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Isolation and identification of *Bacillus Thuringiensis*

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Abstract
The single isolate of *Bacillus thuringiensis* was obtained from the twenty soil samples which were collected from Shahuwadi and Kagal tehsils of Kolhapur district of Western Ghat Zone of Maharashtra. The isolate was futher subjected for morphological and biochemical characterization. The isolate was having circular colonies, rough in nature, wavy margin, the colour of colony was creamy white having diameter 3mm. The isolate was subjected to the biochemical characterization showed positive reaction for catalase, starch hydrolysis, casein hydrolysis, nitrate reduction, voges- proskauer reaction, oxidase test and negative reaction for acid and gas, arginine dihydro lysis and esterase activity. The isolate was gram positive and motile. The cells were found to be producing endospores. The isolate possessed Bipyramidal shaped crystals when observed under microscope. The use of soil bacterium *Bacillus thuringiensis* has became immense potential for use of biopesticide. So there is currently need to isolate and identify *Bacillus thuringiensis* strain.

Keywords: Isolation, identification, *Bacillus thuringiensis*.

Introduction
Bacillus thuringiensis has been used as a successful biological insecticide for more than 40 years and is a uniquely specific, safe and effective tool for the control of a wide variety of insect pests (Nester *et al.*, 2002) [9]. *Bacillus thuringiensis* has been looked into as an alternative to chemical pesticides for many years. Farmers totally rely upon the chemical pesticides and due to indiscriminate use of conventional insecticides have resulted in environmental pollution, risk to human and animal health, adverse effect on the non-target beneficial insect, resistance to chemicals, resurgence of minor pests and residual hazards. One of the solutions for pest insurgence problem is use of biological agents. The greatest successes in microbial pesticides have come from the use of commercial preparations of *Bacillus thuringiensis*. *Bacillus thuringiensis* is rod shaped, gram positive bacterium abundant in soil and other habitat throughout the world (Kumar, 2002) [4]. Biological control of insect pests has become popular and provides a safer means to reduce insect damage (Dhaliwal and Arora, 1998). The pest specific toxicity of *Bacillus thuringiensis* arises from its crystalline inclusion comprised of one or more polypeptides called insecticidal crystal protein or delta endotoxin present in the sporulating cells (Nagamatsu *et al*., 1998) [8]. Among various options available, the use of soil bacterium *Bacillus thuringiensis* has became immense potential for use of biopesticide. Being a ubiquitous soil bacterium, highly potent native isolates with competitive surviving ability are ideal for developing *Bacillus thuringiensis* based formulation. So there is currently need to isolate *Bacillus thuringiensis*.

Material and method
Isolation *Bacillus thuringiensis*
The isolation from soil was carried out by sodium acetate selection method (Travers *et al*., 1987) [13]. Five hundred milligrams of soil sample was added to 10 ml of sterilized Luria Broth buffered with 0.25 M sodium acetate (pH 6.8) in 125 ml conical flask and was incubated for four hr at 30 ºC with shaking at 200 rpm in incubating shaker. After incubation, aliquots of one ml were heated to 80 ºC for 3 minutes and ten old of serial dilutions up to10-5 were prepared and one ml of suspension from each test tube was transferred in sterilized petriplates. The sterilized Luria Agar medium was poured and mix with aliquot gently. The plates were incubated at 28 ± 2 ºC for 24 to 48 hr. The colonies formed were picked up based on their morphological similarities with those of reference *Bacillus thuringiensis*. Selected colonies were purified by repeated streaking on T3 medium and then stored at 4 ºC for further studies.

Identification *Bacillus thuringiensis*

Morphological characterization

Morphological characterization of the isolate was done by comparing the colony morphology with those of the reference strain *Bacillus thuringiensis*. The criteria selected for the study of colony morphology include size, shape, nature of colony margin, appearance and colour of the colony.

Microscopic study

Endospore staining

Endospore staining was done by following the protocol of Cappuccino and Sherman (1992) [2]. The cells from culture grown for 72 hr in modified glucose medium (MGM) broth were smeared on a clean glass slide and allowed to air dry. Smear was heat fixed by passing the slide over flame followed by heating over steam for 15 minutes with continuous addition of five per cent aqueous solution of malachite green. After steam fixation, excess stain was washed under running tap water. The slide was counter stained with 0.5 per cent aqueous solution of safranin for 30 seconds and washed thoroughly under tap water. The slide was blot dried and observed under microscope.

