CHILEAN JOURNAL of AGRICULTURAL RESEARCH

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RESEARCH ARTICLE

Enhancing tomato growth with an endophytic actinomycete and applied planting media as its carriers

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Received: 18 June 2023; Accepted: 9 August 2023, doi:10.4067/S0718-58392023000600725

ABSTRACT

Endophytic actinomycetes play an important role in plant growth-promoting with their ability to live within and benefit plants. Although they are widely studied, application studies are still lacking. Therefore, not only the plant growth-promoting isolates were studied but also materials that act like carriers of these actinomycetes were tested creating its practical application in this study. Three endophytic actinomycetes isolated with the potential of plant growth promotion, TGsL-02-04, TGsL-04-60 and TGsR-03-04 were cultured and inoculated into 10 d-old tomato (*Solanum lycopersicum* L.) seedlings. Plant N, P, K, Ca and Mg uptake was analyzed. To develop planting media as a carrier for the plant growth promoting actinomycetes, the best isolate was cultured and poured into the prepared carriers with 15 mL per 50 g carrier material then incubated at 25-30 °C for 90 d. Carriers were sampled to evaluate viability of the added isolates at day 0, 15, 30, 45, 60, 75 and 90. This study indicated that TGsL_02_04 (*Nocardiopsis* sp.), has a remarkable capacity for helping plant to absorb essential nutrients especially N and P for 87.0 ± 3.71 and 2.98 ± 0.34 mg plant^{-1}, respectively and the presence of TGsL_02_04 enhances nutrient absorption, leading to a positive impact on plant productivity. In addition, TGsL_02_04 shown the highest dry weights as 2.21 ± 0.31 g, correlated with the observed nutrient uptake results. For the carrier development, the coconut coir carrier showed the highest cell count compared to vermiculite, diatomite and perlite. The TGsL_02_04 with the coconut coir carrier showed a promising result that they can be applied to plantation.

Key words: Coconut coir, diatomite, *Nocardiopsis* sp., perlite, planting media, *Solanum lycopersicum*, vermiculite.

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) is one of the economic plants and an important plant in food industries (Hernández Suárez et al., 2008). With carotenoids such as beta-carotene, a substrate of vitamin A and an antioxidant substance, tomatoes are a healthy food and popular as people are more concerned about their health nowadays. To cultivate tomatoes, widespread utilization of synthetic fertilizers has been applied resulting in a decline in soil organic matter levels and suppression of microbial activity (Zhang et al., 2017; Detraksa, 2020). This challenge can be alleviated with the biotic method such as an introduction of beneficial microbes with plant growth ability and one of the potential candidates is endophytic actinomycete. Endophytic actinomycetes play an important role in promoting plant growth by residing within the plant and providing numerous benefits to the host plant. Actinomycetes can produce phytohormones such as auxin, cytokinin and gibberellin which support plant growth promotion in different parts of plants. This can support the stress tolerance of plants and protect the plant from diseases (Narsing Rao et al., 2022). In addition, actinomycetes can produce siderophores binding to Fe, in this way plants can utilize the mineral (Narsing Rao et al., 2022). Endophytic actinomycetes are an actinomycete living inside plant tissue and with the potential to promote plant growth, they are considered to be used as a plant growth promoter (Aamir et al., 2020) and with a suitable planting material that can carry their cells, this would make a promising for their application in plant growth promoting. Using carrier materials as a method to increase the survival of bacteria can protect the microbial cells from soil biotic and abiotic stress (Abd El-Fattah et al., 2013; Detraksa, 2020). Despite extensive research, the application aspect of the term is continuously evolving and developing.

Planting media have been used to replace soil which plants can grow on the media as same as their normal conditions. Not only support plant growth as their usual habitat but planting media also play important roles which are to sustain plant parts, store plant nutrients, maintain moisture for plants and exchange gas on plant roots (Agarwal et al., 2021). A desirable carrier material should have the ability to stimulate and create a favorable environment for the growth and long-term viability of inoculated bacteria during storage (Chromkaew et al., 2023). Planting media used in Thailand are mostly local materials that can be easily found in local area which are coconut coir, sand, perlite, vermiculite and diatomite. The growth of microorganisms can be influenced in varying ways by different planting media and their respective mixing ratios (Van Gerrewey et al., 2020).

The objectives of the study are to select endophytic actinomycetes that exhibit the greatest potential for enhancing tomato growth and nutrient uptake, as well as to determine a suitable carrier for future applications of these beneficial actinomycetes.

