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Isolation Of *Bacillus Thuringiensis* From Soil Sample, Identified By 16s Rrna Analysis And Identify Its Larvicidal Activity On Jasmine Plant

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Abstract

Bacillus thuringiensis (*Bt*) is having a most important role in the field of agriculture and genetic engineering because of its uniqueness in protein production. It supports to control various pest in plants such as green fly, white fly, beetles and few types of larvae by its toxic nature. It works well as insect and pest controller in the genetically modified crops. The Bt toxic genes were used in some crops for the pest control. In this work, we had to isolate the target bacterial strains from agricultural soil. The isolated bacterial strains were identified by macroscopic and microscopic observations. Later the isolated bacterial strains forwarded to 16s rRNA analysis for the bacterial confirmation. The isolated bacterial strain used to control pest in the jasmine plants and positive level of results were collected.

Keywords: Bacillus thuringiensis, 16s rRNA, agriculture, Jasmine plant, Larvicide

INTRODUCTION

Bacillus thuringiensis (BT) is one of the bacteria that lives in soil. It produces some protein like components, that act as toxic to some insects when eaten by insects. That protein component non toxic to human beings because it cannot be activate our body secretions. Bt used as biopesticides in all over the world in the field of agriculture in various forms like granules, sprays and some other types of products (1).
There are many types of Bt available in the society and each type of Bt control different groups of insects. Most of the insect gut having pH in the range of 9.0 to 10.5 pH and this pH will activate the Bt toxin (RED, 1998). As well as human gut having acidic pH so that, the toxic component does not get activation itself.
There are so many numbers of crystals producing *Bacillus thuringiensis* present in the nature. But all are not effectively works in all insects and pest. The *Cry* genes are responsible for crystallization, when it expressed in insects (3). There are more than 700 *Cry* gene producing *Bacillus thuringiensis* were isolated in all over world. According to Alper *et al.,* (2016) (4) research, a single bacteria may contain more than one *Cry* or crystallization genes in itself. There are some transgenic crops also developed for controlling or avoidance of diseases and insect affections such as Coleoptrera, Lipidoptera and Diptera pests by the application of gene

transfermation. Various companies released commercially transgenic plants which was made by the insertion of *Cry* genes and it called as Bt transgenic plants such as Bt cotton, Bt corn and etc., (5).
Bacillus thuringiensis produce bacteriocin component that in food industries for various applications like controlling of contamination and pathogenic bacterial development in food and its products. Specially it works against of *Listeria sp., E.coli, Staphylococcus aureus, Clostridium sp., Bacillus sp., Salmonella sp.,* and some other bacterial pathogens (6).

MATERIALS METHODS

Sample collection

Soil sample were collected from various agriculture location by using glass tubes. Totally 15 samples collected from agricultural lands which not treat by any chemical fertilizers and chemical pesticides. The collected samples were stored in refrigerator at 4°C until it used.

Isolation of bacteria

1g of soil measured and suspended in 10ml of 0.80% Nacl. The mixture of component heated with shaking water bath and the heat applied up to 70°C to 75°C. Later, the tubes were kept for to reach normal temperature and then 100µl of suspension taken and loaded into nutrient agar plates. The loaded plates were incubated at 30°C for 24 to 48 hours. The developed colonies were used to subculture and staining methods. Gram staining and spores staining were done for identification of morphological structure of isolated bacteria.

Gram and Spore staining

Gram staining method used to identify the bacteria whether gram positive or gram negative and the shape will declare that, the isolated bacteria rod or cocci. The spore staining support to find that the isolated bacteria having ability of spore forming or not. Both staining methods were used to identify the bacterial characters.

Culture characters

There are few media and chemical components used to identification of morphological, physiological and biochemical properties of isolated microorganisms. Nutrient agar, EMB agar, Mannitol salt agar, MacConkey agar, Gelatinase agar, Peptone water, MR-VP medium, Simmon citrate agar and Ureases agar used to identification of isolates.

Extraction of DNA

The developed culture was used to extraction of DNA by the application of QIAmp DNA mini kit (Qiagen). According to the protocol and manufacturer instruction, the extraction process was carried out. The extracted bacterial DNA was stored in refrigerator condition or 4°C until it used to PCR process.

PCR amplification

The bacterial DNA extract (1 µl) and control were amplified with the application of 1 µl of 10 µm primers, 3.5 µl of GoTaq green mastermix, 3.5 µl of nuclease free water. The denature process held in 94°C and annealing process held in 55°C and the second extraction carried in 72°C. the agarose gel electrophoresis applied for separation of PCR products and it is identified by ethidium bromide (7)

Identification of toxicity of isolated bacteria

In this study, the isolated bacteria used as pest controlling agent against of pest and insects that present in jasmine plant. Before of application, complete data were collected in the trial field which includes days of infections, any organic and inorganic components applied for the disease management in the plants, any fertilizers or liquids applied to promote the plant growth.

