

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.

ABSTRAK

Penggunaan agen kawalan biologi dalam pengurusan penyakit *Ganoderma* menawarkan pendekatan mesra alam dalam mengurangkan kebergantungan terhadap racun kulat kimia sintetik. Aktinomiset merupakan kumpulan mikroorganisma dengan keupayaan menghasilkan pelbagai bahan bermanfaat yang berpotensi besar dikembangkan sebagai agen kawalan biologi. Aktinomiset pencilan rizosfera, *Streptomyces nigrogriseolus* GanoSA1 (*Streptomyces* GanoSA1) telah menunjukkan aktiviti kawalan biologi *Ganoderma* yang baik dalam mengurangkan jangkitan penyakit *Ganoderma* di peringkat kajian makmal, tapak semaian dan ladang.

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.

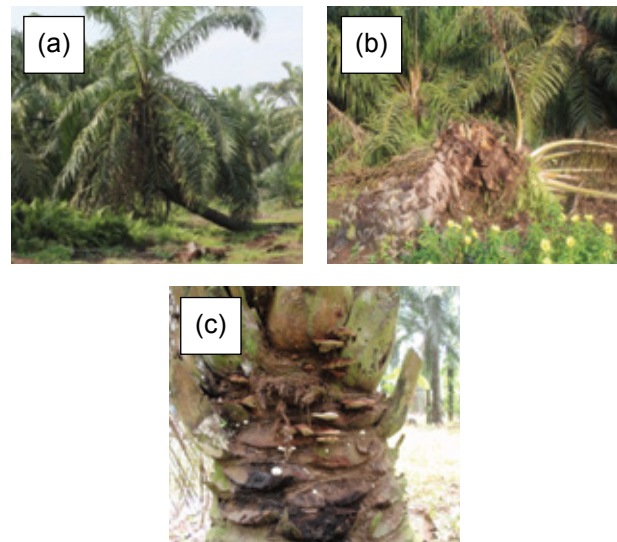


Figure 1. *Ganoderma* disease in oil palm; a) mature palm showing foliar symptoms of *Ganoderma* disease, b) palm collapse due to *Ganoderma*, and c) *Ganoderma* fruiting body observed on the palm bole area.

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),

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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.

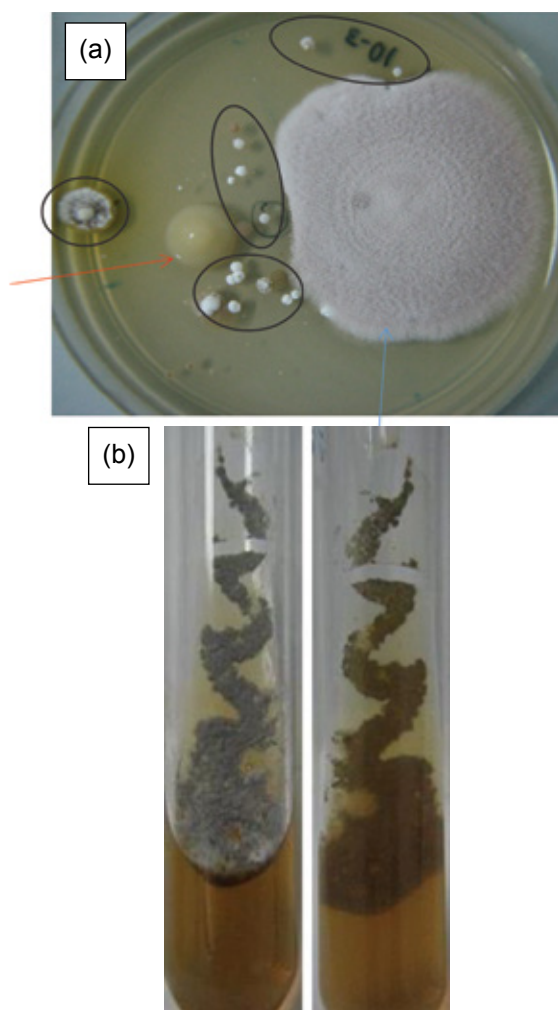


Figure 2. Different morphologies of a) actinomycetes (black circled), bacteria (red arrow) and fungi (blue arrow) observed on isolation plates, b) pure *Streptomyces nigrogriseolus* GanoSA1 on Yeast Malt agar (YMA) after seven days of incubation at 28°C (Idris and Shariffah, 2021).

ROLES AND APPLICATION OF *Streptomyces* spp.