MATERIALS AND METHODS

Endophytic actinomycetes and tomato seedlings preparation

Three endophytic actinomycetes isolates with the potential of plant growth promotion, TGsL_02_04 (*Nocardiopsis* sp.), TGsL_04_60 (*Nocardiopsis* sp.) and TGsR_03_04 (*Streptomyces* sp.) identified in a previous study (Shutsrirung et al., 2013), were cultured in IMA-2 (inhibitory mold agar - 2) at 30 °C for 1-2 d then they were transferred to in IMB-2 broth. The cultures were horizontally shaken at 120 rpm for 7 d.

The tomato seeds (*Solanum lycopersicum* L.) hybrid extra 390 F1 variety (East-West Seed company) were sterilized by soaking in sterile distilled water for 3 min, then wrapped with cheesecloth or muslin cloth and soaked in 3% sodium hypochlorite for 1.5 min. After that seeds were washed with sterile distilled water three times. Seeds were dried on paper and then incubated in the dark on sterile wet tissue paper for 2-3 d or until the germinated roots were 0.5 cm long. Tomato seedlings were planted into sterile corn cob fertilizer and crude sand (10:10 v:v ratio) with 0.5 g leonardite. All planting media were sterilized by autoclaving at 121 °C for 60 min, two rounds with 3 d intervals in each round. The plant materials were added into plant trays with holes for 80 g per hole. One hundred eighty tomato seedlings were planted into plant trays. The experiment was conducted under laboratory conditions.

Plant growth promotion assays of endophytic actinomycetes

Endophytic actinomycete cultures were resuspended on sterile distilled water for a 1:50 ratio (~ 10^7 spores mL^{-1}) then 1 mL cultures were inoculated into each of a 10 d-old tomato seedling. The seedlings were grown for 30 d and watered using distilled water. Six treatments with 30 replicates were separated as follows: Control, TGsL_02_04, TGsL_04_60 and TGsR_03_04. Dry weight was measured. Nutrient uptake of a plant for N, P, K, Ca and Mg was analyzed using the following methods. From each plant tissue, 0.5 g dry biomass was subjected to nitric-perchloric acid digestion to analyze total N, P, K, Ca and Mg content. Total nutrient uptake was based on the nutrient content in the whole tomato plants.

Development of planting media as a carrier of endophytic actinomycetes

Coconut coir (Figure 1a), diatomite (Figure 1b), vermiculite (Figure 1c) and perlite (Figure 1d) were used as a carrier of endophytic actinomycetes. Fifty grams of carrier materials were sterilized by autoclaving at 121 °C for 1 h for two rounds with 3 d intervals in each round.

Figure 1. Type of planting media for the actinomycetes carrier: Coconut coir (a), diatomite (b), vermiculite (c) and perlite (d).

The TGsL_02_04 isolate was cultured in IMA-2 broth and incubated by shaking at 120 rpm for 7 d at ambient temperate. The broths were poured into the prepared carriers with 15 mL 50 g^{-1} carrier material then incubated at 25-30 °C for 90 d. The carrier materials were sampled to evaluate the viability of the added isolates at 0, 15, 30, 45, 60, 75 and 90 d. Ten grams of carrier materials were sampled into a glass bottle with 95 mL sterile distilled water and shaken for 15 min. Then the samples were diluted using a 10-fold dilution technique to get 10^{-2} to 10^{-6} dilution. Each dilution was used for a drop plate on IMA-2 and the plates were incubated at ambient temperature for 7 d then appear colonies on the plates were counted. Morphology of endophytic actinomycetes in four carriers were observed using scanning electron microscope (SEM) at the Electron Microscope Service Center at the Faculty of Science Chiang Mai University. Ten grams of the carriers were added into 100 mL distilled water in an Erlenmeyer flask then shaken for 30 min and filtered with filter No 1. The filtrated solution was measured pH using pH meter.

Statistic data analysis

Data in this experiment were analyzed using ANOVA and compared the similarity using Tukey and linear mixed-effects models (LME) on Statistic for R program version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS AND DISCUSSION

