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DISCOVERY AND PATHOGENICITY OF *PSEUDOMONAS FLUORESCENS* AGAINST VARIOUS SPECIES OF TERMITES

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Abstract: Pathogenicity of *Pseudomonas (P) fluorescens* tested against *Microcerotermes (M) championi, Heterotermes (H) indicola* and *Bifiditermes (B) beesoni.* All the termites were found susceptible to the infection caused by *P. fluorescens.* Their value of LT_{50} and LT_{90} showed a range from about 101 hours to 127 hours and 265 hours to 302 hours respectively along with the slope of regression lines (b) from 3.06 to 3.39. Histopathological studies were carried out to see the mode of action of *P. fluorescens.* It attacked intestinal epithelium of *M. championi*, disintegrated fat body tissues of *H. indicola*, and heavily attacked fat bodies of *B. beesoni* at 72 hours, 48 hours and 120 hours following infection, respectively.

Keywords: *Pseudomonas fluorescens*, pathogenicity, termites, insect control.

INTRODUCTION

Attempts have been made in bacterial control of termites as an alternative to chemical insecticides. *Bacillus thuringiensis* and its preparation have shown a great potentiality as microbial insecticide against various species of termites. Smythe and Coppel (1965) reported that soluble toxin preparation from *B. thuringiensis* was found to be toxic to three species of *Reticulitermes, and Zootermopsis angusticollis.* Vypijack *et al.* (1972) reported the possibility of termite control by microorganisms including *B. thuringiensis.* Khan *et al. (*1977a) isolated *B. thuringiensis)* was pathogenic to *Heterotermes indicola* and *Bifiditermes bessoni.* Studies on pathogenicity and development of *B. thuringiensis* in termites. *Microcerotermes championi* and *B. bessoni* were also carried out by Khan

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*et al. (*1985). *Serratia marcescens* was also considered as a potential pathogen against termites. Toumanoff and Toumanoff (1959) studied the epizootics of *Reticulitermes santonnensis* caused by *S. marcescens.* According to Lund (1965) spore forming bacteria, *S. marcescens,* that can be carried back by termites to their colony, gave 100% mortality to laboratory termite culture within 24 hours. Khan *et al.* (1977b) also observed the susceptibility of various species of termites to *S. marcescens.*

Pseudomonas aeruginosa has been reported to be pathogenic to several species of insects. Lysenko (1963) reported that it was extremely pathogenic when injected into the body cavity of *Galleria mellonella* larvae. It was found pathogenic to laboratory reared cultures of grasshopper, *Melanoplus bivittatus* (Say) and *Camnula pellucida* (Scudder), but natural infection in field population has never been demonstrated (Stephens, 1958; Bucher, 1959, 1963; Bucher and Stephens, 1959). Ashrafi *et al.* (1965) reported that *P. aeruginosa* was pathogenic to desert locust, *Schistocerca gregaria.* The Pathogenicity of *Pseudomonas aeruginosa* against termites was first reported by Khan *et al.* (1992). Similarly, the present study will also provide another record for the pathogenicity of *P. fluorescens* to termites.

MATERIALS AND METHODS

Isolation of Pseudomonas fluorescens

Pseudomonas (P) fluorescens was isolated from various species of termites collected from different localities of Pakistan including Karachi, Lahore and Islamabad. It was identified according tests mentioned in Bergey's Manual of Determinative Bacteriology (Breed *et al.* 1972). The pathogenicity was tested against *M. championi. H. Indicola and B. bessoni.* In preliminary experiments, *P. fluorescens* cultures were grown on nutrient agar medium at 28± 1^{o}C for 24, 48 and 72 hours.

The pathogenicity of these cultures was determined against termites. When it was observed that 72 hours old culture of *P. fluorescens* was more virulent, its pathogenicity was then determined against *M. championi, H. indicola and B. bessoni.* A concentration of about 7×10^{9}/ ml of viable rods of 72 hours old culture of *P. fluorescens* were prepared in sterile distilled water. The workers of *M. championi, H. indicola* and

nymphs of *B. bessoni* were divided into two groups each: the 'control' and the 'test' group. Each group had 25 termites.

