https://doi.org/10.51470/IJNS.2024.01.01.46.

Crossref

International Journal of Nature Science (IJNS)

Regd.No. 114/2017

Estd:2024

Biological control of tea pathogens by Silicon Solubilizing Bacteria (Bacillus nucilaginosus)

P. Ponmurugan

Department of Biotechnology, K.S.Rangasamy College of Technology, Tiruchengode - 637 215, Namakkal District, Tamil Nadu, India.

Article History

Volume:1, Issue:1, 2024

Received: 17th January, 2024

Accepted: 20th January, 2024

Published: 30th January, 2024.

doi.org/10.51470/IJNS.2024.01.01.46-50

Abstract: *Following the collection of 257 soil samples, 123 strains of silicon solubilizing bacteria (SSB) were identified by the use of silicon basal media. These strains were then refined, screened, and their antifungal activity was assessed. Of them, 24 isolates grew quickly, 48 slowly, and the remaining 51 strains could only develop slowly to moderately. Physiological, biochemical, and morphological characteristics identified Bacillus nucilaginosus as the parent organism of all isolated cultures. Additionally, the SSB01 strain was chosen based on its in vitro performance when tested for antagonistic potential against root pathogens like Poria hypolateritia and Fomes noxius, leaf pathogens like Pestalotiopsis theae and Cercosporella theae, and tea stem pathogens like Phomopsis theae and Tunstallia aculeata. The growth pattern, mycelial coloration, exopolysaccharide synthesis, and diffusible pigment of Bacillus nucilaginosus were all observed and recorded. The enzymes lipase, chitinase, gelatinase, cellulase, and amylase, which are helpful in eliminating tea pathogens in vivo, were produced by each isolate.*

Keywords: *Silicon solubilizing bacteria, antifungal activity, biocontrol, Bacillus nucilaginosus, tea soils, tea diseases.*

Authors citation: Ponmurugan.P,. Biological control of tea pathogens by Silicon Solubilizing Bacteria(Bacillus nucilaginosus) .Int.J.Nat.Sci.Vol.1(1). 2024.Pp:24-50.
https://doi.org/10.51470/IJNS.2024.01.01.46.

INTRODUCTION

Numerous microorganisms can be found in tea soil, and the majority of them are good for plant growth (Baby et al., 2002). Silicon solubilizing bacteria (SSB) are a significant group of soil microorganisms that produce a wide range of secondary metabolites, many of which have antibacterial or antifungal characteristics (Al-Falih, 2003). Finding aproductive soil bacterium that could dissolve silicate could allow other vital nutrients to be released into the soil (Muralikannan, 1996). One of the key mechanisms for the biological inhibition of several phytopathogens is induced systemic resistance (ISR) by SSB (Sommer et al., 2006; Sacala, 2009). Vijayapriya and Muthukkaruppan (2010) investigated the effectiveness of SSB in enhancing the ISR of rice plants against Pyricularia oryzae, the most devastating phytopathogen in terms of biotechnological application on plant growth and biocontrol activities.

Fungicides are mostly used to combat tea illnesses. A few tea diseases have been shown to be effectively controlled by soaking the soil with systemic fungicides (Ponmurugan and Baby, 2005; Chandramouli and Baby, 2002). Fungicide-soaked soil has a negative impact on helpful microorganisms. Chemical control is also costly and unpredictable. An effective substitute for treatment of stem and root problems is biological control. Tested against a range of tea illnesses, the antagonistic microorganisms, namely bacterial (Bacillus and Pseudomonas spp.) and fungal (Trichoderma and Gliocladium spp.) were found to be effective (Chandramouli and Baby, 2002; Premkumar and Baby, 2005; Ponmurugan and Baby, 2005). Similar to this, antagonistic microorganisms such as actinomycetes (Streptomyces spp.) secrete chemicals that regulate growth and antibiotics, respectively, which help to arrest the growth of pathogens and improve plant metabolism.

The root pathogens Fomes noxius (brown root rot) and Poria hypolateritia (red root rot disease), as well as Phomopsis theae (cause of collar canker) and Tunstallia aculeata (cause of thorny stem blight) and leaf pathogens Pestalotiopsis theae (cause of grey blight) and Cercosporella theae (cause of bird's eye spot) are significant in tea plantations because they impact the health of the shrubs, which in turn affects the potential for crop yield. In order to remember, a study was conducted.