The result indicated that incorporating actinomycetes into the planting media could enhance the plant's ability to absorb nutrients on average (Figure 2). As mentioned, endophytic actinomycetes can be used for plant growth-promoting which they can produce phytohormones such as auxin, cytokinin and gibberellin to promote plant growth (Shutsrirung et al., 2013; Egamberdieva et al., 2017). In this study, TGsL_02_04 isolate showed the best average N, P, K, Ca and Mg plant uptake which were 87.0 ± 3.71, 2.98 ± 0.34, 2.01 ± 0.08, 5.74 ± 0.17 and 0.62 ± 0.03, respectively (Figure 2; ANOVA: df = 3, $p \leq 0.05$ for all analyzed nutrients). Even though when compared within the same nutrient using Tukey HSD, TGsL_02_04 was not significantly different from TGsL_04_60 for K, Ca and Mg uptake which were 1.82 ± 0.10, 5.71 ± 0.15 and 0.67 ± 0.01 mg $plant^{-1}$, respectively. Both TGsL_02_04 and TGsL_04_60 were from the same genus of *Nocardiopsis* sp. which exhibited similar characteristics (Bennur et al., 2015). However, TGsL_02_04 still shows higher N, P and K uptake (Tukey HSD: p = 0.0000324 and 0.0006400, respectively) which are major elements for plants. Although there was nonsignificant difference in K the trend of TGsL_02_04 is higher than TGsL_04_60. Therefore, TGsL_02_04 stood out as the plant nutrient uptake promoting isolate compared to the others.

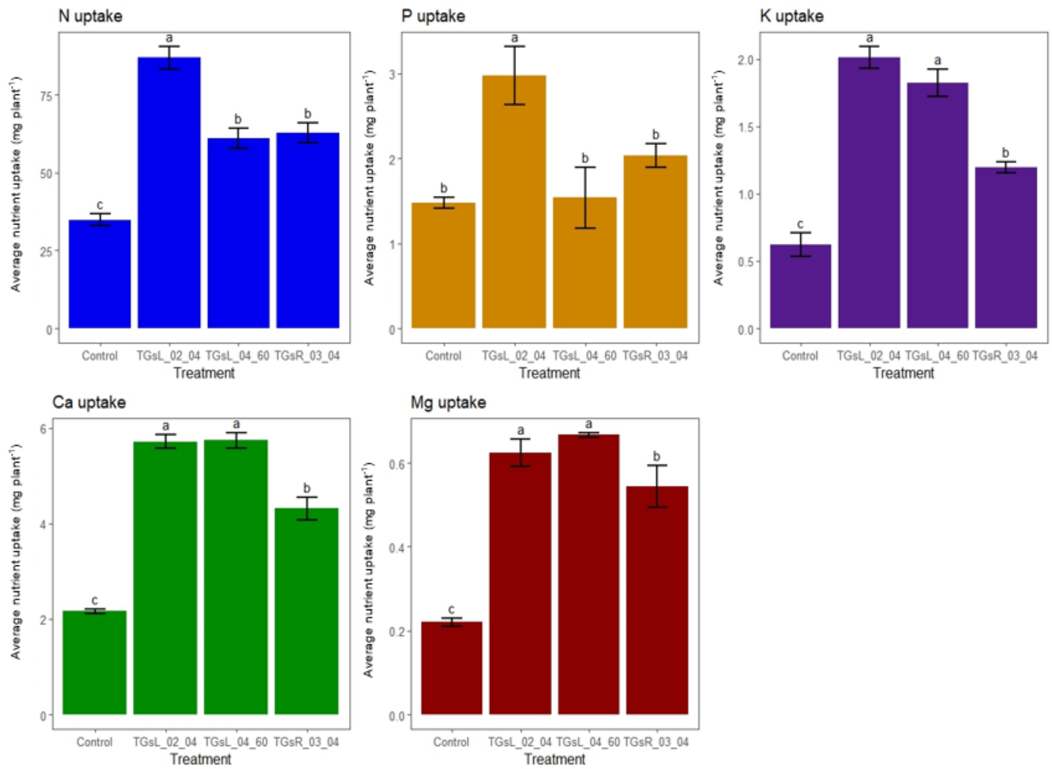

Figure 2. Average nutrient uptake of a plant analysis for N, P, K, Ca and Mg. The treatments were control, TGsL_02_04, TGsL_04_60 and TGsR_03_04. ANOVA for overall treatments and Tukey HSD of each treatment in the experiments were tested (ANOVA: df = 3, $p \leq 0.05$ for all analysed nutrients and Tukey HSD results are showed in letters on the graphs).

Plant weights of tomatoes were measured to confirm the actinomycetes effect on tomato productivity measuring by plant weight (Figure 3). For whole plant (stem and root), treatment with TGsL_02_04 shows the highest dry weights which are compatible with nutrient uptake results. In addition, considering the other treatment, they are also relatable to nutrient uptake results. These two results from nutrient uptake and plant weight are the confirmation that TGsL_02_04 can promote nutrient uptake of the plants which can affect plant productivity. The TGsL_02_04 which was *Nocardiopsis* sp., a genus that can promote plant growth according to the study of *Nocardiopsis lucentensis*, it can promote biomass gain in barley and maize (AbdElgawad et al., 2021).