RESULT AND DISCUSSION

There are totally 15 soil samples were collected from the agricultural lands by the application of glass tubes. The collected samples were processed for the isolation of target bacteria. The processed samples were inoculated into agar plates and it forwarded to gram staining process for microscopic identification isolated bacteria. Gram positive rod types of bacterial cells were identified in sample 2, 5, 11 and 14. Those bacterial colonies were showed in similar morphological appearance.

Culture characters

White colored colonies were appeared in nutrient agar. Dark colored colonies and very rarely colonies developed in EMB agar and Mannitol salt agar. Inverted tree like growth showed in the Gelatinase agar. In the biochemical analysis, indole, VP and citrate test were positive and MR was negative in the analysis. As well as, catalase, oxidase and gelatinase were showed as positive (8).

Gram and Spore staining

Violet colored rod shaped bacteria were appeared in the gram staining and it considered as Gram positive rod. Dark green colored spores and pink colored vegetative cells were appeared in the spore staining and the bacterial strain positive for Spore forming (9).

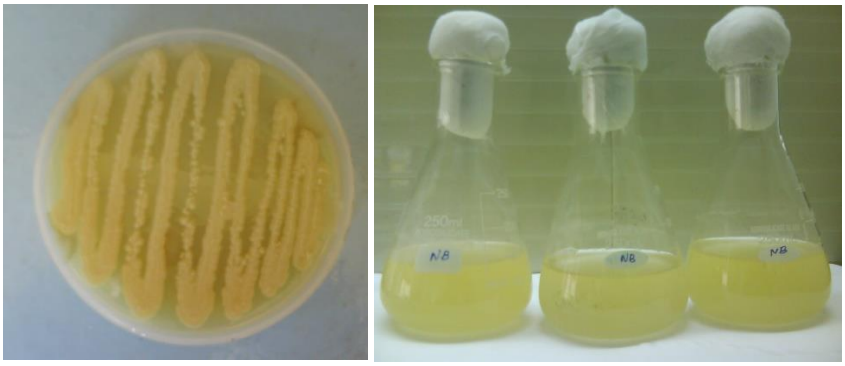

Figure 1: *Bacillus thuringiensis* on Nutrient agar and Nutrient broth 16s rRNA analysis

TGCAAGTCGAGCGAATGGATTGAGAGCTTGCTCTCAAGAAGTTAGCGGCGGACGGGTGAG
TAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACC
GGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGG
ATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGT
AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGA
GTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAA
TAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGT
GGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGG
GAGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGA
TATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGA
AAGCGTGGGGA

Toxicity analysis of *Bacillus thuringiensis*

Bt were cultivated in Nutrient broth for the mass cultivation and used to disease management in infected jasmine plants. Jasmine plants infected by white flies and it didn't treat by any organic and inorganic materials for disease management. Liquid organic fertilizers (humic and aminoacid combination) were applied for plant growth promotion.

According to Choma *et al.*, (1990) (10) at the region of mid-gut and high pH are responsible for the soluble of crystalline proteins and the soluble form of components are called as δ (delta) endotoxins. The toxin which produced by *B. thuringiensis* mainly target mid gut of the insects and pests (11). Milne and Kaplan (1993) (12) reported that, the Bt crystalline proteins are inactive till it reacted with enzymes of either insects or pest. Soberon *et al.*, reported that, Bt delta toxins (crystal proteins) has been isoloated from insects and pest, these type of crystal were formed by *Cry* proteins and theses types of components are toxic to the insects and pest.

In 1994, the Bt toxin detailed by Knowles (11) and he reported that, the different types of proteins were identified and all of the not works in the same manner, it works depending upon the pest and insects in the plants (13,14). As well as, Lee *et al.*, (2004) (15) reported that, active toxic component initially bind with glycoprotein and then only it gets activation itself.

CONCLUSION

Fifteen samples were collected from the various locations and four *Bacillus sp.*, were confirmed by Gram staining, spore staining and microbial culture characters. Well developed one isolate were forwarded to 16s rRNA analysis and it confirmed as *Bacillus thuringiensis*. The isolated bacterial suspension applied to the plants which was affected by white flies and rubber worms. Positive results were showed after five days. *Bacillus thuringiensis* widely used to control insects and pest in various plants. *Bt* is considered as entamopathogenic and entomotoxic bacteria and these bacterial strains used to control insect and pest in the

plants. The insecticidal proteins including *Cry* and *Cyt* crystallin proteins synthesized by *Bacillus thuringiensis* that support to control pest and insects in the plants.

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