Short Communication

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Serotypes of *Bacillus thuringiensis* in Pakistan

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Occurrence of bacteria in connection with a disease in silkworms was reported for the first time by Pasteur 1870. In 1902 Ishiwata isolated a *bacillus, Sotto bacillus* from silk worms suffering from severe dysentry. Akoi and Chigasaki 1915 reported the pathogenicity of *Bacillus sotto* in experimentally infected worms; the name of the bacterium was changed to *Bacillus thuringiensis*. Commercial preparations based on spores and / or crystals of this bacterium are used to control insects in many countries [1-15]. Search for new promising strains is also in progress. Shiekh *et al.* [6] isolated a new strain known as variety Pakistani. The present communication indicates the results of serotyping of the local isolates. The experimental set up used was the same as described by Sheikh *et al.* [12].

Samples of Lepidopterous larvae and pupae infected with bacteria were collected from various farms of the country including Azad Kashmir. The specimens were taken in sterile vials and brought to the lab. The vials were labeled and stored frozen.

Selection was made on the basis of blackish discharge staining, the outer surface of the cocoons, likewise the larvae also get black and brownish while infected (Fig. 1). The internal material that oozed out was streaked on nutrient agar plates. After 48 hrs colonies were checked (Fig 2) for the presence of endospores by Gram's method and phase contrast microscopy (Fig 3).

Two hundred and fifty specimens of diseased Lepidopterous larvae were studied (Fig 1), 150 were characterized and identified as infected with *B. thuringiensis* based on morphological, cultural, biochemical and serological characteristics of these isolates. Parasporal bodies, endotoxin or crystals started appearing between 48 to 72 hrs. Maximum liberation of crystals and spores was observed after 120 hrs. (Fig 4).

Table 1 indicate the results of serotyping. Serotype 4 ab dominates over other serotypes. The other serotypes identified/isolated are 3ab, 5ab, 8ab, 2a and 13a.

Fig. 1.

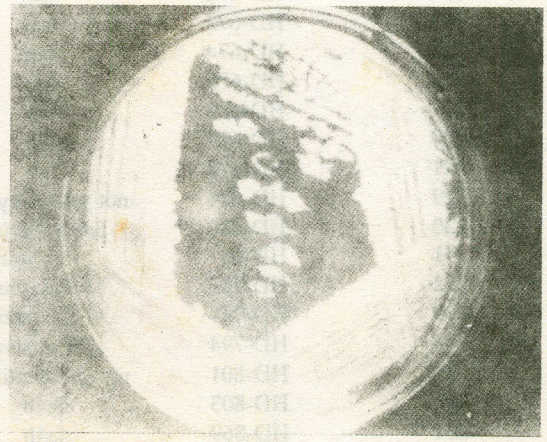

Fig. 2.

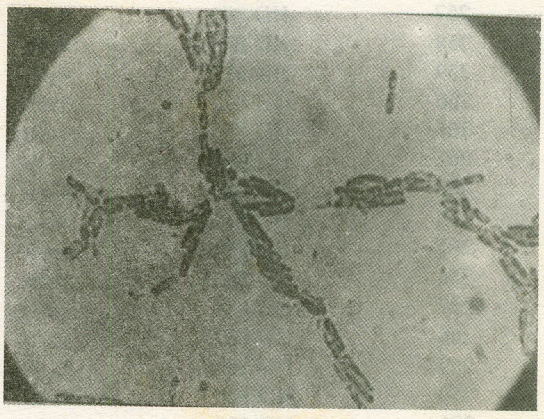

Fig. 3.

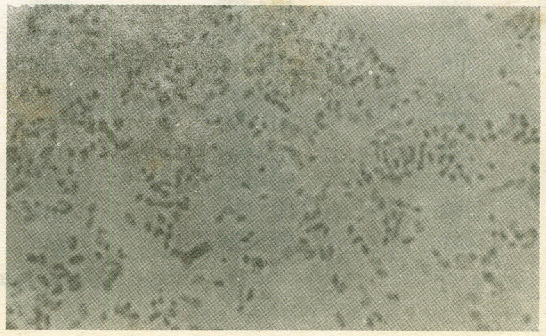

Fig. 4.

TABLE 1. RESULTS OF SEROTYPING OF SUSPECTED CULTURES OF BACILLUS *THURINGIENSIS* (SOTTO).