RESOURCES AND TECHNIQUES

The way that SSB strains performUsing silicon basal (Bunt and Rovira, 1955)medium (g/l: 1.0 peptone, 1.0 yeast extract, 20.0 glucose, 0.05 ammonium sulphate, 0.1 magnesium chloride, 25.0 magnesium trisilicate, 20.0 agar, 250 ml soil extract, 750 ml distilled water, pH 6.6), attempts were made to isolate SSB in the soil samples. Every SSB isolate was cultured on silicon basal medium at 37°C, with daily observations of the growth rate made for a maximum of five days. The isolates were divided into three growth categories: moderate (growing between four days), sluggish (growing more than five days), and quick (growing well in three days). From genus to species, all of the isolated cultures were identified using morphological, biochemical, and physiological features (Williams and Wilkins, 1994). Diffusible pigment production was also monitored (Keiser et al., 2000). checking SSB strains for the production of extracellular enzymes (Dubey and Maheshwari, 2002)Invertase, amylase, cellulose, chitinase, protease, and lipase are six significant enzymes that were qualitatively screened for in all the identified SSB strains. Each of the 20 altered agar plates' four corners was streaked with a different strain of SSB, and the substrates—such as starch, sucrose, carboxymethyl cellulose, chitin, casein hydrolysate, and tween 20—were then cultured for five days at room temperature. After that, the plates were flooded with the appropriate indicator solution, and the formation of a clear zone surrounding the organism's growth was interpreted as evidence of positive enzyme activity.

Production of antifungal metabolites (Bauer et al., 1996) Antifungal chemicals were extracted from SSB cultures using an antibiotic production medium (25 g starch, 10 g glucose, 2 g yeast extract, 3 g calcium carbonate, and 1 ml of trace solution containing $ZnSO_4$, $MnCl_2$, $CuSO_4$, $FeSO_4$, pH 7.5). The pure culture of the SSB01 strain was chosen for the investigation based on its in vitro performance, including growth pattern and colour, together with the culture parameters as reported by Goodfellow et al. (1987). It was infected with the SSB01 strain into a 250 ml conical flask containing 25 ml of seed media. It was then maintained for five days at 220 rpm in a rotary shaker. The culture filtrate was sterilised and obtained a clear solution by centrifuging it at 11,000 rpm.

checking for antifungal activity in SSB strainsUsing dual culture (Huang and Hoes, 1976) and antibiosis (Dennis and Webster, 1971) methods, the biocontrol potential of SSB strains against fungal tea pathogens was investigated. The SSB01 strain culture filtrate was bioassayed in vitro at a 10% level in the antibiosis method. To achieve the desired concentration, the culture filtrate was combined with melted and cooled PDA media and evenly distributed into petriplates. A 5 mm mycelial disc containing tea pathogens was used to inoculate the plates. For this investigation, tea stem pathogens like Poria hypolateritia and Phomopsis theae, leaf pathogens like Pestalotiopsis theae and Cercosporella theae, and root pathogens like Fomes noxius and Cercosporella they were used. As a control, pathogens inoculated in an unaltered media were used. In order to determine the percentage of inhibition, the pathogens' radial growth was measured until the pathogens in the control plates had fully covered the plates. However, modified potato dextrose and casein nitrate agar medium (50 percent PDA and fifty percent silicon basal media) was utilised to explore the dual culture approach. Up to 11 days of incubation were used to measure the zone of inhibition (ZOI) against tea pathogens. The test pathogens' mycelial discs were also maintained on control plates without an SSB strain inoculation.

Calculating the SSB strains' silica solubilizing effectivenessFor this work, petri dishes with silicon basal media supplemented with 10% silica sources (w/v) were used. The commercially accessible forms of silica, namely sodium, potassium, calcium, and magnesium trisilicate, were selected. It was injected with the B. nucilaginosus strain (SSB01 strain) and kept in an incubator for five days at 37°C. A metric scale was used to measure the halo zone of the infected area with the SSB01 bacterium after incubation.

