Pakistan J. Sci. Ind. Res., Vol. 29, No. 4, August 1986

DISTRIBUTION OF *BACILLUS THURINGIENSIS* SEROTYPES IN PAKISTAN

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(Received 5 March 1985; revised 5 May 1986)

Distribution of *Bacillus thuringiensis* in Pakistan was investigated and it was found that most of the crystalliferous isolates belong to *Bacillus thuringiensis* sub sp. sotto i.e. 129 out of 150 isolates. Other isolates belong to serotype B.t. alesti (3) B.t. dendrolimus (3), B.t. kurstaki (4) B.t. kenyae (1) and B.t. pakistani (10).

INTRODUCTION

Bacillus thuringiensis an entomopathogenic bacterium, is known to occur in soil and in dead/diseased larvae of susceptible insect species [1-8]. The present study deals with extensive survey work for isolation, identification and serotyping of the isolates occurring in various regions of Pakistan.

MATERIAL AND METHODS

Collection of specimens. Dead/diseased larvae were collected from various parts of Pakistan, including the areas famous for raw silk production. The larval material was taken in sterile vials and stored frozen.

Isolation of crystal forming bacteria. A back exudate invariably comes out of the body of the infected larvae as a result of bacterial infection, making them soft and pulpy. The larval stuff, was cleaned from outside gently with 75% alcohol. The larva was punctured with sterile needles in a sterile petri dish. The oozing exudate was plated out on the solid medium (0.5% peptone, E. Merck, 0.5% NaCl, E. Merck, 10% beef extract Difco, and 2% Agar Difco. pH 7.00). Dry specimens were cleaned, similarly and after evaporation of alcohol, were crushed in sterile saline. The suspension was used for isolation.

Plates were incubated initially for 24 hr. at 30°. A selection of spore formers was then made, which were transferred on the fresh medium. After 48 hrs. incubation the culture was examined under phase contrast microscope (Fig. 1). Crystal forming bacteria were isolated and acid fermentation of sugars was done, using glucose, sucrose, lactose, maltose, mannitol and xylose (1% w/v sugar E. Merck) in peptone water (sterlized at 10 lbs pressure for 20 min). Lecithinase production was studied in meat extract agar containing 2% fresh egg yolk.

RESULTS AND DISCUSSION

The results indicate that B.t. alesti, B.t. dendrolimus, B.t. kurstaki, B.t. kenyae, B.t. pakistani and B.t. sotto are the serotypes of crystalliferous bacteria found in Pakistan. The serotyping of suspected B.ts. were carried out at the Pasteur Institute Paris using the technique by H.de Barjac

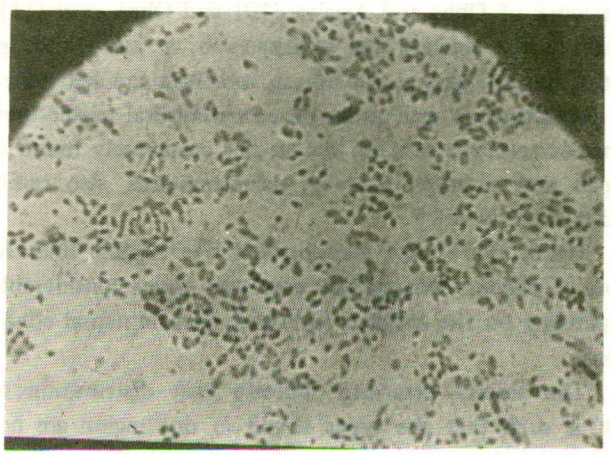

Fig. 1. Liberated spores and crystals of B.t. after 120 hrs. x 1200

[9]. The details of the geographical distribution of these serotypes according to the place (province) of collection and simultaneous existence of different serotypes are given in Table 1. The microscopic studies of the isolates indicate that they produce spherical or dipyramid shaped parasporal bodies (Fig. 1). Isolates non-toxic to *Heliothis armigera* were also isolated.

The serological studies of 150 isolates indicate that 127 isolates belong to B.t. sub sp. sotto and hence it is widely distributed in the country while the distribution of serotypes sub. species namely B.t. sub. sp. alesti, B.t. sub. sp. dendrolimus, B.t. sub. sp. kenyae, B.t. sub. sp. kurstaki and B.t. sub. sp. Pakistani have also been found.

Table 1. Serological distribution of crystalliferous bacteria isolated in Pakistan

Province	*B.t. alesti*	*B.t. dendrolimus*	*B.t. kenyae*	*B.t. sotto*	*B.t. kurstaki*	*B.t. pakistani*	Total No. of serotype in each province
Azad Kashmir and NWFP	2	1	1	54	–	–	58
Punjab	1	1	–	73	2	1	78
Sind	–	–	–	–	–	4	04
Baluchistan	–	1	–	–	2	7	10
Total	3	3	1	127	4	12	150

Table 1 indicates interesting results. B.t. sub sp. sotto is present in N.W.F.P. and Punjab province whereas it is totally absent in Sind and Baluchistan. Similarly Bt. sub. sp. pakistani which had been isolated from this part of the world (Sind) [9, 12] is present only in Sind and Baluchistan and is not found in Punjab and N.W.F.P. Yet another finding is that no species of B.t. was found in Sind other than B.t. sub. sp. pakistani. The present results confirm the work of Ohba and Aizawa [10] and Ohba *et al* [11]. Their results indicate that serotype sub. sp. alesti, sub. sp. sotto, sub. sp. aizawai and sub. sp. morrisoni are distributed in prefecture as well as in other sericultural prefectures, whereas serotype sub. sp. kenyae has been rarely found in Japan and serotype sub. sp. darmstadiensis also rarely exists in the country.

Hence the results mentioned above suggest that the flora of *Bacillus thuringiensis* serotypes varies considerably depending on the locality and other environmental conditions.

The results described indicate that distribution of B.t. is such that the types found in one region are not found elsewhere. This is in conformity with the results of a similar study done in Japan. Regionwise the sharp distribution of the organisms renders an interesting situation which need further investigation.

Acknowledgement. The authors wish to thank Dr. H. de Barjac, Institute Pasteur Paris, for serotyping the cultures and USDA for providing us the research grant No: FG-PA317 (PK. ARS. 146) under PL-480 Programme.

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