Crystal protein staining

Crystal protein staining was done by following the protocol given by Sharif and Alaeddinoglu (1988) [10]. Smear of the cells from culture grown for 64 hr in Modified Glucose medium (MGM) broth were heat fixed and dipped in 0.25 per cent coomassie brilliant blue solution for three minutes. The slides were then washed under running tap water, air dried and observed for dark blue coloured crystals under microscope and recorded.

Biochemical characterization

The isolate was subjected to various biochemical tests viz., catalase, starch hydrolysis, casein hydrolysis, nitrate reduction, voges- proskauer reaction, oxidase test, acid and gas, arginine dihydro lysis and esterase activity as per the procedures outlined by Cappuccino and Sherman (1992) [2].

Result

Out of 20 soil samples, Single isolate of *Bacillus thuringiensis* from Bambavada region of Shahuwadi Tehsil was found. This attributed to the diversity of fauna and flora existed in Shahuwadi. The isolate was having circular colonies, rough in nature, wavy margin, the colour of colony was creamy white having diameter 3mm (Table 1). The isolate was subjected to the biochemical characterization. The native isolate showed positive reaction for catalase, starch hydrolysis, casein hydrolysis, nitrate reduction, voges- proskauer reaction, oxidase test and negative reaction for acid and gas, arginine dihydro lysis and esterase activity (Table 2).

The isolate was observed for gram staining, motility, endospore and crystal formation. It was observed that the isolate was gram positive and motile. The cells were found to be producing endospores. The isolate possessed bipyramidal shaped crystals when observed under microscope (Table 3).

Table 1: Morphological characteristics of native *Bacillus thuringiensis* isolate

S. No.	Colony Morphology	
1	Shape	Circular
2	Colour	Creamy white
3	Nature	Rough
4	Margin	Wavy
5	Size	3mm
6	Elevation	Flat

Table 2: Biochemical characterization of the native *Bacillus thuringiensis* isolate

S. No.	Enzymatic test	Result
1	Catalase	+
2	Starch hydrolysis	+
3	Casein hydrolysis	+
4	Nitrate reduction	+
5	V-P reaction	+
6	Acid and gas production	-
7	Arginine dihydrolase	-
8	Esterase avtivity	-
9	Oxidase test	+

+ = positive Test, - = negative Test

Table 3: Gram reaction, motility, endospore formation and crystal staining of native *Bacillus thuringiensis* isolate

S. No.	Microscopic Morphology	
1	Gram reaction	Gram positive
2	Motility	Motile
3	Endospore production	+
4	Crystal formation	+

Discussion

Theunis *et al.* (1998) [12] reported similar procedure of Travers *et al*. (1987) [13] has been extensively used to isolate *Bacillus thuringiensis* from different ecological niches *viz.*, soil, grain dust, rice straw, compost and mammalian faeces. Similarly, isolation procedure was reported by Lee *et al.* (2001) [6] isolated many *Bacillus thuringiensis* subsp. *kurstaki* from Korean soil samples using sodium acetate selection method. Kaur *et al.* (2006) [5] subjected *Bacillus thuringiensis* isolates for both morphological and biochemical characterization and observed all the isolates to be gram positive, rod shaped, spore forming and showed typical colony morphology with mucoid or glistering surfaces having entire edges and density ranging between translucent to opaque who also reported that the strains of *Bacillus thuringiensis*, besides producing parasporal crystal bodies, were positive for catalase production, oxidase activity, nitrate reduction, starch and casein hydrolysis. Similar observations made by Starr (1981) [11] reported that the strains of *Bacillus thuringiensis*, were positive for catalase production, V-P reaction, nitrate reduction, starch and casein hydrolysis but were negative for acid and gas production. Similarly, Aramideh *et al.* (2010) [1] isolated 48 native *Bacillus thuringiensis* strains were characterized by crystal morphology majority of strains (58%) had bipyramidal crystals. Lele and Nabar (2010) [7] obtained 82 isolates of *Bacillus thuringiensis* These isolates were identified and characterized for production of crystal protein microscopically. The isolates showed three types of parasporal crystal proteins. Thirty two isolates showed cuboidal and spherical shaped crystal proteins and 18 produced bipyramidal crystals.

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