AN OVERVIEW OF BIOCONTROL AGENT EFFICACY IN CLINICAL LABORATORY

Nasser Badi K Almutairi[1*], Majed Badi K Almutairi[2], Nasser Salem Mansour Alalyani[3], Mamdouh Saed M Alharbi[4], Ayman Mohammedamin A Qashgari[5], Mohammed Abdulrahman Ahmed Alattas[6], Muaffag Hamed M Abualreesh[7], Mohammed Jarman Alotaibi[8], Saad Fawaz Bin Khatim Alghufairy[9], Mohammed Hamzah Mubarakalkhuzaee[10], Naif Eid Saleh Alotaibi[11], Ayman Abdullah Soqaty[12]

Abstract:
Bacillus subtilis and other Bacilli are commonly utilized as biological control agents to combat plant bacterial infections, however the specific processes via which they provide protection remain unclear. The aim of our work was to extract B. subtilis strains with high biocontrol efficiency from natural habitats and to understand the mechanisms via which these strains provide plant protection. 4PHCISA25 shown superior biofilm removal capabilities compared to 4PHSA25 against MRSA strain biofilms, as indicated by biofilm removing tests. Mouse tests showed that injecting 4PHCISA25 was safe and effectively prevented abscess formation caused by MRSA within 24 hours, while also aiding in the recovery from clinical symptoms. This study emphasizes the utilization of phages to battle MRSA.

[1]*Specialist Laboratory, Sajer General Hospital
[2]specialist Laboratory, Sajer General Hospital
[3]specialist Clinical Physiology, Maternity And Children’s Hospital, Laboratories And Medical Technology
[4]technician-Laboratory, Maternity And Children’s Hospital, Mecca Saudi Arabia
[5]technician-Laboratory, Maternity And Children’s Hospital, Mecca Saudi Arabia
[6]technician-Laboratory, Maternity And Children’s Hospital, Mecca Saudi Arabia
[7]Technician-Laboratory, Maternity And Children ‘S Hospital, Mecca Saudi Arabia
[8]technician-Laboratory, Maternity And Children’s Hospital, Mecca Saudi Arabia
[9]medical Laboratory Technician, Laboratory And Blood Bank, Maternity And Children Hospital , Makkah Health Cluster , Makkah, Saudi
[10]technician-Laboratory, Maternity And Children’s Hospital, Mecca Saudi Arabia
[11]laboratory Specialist, Albajadia General Hospital
[12]laboratory Technician, Poison Control And Forensic Chemistry Center

***Corresponding Author: -** Nasser Badi K Almutairi
*Specialist Laboratory, Sajer General Hospital

DOI: 10.53555/ecb/2022.11.7.48

Introduction:

Biofilms are groups of specialized cells surrounded by a matrix produced by themselves. It is now commonly acknowledged that the majority of microorganisms develop biofilms on both non-living and living surfaces in various environments such as natural, clinical, and industrial settings. Rhizosphere bacteria, including Plant Growth Promoting Rhizobacteria (PGPR), are often observed to create small colonies or structures resembling biofilms on plant roots. Research indicates that biofilm production by PGPR is crucial for plant protection. Paenibacillus polymyxa, a PGPR bacterium, colonizes plant root tips, creating biofilm-like structures, and protects plants from pathogen infections. For instance, highly mucoid mutants of the Pseudomonas fluorescens CHA0 strain, which frequently indicate increased biofilm formation, are viewed as effective biocontrol agents. These mutants demonstrated a significantly improved ability to colonize carrot roots compared to the original wild type strain. A recent study showed that a B. subtilis strain (ATCC 6051) may create biofilm-like structures on Arabidopsis plant roots and shield them against Pseudomonas syringae infections. Most of the previous studies have not provided sufficient direct data to prove the causal relationship between biofilm development and biocontrol effectiveness.

Cucumber, scientifically known as Cucumis sativus L., is a significant vegetable in China. Fusarium oxysporum f. sp. cucumerinum is considered a highly devastating soil-borne plant disease in cucumber farming. Consistent and heavy use of chemical pesticides on cucumber crops can have negative effects on the environment and human health. Microbial antagonists are becoming increasingly recognized as a promising management method due to their ability to prevent soil pathogens. Actinomycetes, particularly Streptomyces, exhibit latent traits as biocontrol agents against soil-borne plant diseases through the formation of various secondary metabolites and biologically active compounds, which enhance crop development and yield. Some actinomycetes exhibit remarkable antifungal action against F. oxysporum f. sp. cucumerinum [6]. Actinobacteria are typically isolated for managing fusarium wilt of cucumber in traditional settings like agriculture and forestry. Recent reports indicate the potential for isolating actinomycetes from unexplored environments like marine sediments in Chile and Loktak Lake in Manipur, India. This has led many researchers to begin selecting and isolating microbes from untapped habitats.