Plant growth promotion (PGP)

The actinomycetes influence soil fertility through the involvement of many mechanisms and

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 griseocarnus* R132 to control phytopathogen growth and promote the growth of pepper (*Capsicum annum*) was reported by Liotti *et al.* (2019).

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 (Doubou *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

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), oak (Kurth *et al.*, 2014), 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 plantae* 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 10^9 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

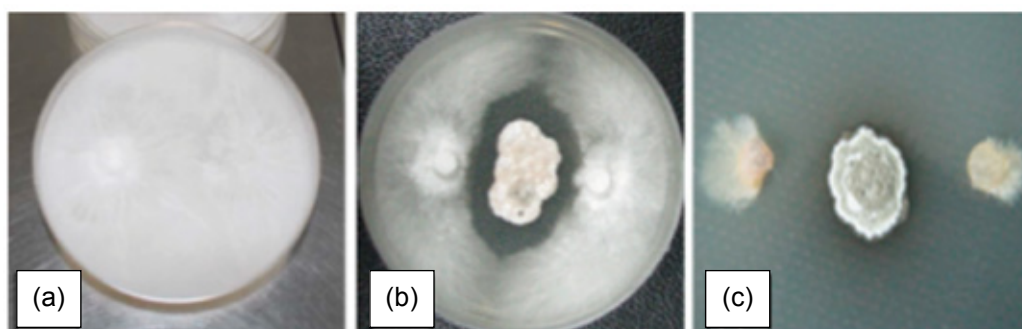


Figure 3. In vitro assay plates of actinomycetes isolates against *G. boninense*; a) control plate of *G. boninense*, b) Positive inhibition observed between actinomycetes during in vitro assay and, c) Inhibition of *G. boninense* by *Streptomyces nigrogriseolus* GanoSA1 (Shariffah Muzaimah *et al.*, 2020).



Figure 4. Mass production of *Streptomyces nigrogriseolus* strain GanoSA1; a(i-iii) from pure culture of GanoSA1 to a 100L biofermentor and b) packaging of the final product d) *S. nigrogriseolus* strain GanoSA1 commercial product 'EMBIO™ actinoPLUS' in, b(i) 300 g/sachet, b(ii) 5 kg/bag and (biii) 10 kg/bag, packaging.

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 Sprague-Dawley rats (Female, nulliparous and non-pregnant). The LD₅₀ 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.*

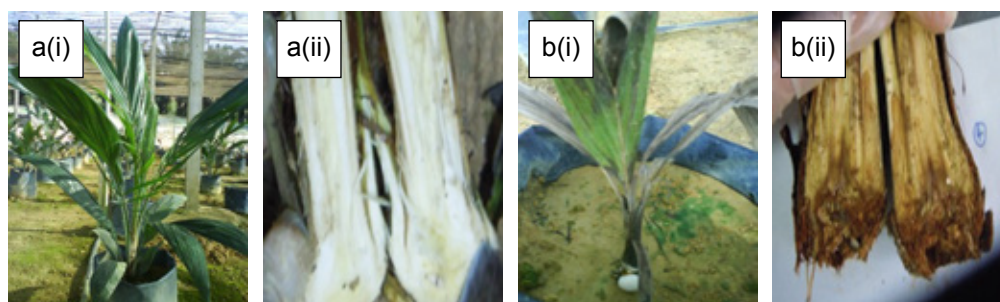


Figure 5. In plantae trial to study the effectiveness of *Streptomyces nigrogriseolus* GanoSA1 to control Ganoderma disease incidence in nursery experiment, a(i) seedling treated with *Streptomyces* GanoSA1, a(ii) cross-section of seedling treated with *Streptomyces* GanoSA1, b(i) untreated (control) seedling and b(ii) cross-section of untreated (control) seedling showing lesion and severe rotting due to Ganoderma infection.

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 7. Application of commercialised *Streptomyces* GanoSA1 powder in a) nursery, b) immature/mature palm of field planted oil palm and c) planting hole.



Figure 6. Field evaluation using seedling baiting trial in field, a) palm treated with *Streptomyces* nigrogriseolus Gano SA1 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).

(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.

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

States	Area involved (ha)	Number of sachet distributed (300 g/sachet)
Negeri Sembilan	379.01	56 094
Melaka	106.24	15 724
Selangor	34.31	5 078
Johor	1 265.58	187 305
Perak	1 113.86	164 852
Pahang	16.06	2 377

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