S. No.	Original suspected B.T.No	HD-number	Serotype
1	177	HD-662	4ab
2	180	HD-663	4ab
3	181	HD-664	1 thuringiensis
4	185	HD-665	8 ab morrisoni
5	191	HD-666	4ab
6	193	HD-667	4ab
7	195	HD-668	4ab
8	201	HD-669	4ab
9	202	HD-670	4ab
10	204	HD-671	4ab
11	212	HD-672	4ab
12	213	HD-673	4ab
13	215	HD-674	4ab
14	216	HD-675	4ab
15	217	HD-676	4ab
16	220	HD-677	4ab
17	221	HD-678	4ab
18	229	HD-679	not yet serotyped
19	230	HD-680	5ab golleriae
20	231	HD-681	2 finitimus
21	232	HD-783	13-Pakistani
22	233	HD-789	13-Pakistani
23	234	HD-794	13-Pakistani
24	235	HD-801	not yet identified
25	236	HD-803	4a4b
26	254	HD-869	4a4b
27	255	HD-870	not identified
28	256	HD-872	4a4b
29	257	HD-873	4a4b
30	258	HD-875	4a4b
31	259	HD-877	4a4b
32	260	HD-878	4a4b
33	261	HD-879	4a4b
34	262	HD-880	4a4b
35	263	HD-881	4a4b
36	264	HD-883	4a4b
37	265	HD-884	4a4b
38	266	HD-885	4a4b
39	267	HD-886	4a4b
40	268	HD-887	4a4b
41	269	HD-888	4a4b
42	270	HD-889	4a4b
43	271	HD-890	4a4b
44	272	HD-892	4a4b
45	273	HD-895	4a4b
46	274	HD-896	4a4b
47	275	HD-897	4a4b
48	276	HD-898	4a4b
49	277	HD-899	4a4b
50	278	HD-900	4a4b
51	279	HD-902	4a4b
52	280	HD-903	4a4b
53	281	HD-904	4a4b
54	282	HD-907	4a4b
55	284	HD-922	4a4b
56	285	HD-924	4a4b
57	287	HD-926	4a4b
58	288	HD-930	4a4b
59	289	HD-931	4a4b
60	290	HD-946	4a4b
61	292	HD-952	4a4b
62	293	HD-958	4a4b
63	294	HD-981	4a4b
64	295	HD-982	4a4b
65	296	HD-984	4a4b
66	297	HD-987	4a4b
67	298	HFD-989	4a4b
68	299	HD-990	4a4b
69	300	HD-832	4a4b
70	303	HD-835	4a4b
71	304	HD-836	4a4b
72	305	HD-839	4a4b
73	306	HD-866	4a4b
74	307	HD-891	4a4b
75	308	HD-908	not identified
76	309	HD-909	4a4b
77	310	HD-914	4a4b
78	311	HD-916	4a4b
79	312	HD-917	4a4b
80	313	HD-918	4a4b
81	314	HD-932	4a4b
82	915	HD-933	4a4b
83	316	HD-934	4a4b
84	317	HD-935	4a4b
85	318	HD-936	4a4b
86	320	HD-939	4a4b
87	321	HD-941	4a4b
88	322	HD-942	4a4b
89	324	HD-964	4a4b
90	325	HD-965	4a4b
91	326	HD-972	4a4b
92	327	HD-971	4a4b
93	328	HD-973	4a4b
94	929	HD-974	4a4b
95	330	HD-978	4a4b
96	331	HD-979	4a4b
97	332	HD-1008	4a4b
98	333	HD-1028	4a4b
99	334	HD-1030	4a4b
100	335	HD-1031	4a4b
101	336	HD-1084	4a4b
102	337	HD-1085	4a4b
103	339	HD-1140	4a4b
104	342	HD-1156	4a4b
105	343	HD-1211	4a4b
106	344	HD-1212	4a4b
107	345	HD-1213	4a4b
108	346	HD-1215	4a4b
109	347	HD-1219	4a4b

Contd. Table 1

(Table 1 Contd.)

Contd. Table 1

Table 1 Contd.....)

110	355	HD-1228	4a4b
111	368	HD-1288	4a4b
112	369	HD-1295	4a4b
113	370	HD-1290	not identified
114	371	HD-1026	4a4b
115	372	HD-1080	4a4b
116	373	HD-1091	4a4b
117	374	HD-1095	4a4b
118	375	HD-1095	4a4b
119	376	HD-1119	4a4b
120	362	HD-1257	4a4b
121	365	HD-1260	4a4b
122	366	HD-1261	4a4b
123	367	HD-1287	4a4b
124	368	HD-1288	4a4b
125	392	HD-1199	4a4b
126	396	HD-1210	4a4b
127	398	HD-1228	4a4b
128	402	HD-1234	4a4b
129	405	HD-1236	4a4b
130	406	HD-1237	4a4b
131	410	HD-1249	4a4b
132	424	HD-1304	4a4b
133	427	HD-1307	4a4b
134	428	HD-1310	4a4b
135	429	HD-1311	4a4b
136	435	HD-1320	4a4b
137	436	HD-1321	4a4b
138	809	HD-682	2 finitimus
139	812	HD-683	5 ab galleriae
140	814	HD-684	4ab
141	819	HD-685	4ab
142	822	HD-686	4ab
143	825	HD-687	4ab
144	850	HD-689	*
145	851	HD-690	*
146	867	HD-691	*
147	868	HD-692	*
148	424	HD-693	4ab
149	448	HD-694	3ab
150	449	HD-695	3ab

The details of geographical distribution of these serotypes according to place (province) of collection has been reported by Shiekh *et al* . [12].

Present results are in complete conformity with previously published data in respect of presence of crystal forming bacteria in Lepidopterous insects in natural conditions. In Pakistan serotype 4a,b infection is more prevalent than any other.

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