The test group for each species of termite was infected by 1 ml of suspension of *P. fluorescens;* while the control was supplied with 1ml sterile distilled water only. The experiment was replicated thrice. The mean percentage mortality was calculated.

The response of pathogenicity of *P. fluorescens* was also plotted as regression lines, which were calculated by Probit analysis; the detail of which is already mentioned (Khan *et al.,* 1992). LT_{50} and LT_{90} were determined for comparative study, while the slopes of regression lines (b) were determined in order to confirm the pathogenicity. When the control mortality was 8% or more, the percentage moratalities of test groups were corrected by Abbots's formula (Abbott, 1925) as modified by Krejzova (1975).

Periodically dead termites were examined in smears after beings stained by Gram's method. The causative agent was isolated by Streak plate method and identified as *P. fluorescens.* These tests were in accordance with Koch's postulates as described by Bucher (1973).

Histopathological studies were also were also carried out (Vago and Amargier, 1963) to see the mode of action of *P. fluorescens* in various species of termites. The histopathological sections were strained by the techniques given in Hotchkiss (1948).

RESULTS

Pseudomonas fluorescens was isolated from various species of termites collected from different parts of Pakistan (Karachi, Lahore and Islamabad). It was cocco-bacilli, gram-negative and non-spore former. The mortility test was variable. The termites particularly *B. beesoni,* turned dark green in color due to the infection of *P. fluorescens.* On the nutrient agar Petri dishes the colors were grayish brown having a dark green center. The back of each colony was also green. The green pigment was seen diffused throughout the medium. The pigmentation changed from green to brownish green and became brown as the age of the culture increased. A 72 hours old culture on nutrient agar slant was pure green and transparent. The

bacterium equally fermented glucose and mannitol with the production of acid only. While maltose, lactose, sucrose and starch were not fermented.

The green pigmentation was so dominant that it gave bluish green color to lactose and blue color to sucrose broths. This color may be due to pigmented material in combination with bromocersol purple or bromothymol blue used as an indicator in sucrose and lactose, respectively. Simmon's citrate was utilized; gelatin was liquefied and nitrate was reduced to nitrite. Methyl red, Voges-Proskauer and Kovac's Indole were negative. The tryptone broth, used for the test of Indol production culture for 72 hours. Thus it was identified as *P. fluorescens* (Breed *et al.,* 1972).

The pathogenicity of *P. fluorescens* was tested against workers of *M. championi, H. indicola* and nymphs of *B. beesoni.* At 72 hours following infection, there was about 18%, 20% and 28% mortality in the test groups of *M. championi*, *H. indicola* and *B. beesoni*, respectively. Their 100% morality occurred at 288, 264 and 240 hours in the test groups of *M. championi, H. indicolla* and *B. beesoni*, respectively (Table I).

Table I: Mortality percentage of *M. championi, H. indicola, and B. beesoni* infected by *Pseudomonas fluorescens* along with LT_{50}/LT_{90} and slope of Regression lines

Hrs after infection	*M. championi*		*H. indicola*		*B. beesoni*	
	Gr.1	*Gr. 2*	*Gr.1*	*Gr.2*	Gr. 1	Gr. 2
	Control	*Test*	*Control*	*Test*	Control	Test
24	2.67	06.67	00.00	6.67	2.67	09.33
48	4.00	10.90	00.00	13.33	4.00	16.00
72	5.33	17.68	00.00	20.00	4.00	28.00
96	5.33	26.03	02.67	36.00	4.00	42.67
120	6.67	36.40	06.67	42.31	4.00	49.33
144	8.00	46.37	08.00	50.72	4.00	60.00
168	8.00	57.96	09.33	60.34	4.00	68.00
192	8.00	69.56	10.67	72.90	4.00	82.67
216	8.00	79.7	10.67	84.98	4.00	92.00
240	8.00	86.95	10.67	93.94	4.00	100.00
264	8.00	95.65	10.67	100.00		101
288	8.00	100.00		117		265
*LT_{50}		127		290		3.06±1.13
*LT_{90}		302		3.24±1.67		
Slopes (b)		3.39±2.13				

* The LT_{50} and LT_{90} were taken as whole number.