After inoculation for 90 d, the actinomycete TGsL_02_04 showed decreasing trends in all planting media and a significant influence of time (day) on the amount of actinomycete (log10) was detected (LME: df = 101, t = -3.3922, p = 0.0010; Figure 4a). The study showed each planting media can significantly maintain different amounts of the actinomycete (ANOVA: df = 3, F = 6.440, p = 0.000483) which coconut coir showed the highest amount of actinomycete (log10) after 90 d. These findings corroborate the previous studies (Rajesh et al., 2013) which demonstrated the successful isolation of actinomycetes from coconut husk. This suggests that the properties inherent in coconut husk or coconut coir provide an environment conducive to actinomycete growth. Furthermore, according to a study conducted on mustard plants, it was found that the incorporation of coconut coir resulted in accelerated plant growth. Specifically, the treatment with the highest percentage of coconut coir exhibited the most average leaf width, outperforming the treatment with lower coconut coir content (De Side et al., 2023). For future application, to improve the tomato growth and development, grafting plant can be used instead of ungrafted plant in the study (Sun et al., 2021) and together with organic farming system can increase tomato mineral content such as K, Ca, Mg (Dinu et al., 2023).

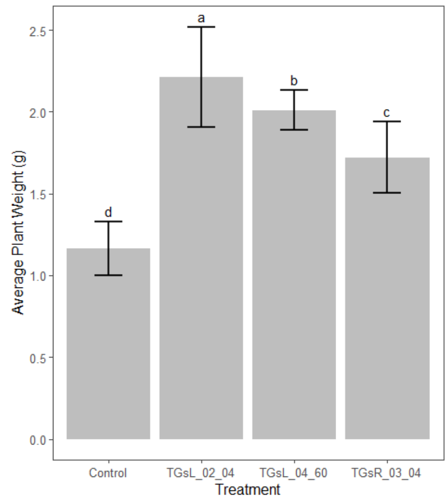

Figure 3. Dry weight of tomatoes in different treatments. The treatments were control, TGsL_02_04, TGsL_04_60 and TGsR_03_04. Tukey HSD of each treatment in the experiments were tested and results are showed in letters on the graph.

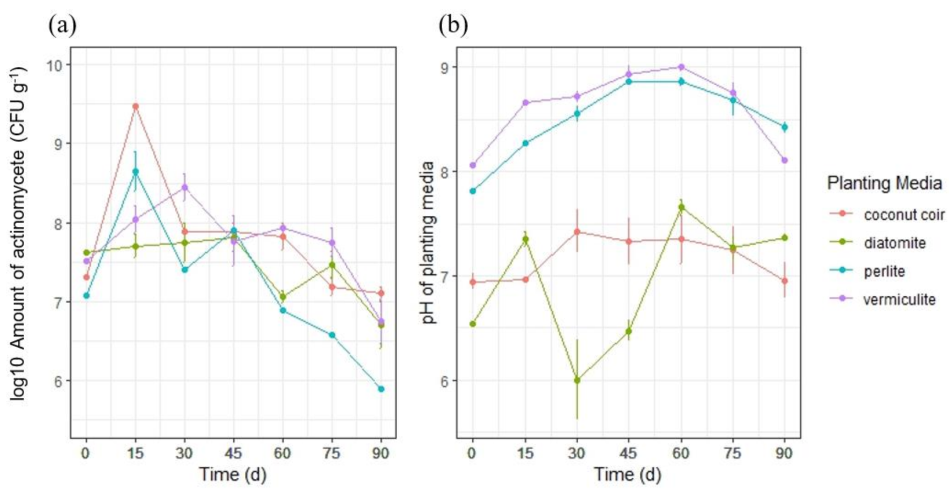

Figure 4. Amount of actinomycete (log10) in different planting media which are coconut coir, diatomite, vermiculite and perlite of isolate TGsL 02 - 04 with LME: df = 101, t = -3.3922, p = 0.0010 (a) and pH of planting media with the isolate with LME: df = 101, t = 0.46347, p = 0.6440 (b).

According to pH we can see the average pH of vermiculite and perlite (average pH 8.5) were higher than coconut coir and diatomite (average pH 7.0) (Figure 4b). Nonsignificant of time (day) was detected (LME: df = 101, t = 0.46347, p = 0.6440; Figure 4b). Therefore, it can be concluded that all planting media successfully maintained a stable pH throughout the entire 90 d experiment period (Figure 4b), making them ideal for plantation purposes. However, a neutral pH range is particularly favorable for plants, especially tomatoes. From Figure 4b, it can be observed that both coconut coir and diatomite fell within the desired pH range. However, the pH of coconut coir remained more stable over time compared to diatomite, which declined at day 30. In addition, coconut coir stands out as an excellent choice for a planting medium to accommodate the actinomycete, due to its porous texture that facilitates air exchange and sunlight exposure (De Side et al., 2023). This study also revealed that coconut coir exhibited the highest capacity for retaining TGsL_02_04, as previously mentioned.