Review:

Staphylococcus aureus is a major global cause of endocarditis, bacteremia, osteomyelitis, skin and soft tissue infections, pleuropulmonary infections, and device-related infections. S. aureus has become a prominent source of health-care-associated infections due to the increase in hospital-based medicine. Managing these infections is difficult because of the introduction of multi-drug resistance strains, especially the methicillin-resistant ones. The emergence of methicillin-resistant Staphylococcus aureus is frequently linked to biofilm formation facilitated by polysaccharide intercellular adhesion (PIA), a crucial stage in S. aureus infection. Methicillin resistance and biofilm-forming capabilities enhance the ability of S. aureus to thrive as a human pathogen in many environments, posing a challenge for treating bacterial infections with antibiotics. Hence, it is crucial to create innovative approaches to address methicillin-resistant S. aureus (MRSA).

Bacteriophages, commonly known as phages, are recognized as natural enemies of bacteria and have been suggested as an effective weapon against bacterial illnesses since they were first identified in 1915. A phage can be categorized as either lytic (virulent) or lysogenic (temperate) depending on its life cycle after attachment to a bacterium and insertion of its genetic material into the cell. Lytic phages are often favored for eliminating bacterial phages and have been successful in combating multidrug-resistant bacteria, including those in biofilms, in both laboratory and clinical settings. A temperate phage can enhance disease and fitness by disseminating damaging genes, such as virulence factor genes (VFGs) and antibiotic resistance genes (ARGs), or by boosting the expression of VFGs present in the host organisms. Converting a thermal phage to a lytic one might increase its safety for use.

MRSA strain biofilms were measured using crystal violet tests, following earlier protocols. Overnight cultures of MRSA strains were injected in NaCl-free Luria-Bertani (LB) Broth (Thermo-Fisher, US) at a volume ratio of 1:100. Then, 200 µl of the bacterial culture was placed into the wells of a 96-well microtiter polystyrene plate located at Trasadingen, Switzerland. Controls consisted of wells containing an identical volume of soup. Following a 24-hour incubation at 37 °C, the medium was removed and the biofilm in each well of the plate was washed once with 200 µl of PBS. The biofilms in each well were fixed with 200 µl of methanol for 30 minutes and then washed three times with 200 µl of PBS. The plate was air-dried, and the biofilms in each well were dyed with 200 µl of 1% crystal violet solution for 15 minutes. The surplus crystal violet solution in each well of the plate was eliminated by three washes with 200 µl of PBS. The bound crystal violet was then freed by adding 150 µl of 33% glacial acetic acid. The biofilm in each well of the plate was assessed by measuring the absorbance at 540 nm. Each strain underwent the experiment a minimum of three times [10].

Bacillus subtilis and other Bacillus species are commonly utilized as biological control agents (BCAs) in agriculture. For instance, many strains of B. subtilis have been effectively used in programs for managing pests and diseases. We identified wild strains of B. subtilis with potent biocontrol properties against several fungal diseases and the soil-borne bacterial pathogen R. solanacearum in laboratory and greenhouse tests. The mechanism by which B. subtilis demonstrates potent biocontrol effects in the rhizosphere is not fully comprehended. Previous studies have shown that the biocontrol activities of B. subtilis are influenced by the generation of antimicrobial compounds, biofilm development, and induction of host systemic resistance.

Bacillus subtilis is widely and extensively found in a variety of settings such as soils, plant roots, animal gastrointestinal tracts, and aquatic ecosystems. Different strains of B. subtilis isolates frequently exhibit varied physical characteristics. I examined the genetic differences between various strains of B. subtilis using microarray-based comparative genomic hybridization (M-CGH). I discovered that approximately 30% of the expected coding sequences in B. subtilis 168 were either missing or different in the other 17 B. subtilis strains that were tested. Genetic variations were identified in genes linked to antibiotic production, cell wall synthesis, sporulation, and germination [12].