Long time mortality curves of the termites infected by *P. fluorescens* are plotted in fig. I. The value of regression slops is presented in the same figure. The data pertaining to LT_{50} and LT_{90} of each species of termites was calculated by Probit analysis. The value of slopes of regression line along with its fiducial limit is also given in Table I.

The data pertaining to LT_{50} and LT_{90} showed *P. fluorescens* caused 50% death of *M. championi, H. indicola and B. beesoni* at 127, 117 and 101 hours; and their 90% mortality occurred at 302 hours, 290 and 265, respectively (Table I). The value of regression slopes (b) of *M. championi, H. indicola* and *B. beesoni* was calculated as 3.4, 3.2 and 3.1, respectively. These values showed that *P. fluorescens* was fairly pathogenic to all the three mentioned species of termites. The value of slopes also indicated that some toxins might be involved for the increase in virulence of *P. fluorescens.*

In order to see the mode of action *P. fluorescens* in various species of termites, histopathological studies were carried out. *Pseudomonas fluorescens* attacked intestinal epithelium of *M. championi* at 72 hours following infection (Fig. 3a). In case of *H. indicola, P. fluorescens* disintegrated fat body tissues at 48 hours following infection (Fig-2a).

The villi of gizzard showed attack of *P. fluorescens* at 72 hours following infection (Fig. 3a). In case of *H. indicola, P. fluorescens* disintegrated fat body tissues at 48 hours following infection (Fig. 2a). The villi of gizzard showed attack of *P. fluorescens* at 72 hours following infection (Fig. 2b). A large number of *P. fluorescens* rods were seen mixed with food materials in the lumen of *H. Indicola* at 120 hours following the infection (Fig. 2c). In case of *B. beesonai, P. fluorescens* attacked on the villi and circular muscles of gizzard at 48 hours following infection. (Fig. 3b).

The fat bodies of *B. beesoni* were heavily attacked by *P. fluorescens* at 120 hours following infection (Fig. 3c). These observations suggested that *P. fluorescens* penetrated through the guts into the body cavity of termites.