The image from scanning electron imaging shows planting media structures which are coconut coir (Figure 5a), diatomite (Figure 5b), vermiculite (Figure 5c) and perlite with actinomycetes cells (Figure 5d). According to the images, it had been confirmed that all types of planting media in this study can hold the actinomycetes cells.

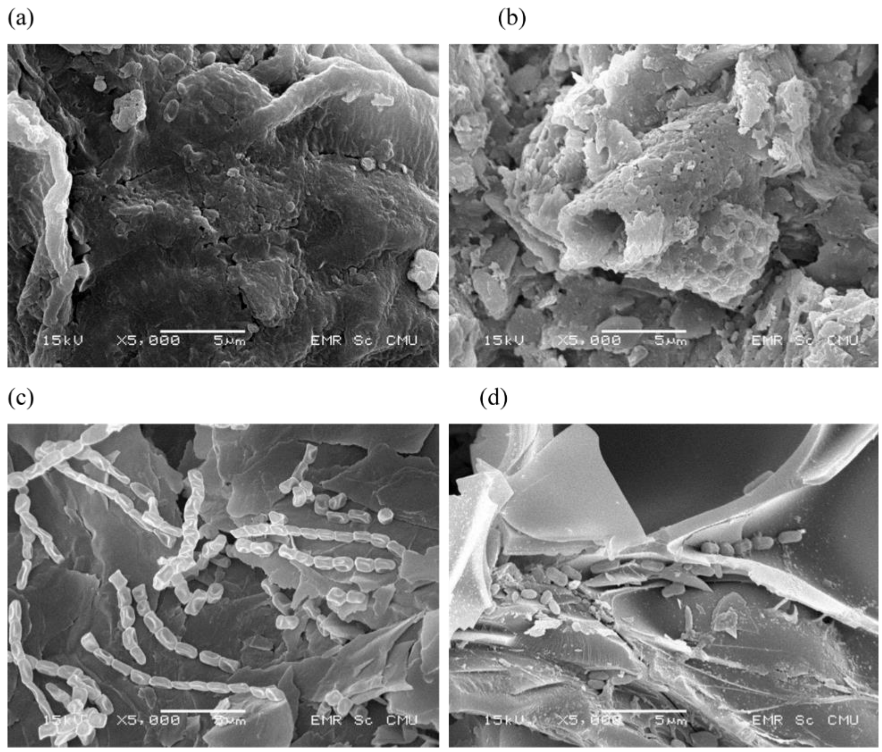

Figure 5. Scanning electron imaging of planting media structures: Coconut coir (a), diatomite (b), vermiculite (c) and perlite (d) with actinomycetes cells.

CONCLUSIONS

This study demonstrated that the TGsL_02_04 isolate had a remarkable capacity for helping the plant to absorb essential nutrients such as N, P, K, Ca, and Mg and the presence of TGsL_02_04 enhanced nutrient absorption, leading to a positive impact on plant productivity. Moreover, TGsL_02_04 exhibited the highest dry weights correlated with the observed nutrient uptake results. Throughout the 90 d inoculation period, TGsL_02_04 consistently showed a decline across all planting media, with the passage of time significantly influencing this trend. The pH of all media remained stable throughout the 90 d experiment which could be implied that the only significant influence factor was time. Therefore, additional of isolate during the plantation is needed to maintain the amount of the isolate in the carrier. Visual evidence by scanning electron imaging confirms that all types of media employed in this study effectively retain actinomycetes cells.

Author contribution

Conceptualization: Y.C. Methodology: Y.C. Validation: T.K., N.M., N.K., N.Mu. Formal analysis: N.M., N.K., N.Mu. Investigation: Y.C., T.K. Resources: Y.C., T.K. Data curation: N.M., N.K., N.Mu. Writing-original draft: Y.C., N.Mu. Writing-review & editing: Y.C., N.M., N.K., N.Mu. Visualization: Y.C., N.Mu. Supervision: T.K., Y.C. Project administration: Y.C. Funding acquisition: Y.C., T.K. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgements
Thank you to Miss Ratchaneekorn Kamsai, Mr. Hiran Rakna and Mr.Passakorn Tong-Uthai for your assistance in data collection throughout the experiments.

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