OUTCOMES: From tea soils collected from various agroclimatic zones of southern India, 123 strains of SSB were identified. For this investigation, 257 soil samples in total were gathered. It was discovered that roughly 24 strains grew quickly, 48 strains slowly, and the remaining 51 strains grew somewhat. A maximum number of the SSB strains were discovered to be moderate to sluggish growth (Fig. 1). Morphological, physiological, and biochemical traits suggested that Bacillus nucilaginosus was the source of the refined isolates of SSB. Extracellular enzymes like invertase, amylase, cellulase, chitinase, protease, and lipase could be produced by any strain of SSB. On the other hand, there were differences in their levels of these enzymes' synthesis. Of the six enzymes, the majority of the strains produced more protease, which was followed by chitinase and amylase. Protease was produced by about 87 strains, chitinase by 73 strains, and amylase by 70 strains.

A well-developed inhibitory zone between the pathogens and B. nucilaginosus was observed, according to the results of the antifungal activity against tea pathogens in dual culture procedures. Pestalotiopsis theae had a linear development of 85.5 mm, and on the eleventh day, Fomes noxius measured 62.3 mm (Table 1). During the first two to three days of growth, all root (P. theae and T. aculeata) and stem (F. noxius and P. hypolateritia) pathogens were completely inhibited. One day of incubation yielded 100% inhibition in the case of leaf pathogens like Pestalotiopsis and Cercosporella.

Utilising several silica sources, including magnesium trisilicate, calcium trisilicate, potassium trisilicate, and sodium trisilicate, a study was conducted to determine the solubilizing efficacy of SSB strains. The highest solubilizing zone development among the several silica sources studied was found to be optimum for magnesium trisilicate, with a solubilizing zone of 95 mm, followed by 88 mm for potassium trisilicate. In sodium trisilicate, a zone with a dimension of 70 mm was found to be the least soluble (Table 2).

TALK: In order to isolate SSB strains, tea soil samples were gathered from many agroclimatic zones in southern India. Approximately 257 tea soil samples were collected, from which 123 strains of SSB were identified. A total of 48 slow-growing, 51 moderately- growing, and 24 quickly developing strains were selected (Fig. 1). According to Ponmurugan et al. (2007) and 2011, the results unequivocally showed that none of the SSB were slow- growing microorganisms, and the majority of their development was comparable to that of filamentous microorganisms like actinomycetes. Numerous in vitro studies revealed that the SSB strains belonged to Bacillus nucilaginosus. Furthermore, to varied degrees, all of the native SSB strains could generate extracellular enzymes as lipase, amylase, chitinase, cellulase, protease, and invertase (Fig. 2). According to Figuera et al. (2005), all of these enzymes have additional

uses besides the biological control of phytopathogens, such as the solubilization of salts, minerals, and metal ions in the soil. It is noteworthy that Streptomyces fungicidicus produced an alkaline protease that is thermostable and has numerous industrial uses (Ramesh et al, 2009). According to Ponmurugan et al. (2011), filamentous bacteria such as actinomycetes (Streptomyces spp.) have the ability to create several extracellular enzymes in vitro, which are highly beneficial for managing various plant diseases.

A well-developed inhibitory zone surrounding the pathogens was observed by the antifungal activity of SSB strains against tea pathogens (Table 1). When root and stem pathogens grew for the first two to three days, there was 100% inhibition, indicating the pathogens' slow growth. When other tea pathogens were tested against Streptomyces spp., Ponmurugan et al. (2007, 2011) observed comparable results. The findings also support the findings of Zahner et al. (1989), who found that in rapidly proliferating infections, inhibition was remarkably low. Using antifungal compounds derived from Streptomyces spp., the growth of fungal pathogens such as Aspergillus niger, Fusarium oxysporum, Candida albicans, and Crytococcus humicolus was greatly reduced (Augustine et al, 2004). Because B. nucilaginosus strains produce secondary metabolites, an inhibitory zone forms around the pathogenic strain (Sanglier et al., 1993). The most widely recognised view, according to Demain and Fang (1995), holds that antibiotics are utilised to compete with competing organisms in environments that are depleting of nutrients.
Magnesium trisilicate was discovered to be the best in terms of the highest solubilizing zone creation when the solubilizing performance of SSB strains employing various silica sources was examined (Table 2). Due to differences in chemical nature, molecular weight, pH, solubility type, and purities, SSB strains formed different solubilizing zones in different silica sources.

