditions. Fundamental works were essential to identify the different mechanisms of BCA action and other prospects to diversify the potential applications for the BCA. Based on our study, Streptomyces GanoSA1 showed the potential to be used as a biocontrol agent in reducing the effect caused by Ganoderma. The usage of this powder contributed positively towards controlling and prevention of the Ganoderma disease in oil palm plantations.

**REFERENCES**


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**TABLE 1. DISTRIBUTION OF COMMERCIALISED Streptomyces GanoSA1 POWDER IN MALAYSIA**

<table>
<thead>
<tr>
<th>States</th>
<th>Area involved (ha)</th>
<th>Number of sachet distributed (500 g/sachet)</th>
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<tr>
<td>Negeri Sembilan</td>
<td>379.01</td>
<td>56 094</td>
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<tr>
<td>Melaka</td>
<td>106.24</td>
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<td>Selangor</td>
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<td>Johor</td>
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</tr>
<tr>
<td>Pahang</td>
<td>16.06</td>
<td>2 377</td>
</tr>
</tbody>
</table>


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