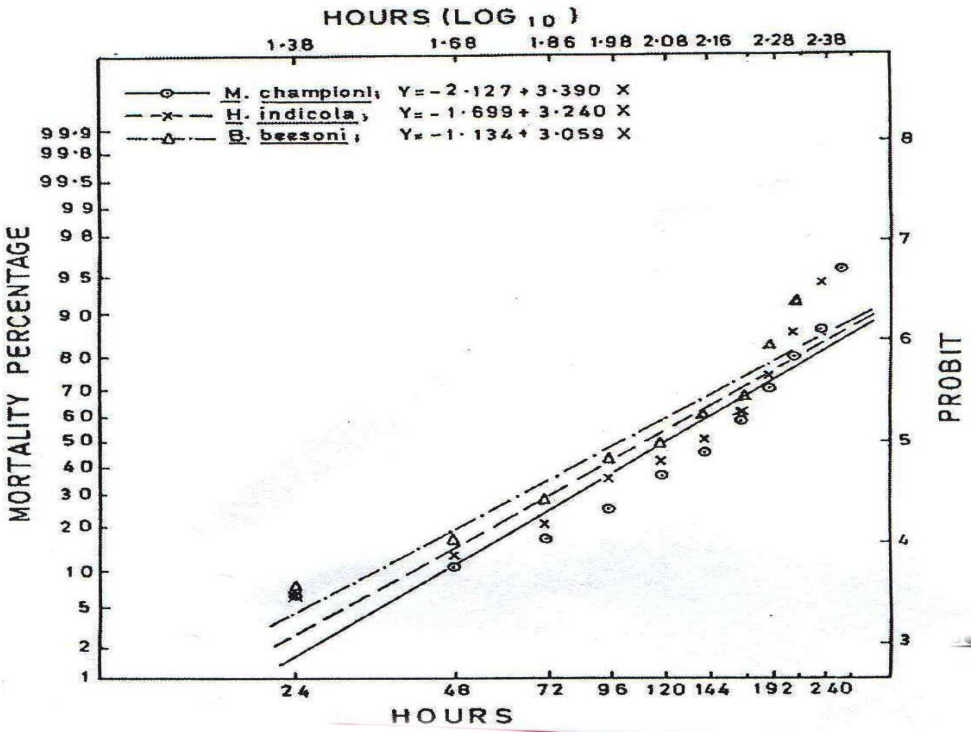

Figure 1: Regression lines of martially percentage of *M. championi* and *H. indicola,* of *B. beesoni* course by locally discovered *P. fluorescens.*

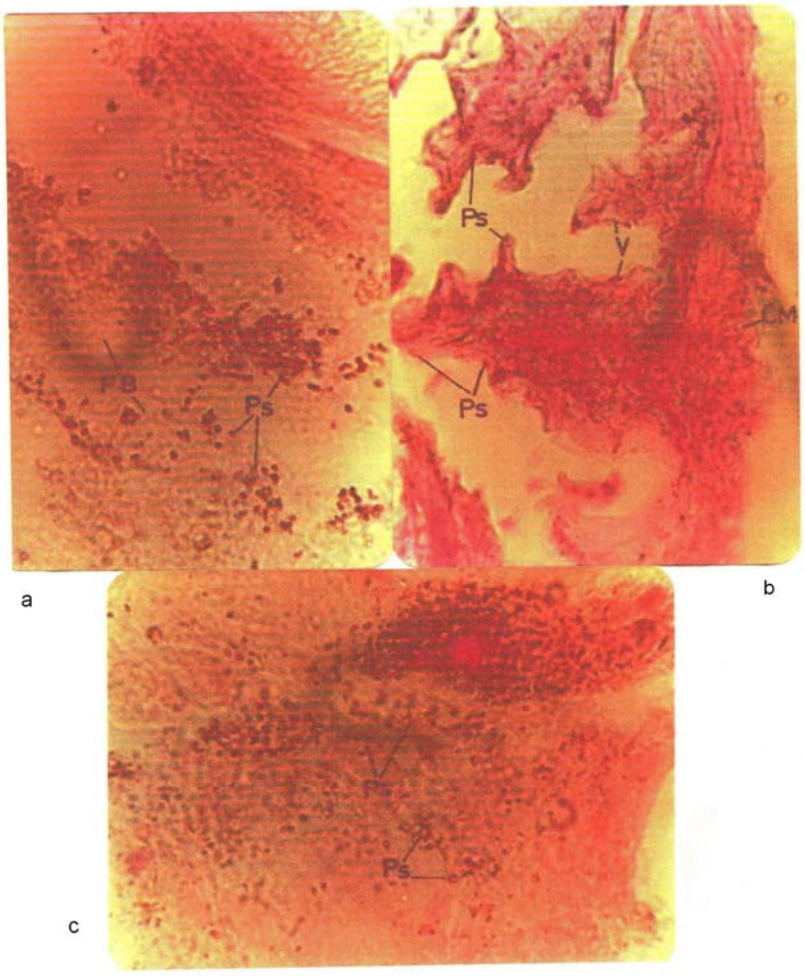

Figure 2: *Pseudomonas fluorescens*. a, The intestinal epithelium of 0*Microcerotermes championi* is attacked by *P. fluorescens*, × 268; b, The villi and circular muscles of gizzard of *Bifiditermes beesoni* are attacked by *P. fluorescens* × 268; c, The fat body tissues of *B. beesoni* are heavily attacked by *P. fluorescens* × 268. CM, circular muscles. EP, epithelial cells. FB, fat body tissues, Ps, *P. fluorescens*. V, villi.