CONCLUSION

The majority of B. nucilaginosus strains identified in this investigation were demonstrated to be potential antagonists against tea pathogens, indicating that the synthesis of secondary metabolites may be able to regulate tea pathogens. After creating the carrier- based bioformulation, field tests should be started to verify the strains' effectiveness against tea illnesses in vivo.

ACKNOWLEDGEMENT

The author is thankful to Dr.S.Marimuthu, Assistant General Manager, Parry Agro Industries, Valparai, Tamil Nadu, India and Dr.K.Thyagarajah, Principal, K.S.Rangasamy College of Technology, Tiruchengode, Tamil Nadu, India for their magnanimous support, necessary facilities and constant encouragement for the study.

REFERENCES

[1] Al-Falih, A.M. 2003. Effect Of Silicon Compounds On Oligotrophic Soil Bacteria. Saudi. J. Biol. Sci. 10: 131-136.
[2] Augustine, S.K. Bhavsar, S.P. Baserisalehi, M. Kapadnis, B.P. 2004. Isolation, characterization and optimization of antifungal activity of an actinomycete of soil origin. Indian J. Expri. Biol. 42: 928-932.
[3] Baby, U.I. Tensingh Baliah, N. Ponmurugan, P. Premkumar, R. 2002. Population level of certain beneficial microorganisms in tea soils. UPASI Tea Res. Found. Newsletter 12 (1): 3.
[4] Bauer, A. Kirby, A.W. Sherries, J.C. Trunk, M. 1996. Antibiotic susceptibility by standard disc method. J. Clinical Patho1. 45: 493.
[5] Bunt, J.S. Rovira, A.D. 1955. Microbiological studies of some sub Antarctic soils. J. Soil Sci. 6: 119-128.
[6] Chandramouli, M.R. Baby, U.I. 2002. Control of thorny stem blight disease of tea with fungicides and biocontrol agents. pp. 531-534. In: Proceedings of Plantation Crops and Development in the new Millennium, (Eds.) P.Rethinum, H.H.Khan, V.M.Reddy, P.K.Mandal and K.Suresh, Coconut Development Board Publication, Kochi, Kerala,
[7] Demain, A.L. Fang, A. 1995. Emerging concepts of secondary metabolism in actinomycetes. Actinomycetologica 9: 98-99.
[8] Dennis, C. Webster, J. 1971. Antagonistic properties of species groups of Trichoderma. II. Production of volatile antibiotics. Trans. Br. Mycol. Soc. 57: 41-48.
[9] Dubey, R.C. Maheshwari, D.K. 2002. Practical Microbiology. S.Chand and Company, New