Conclusion:

Multiple crops are impacted by different diseases. PGPM for pests and diseases in crops are commonly seen as a sustainable substitute for traditional chemical plant protection. These PGPR and PGPF, functioning as MBCAs, are a safe, efficient, and eco-friendly method of pest control that poses no risk to the environment or human health. PGPR/PGPF are hostile microorganisms that may be utilized as biopesticides and biofertilizers to enhance plant health and promote development. Implementing PGPR/PGPF-based biopesticides/biofertilizers commercially might significantly enhance sustainable agriculture and promote a safe environment. This review has summarized studies on PGPMs, focusing on their advantages and impacts as possible bioinoculants for plant growth and biological control. To effectively utilize PGPMs, it is essential to accurately pick advantageous PGPR/PGPF strains and consortia, understand the processes of PGPM-plant interactions, and be prepared for upcoming agricultural difficulties.

Biological control management is a very promising application for sustainable agriculture. It is a verified environmentally benign method for controlling agricultural pests. This technique utilizes live microorganisms to decrease insect populations in a sustainable, reliable, and environmentally friendly way. Biological control is an effective and cost-efficient method used in industrialized nations for pest management, providing advantages to both breeders and consumers over synthetic pest control methods.

References:

1. Jia, K., Gao, Y.H., Huang, X.Q., Guo, R.J., et al., 2015. Rhizosphere inhibition of cucumber fusarium wilt by different surfactin-excreting strains of Bacillus subtilis. Plant Pathol. J., 31, 140–151.
2. Monciardini, P., Iorio, M., Maffioli, S., Sosio, M., et al., 2014. Discovering new bioactive molecules from microbial sources. Microb. Biotechnol., 7(3), 209–220.
3. Taechowisan, T., Lu, C., Shen, Y., Lumyong, S., 2005. Secondary metabolites from endophytic Streptomyces aureofaciens CMUAc130 and their antifungal activity. Microbiology, 151(Pt5), 1691–1695.
4. Rebets, Y., Brotz, E., Tokovenko, B., Luzhetskyy, A., 2014. Actinomycetes biosynthetic potential: how to bridge in silico and in vivo? J. Ind. Microbiol. Biotechnol., 41(2), 387– 402.
5. Shimizu, M., Yazawa, S., Ushijima, Y., 2009. A promising strain of endophytic Streptomyces sp. for biological control of cucumber anthracnose. J. Gen. Plant Pathol., 75, 27–36.
6. Kang, M.J., Strap, J.L., Crawford, D.L., 2010. Isolation and characterization of potent antifungal strains of Streptomy- ces violaceusniger clade active against Candida albicans. J. Ind. Microbiol. Biotechnol., 37, 35–41.
7. Zhao, S., Du, C.M., Tian, C.Y., 2012. Suppression of Fusarium oxysporum and induced resistance of plants involved in the biocontrol of Cucumber Fusarium Wilt by Streptomyces bikiniensis HD-087. World J. Microbiol. Biotechnol., 28, 2919–2927.

8. Li, X., Huang, P., Wang, Q., Xiao, L., et al., 2014. Staurosporine from the endophytic Streptomyces sp. strain CNS-42 acts as a potential biocontrol agent and growth elicitor in cucumber. Antonie van Leeuwenhoek, 106, 515–525.
9. Liu, Y.X., Shi, J.X., Feng, Y.G., Yang, X.M., et al., 2013. Tobacco bacterial wilt can be biologically controlled by the application of antagonistic strains in combination with organic fertilizer. Biol. Fertil. Soils, 49(4), 447–464.
10. Mingma, R., Pathom-Aree, W., Trakulnalemsai, S., Tham- chaipenet, A., et al., 2014. Isolation of rhizospheric and roots endophytic actinomycetes from Leguminosae plant and their activities to inhibit soybean pathogen, Xantho- monas campestris pv. glycine. World J. Microbiol. Biotechnol., 30(1), 271–280.
11. Zhang, N., Song, Z., Xie, Y., Cui, P., et al., 2013. Identification and characterization of antifungal active substances of Streptomyces hygroscopicus BS-112. World J. Microbiol. Biotechnol., 29(8), 1443–1452.
12. Cao, L.X., Qiu, Z.Q., You, J.L., Tan, H.M., et al., 2005. Isolation and characterization of endophytic streptomy- cete antagonists of Fusarium wilt pathogen from surface- sterilized banana roots. FEMS Microbiol. Lett., 247, 147–152.