Delhi, 397p.
[10] Fguira, L. Fotso, S. Ben Ameur-Mehdi, R. Mellouli, L. Laatsch, H. 2005. Purification and structure elucidation of antifungal and antibacterial activities of newly isolated Streptomyces sp. Res. Microbiol. 156: 341-347.
[11] Goodfellow, M. Lacey, J. Todd, C. 1987. Numerical classification of thermophilic Streptomyces.
J. Gen. Microbiol. 133: 3135-3149.
[12] Huang, H.C. Hoes, J.A. 1976. Penetration and infection of Sclerotina sclerotiorum by
Coniothyrium minitans. Can. J. Bot. 54: 406-410.
[13] Keiser, T. Bibb, M.J. Buttner, M.J. Chater, F.K. Hopwood, D.A. 2000. General introduction to actinomycetes biology. pp 1-21.In: Practical Streptomyces Genetics. The John Innes Foundation, England.
[14] Muralikannan, N. 1996. Biodissolution of silicate, phosphate and potassium by silicate solubilizing bacteria in rice ecosystem M.Sc. (Ag.). Thesis, submitted to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, p.115.
[15] Ponmurugan, P. Baby, U.I. 2005. Management of Phomopsis canker of tea with fungicides and biocontrol agents. J. Plantn. Crops 33: 175-178.
[16] Ponmurugan, P. Gopi, C. Maripandi, A. 2007. Studies on Actinomycetes diversity in southern
Indian tea soils for antifungal activity. J. Plantn. Crops 35: 28-32.
[17] Ponmurugan, P. Elango, V. Marimuthu, S. Chaudhuri, T.C. Saravanan, D. Gnanamangai, B.M.
Karunambika, K.M. 2011. Evaluation of actinomycetes isolated from southern Indian tea plantations for the biological control of tea pathogens. J. Plantn. Crops 39: 239-243.
[18] Premkumar, R. Baby, U.I. 2005. Recommendations on the control of root and stem diseases of tea. pp.15-16. In: Hand book of tea culture, UPASI Tea Research Institute, Valparai, Tamil Nadu.
[19] Ramesh, S. Rajesh, M. Mathivanan, N. 2009. Characterization of a thermostable alkaline protease produced by marine Streptomyces fungicidicus MML1614. Bioprocess Biosyst. Eng. doi: 10.1007/s00449-009-0305-1
[20] Sacala, E. 2009. Role of silicon in plant resistance to water stress. J. Elementol. 14: 619-630.
[21] Sanglier, J.J. Haag, H. Huck, T.A. Fehr, T. 1993. Novel bioactive compounds from actinomycetes. A short review. Res. Microbiol. 144: 633-642.
[22] Sommer, M. Fuzyakov, D. Breuer, J. 2006. Silicon pools and fluxes in soils and landscapes-a review. J. Plant Nutr. Soil Sci. 169: 310-329.
[23] Vijayapriya, M. Muthukkaruppan, S.M. 2010. Isolation and screening of silicate solubilizing bacteria and its biocontrol nature against Pyricularia oryzae. Inter. J. Recent Sci. Res. 4: 87-91.
[24] Williams, S.T. Wilkins. S. 1994. Bergey's Manual of Determinative Bacteriology, 9th edition, Williams and Wilkins, Baltimore, Cambridge University Press, UK.
[25] Zahner, H. Weber, W. Siebers, J. Schroder, K. Zeeck, A. 1989. Streptomyces. Arch. Microbiol.
124: 111-116.
[26] Banu, D. A. S., Sheela, S., Muralimohan, N., Saranya, N., Kolar, A. B., & Santhya, V. S. (2023, December 19). Importance and Benefits of Green Audits to Education Institutions and Industrial Sectors. International Journal of Environment and Climate Change, 13(12), 349–356. https://doi.org/10.9734/ijecc/2023/v13i123690
[27] Kavitha, R., Sathish, S. S., Vinoliya, V., Jahirhussain, G., & Basha Kolar, A. (2023, August 24). Green synthesis, characterization and biological studies on in vitro cultured fern – Lygodium microphyllum (Cav.) R. Br. Biochemical and Cellular Archives, 23(2). https://doi.org/10.51470/bca.2023.23.2.975
[28] Saminathan, M., Muruganandam, A., Arumugam, M., & Basha Kolar, A. (2023, September 14). Genetic diversity analysis of Madhuca longifolia (Koen) Macbr. populations by using RAPD markers. Biochemical and Cellular Archives, 23(2). https://doi.org/10.51470/bca.2023.23.2.751
[29] Kolar, A. B., Sheik Mohamed, S., Shareef Khan, M., & Ghouse Basha, M. (2021, January 15). DIRECT PLANT REGENERATION FROM MATURE NODAL EXPLANTS OF ANDROGRAPHIS ECHIOIDES (L.) NEES – A VALUABLE MEDICINAL PLANT. PLANT ARCHIVES, 21(Suppliment-1), 1842–1848. https://doi.org/10.51470/plantarchives.2021.v21.s1.296
[30] Amzad Basha Kolar*, Palanivel.S, M. Sheik Noor Mohamed, S. Sheik Mohamed, M. Shareef Khan, Aakash Raj.S.G., Mohammed Ibrahim.V and Mohammed Nowshath. A. (2021). FLORISTIC STUDY ON ANGIOSPERMS SURROUNDING THE MEDAVAKKAM LAKE, CHENGALPATTU DISTRICT, TAMIL NADU, INDIA. Plant Archives, 21(1), 1953–1962. https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.271
[31] Amzad Basha Kolar , Edwin Raj Esackb , L. Vivekanandanb , M. Ghouse Basha. (2015). K. R. Kavitha , A.K. Bopaiah and Amzad Basha Kolar. (2016). CHEMICAL COMPOSITION OF THE ESSENTIAL OIL FROM THE LEAVES OF CIPADESSA BACCIFERA (ROTH.) MIQ. International Journal of Pharmaceutical Sciences and Research, 7(1), 392–396. The Journal of Ethnobiology and Traditional Medicine, 936–957