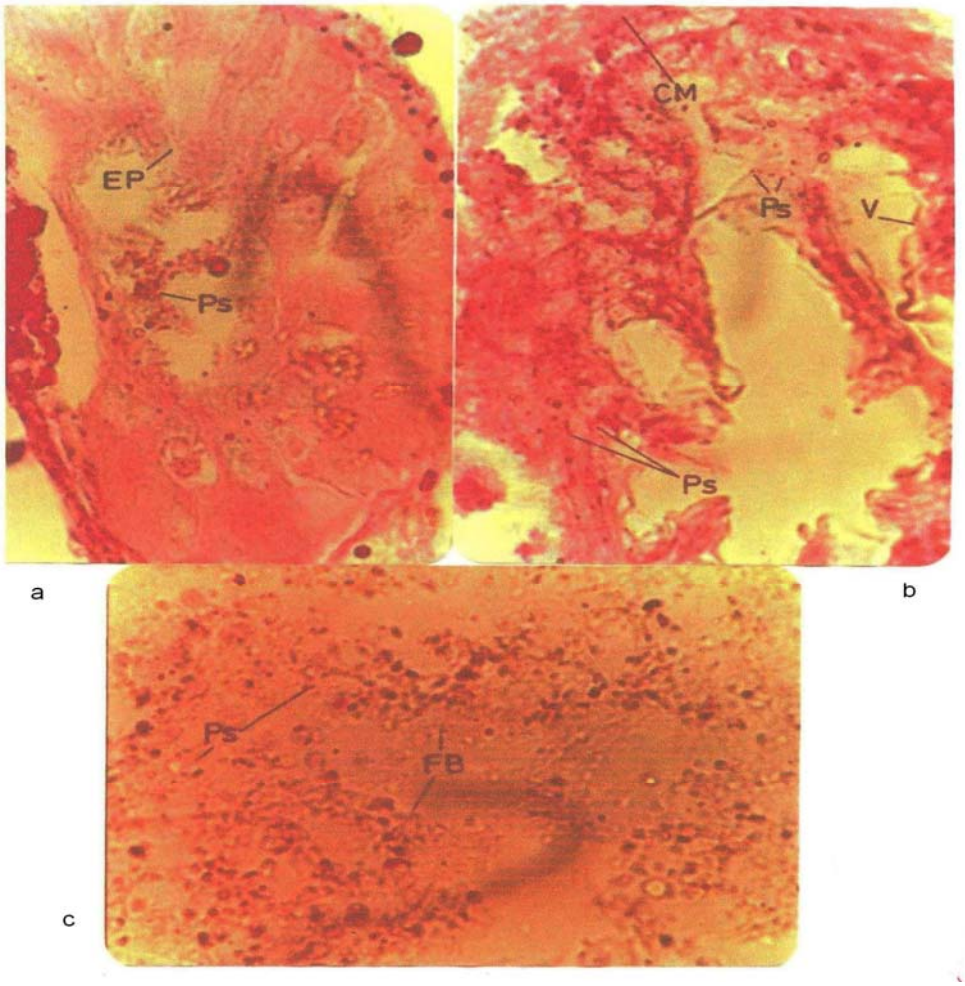

Figure 3: *Pseudomonas fluorescens*. a, Fat body tissues of *Heterotermes indicola* are disintegrated by *P. fluorescens* × 268; b, *P. fluorescens* attacking on the villi of gizzard × 268; c, A large number of *P. fluorescens* rods with food material in the lumen of *H. indicola* × 268. CM, circular muscles; FB, fat boy tissues Ps, *Pseudomonas fluorescens;* v, villi.

DISCUSSION

Pseudomonas fluoresces was isolated from various species of termites which were collected from divers habitats of Pakistan. It was found pathogenic to *M. championi, H. indicola and B. beesoni*. The data pertaining to LT_{50} and LT_{90} and slopes of regression lines showed that there was a marked difference in the susceptibility of each species of termites to *P. fluorescens* the workers of *M. championi* and nymphs of *B. beesoni* were more susceptible to infection as compared to the workers of *H. indicola.* Histopathological studies showed that tissues of alimentary tract and body cavity of *H. Indicola* were very susceptible to *P. fluorescens* infection. Angus (1965) reported that only a few species of the family Pseudomonadales were pathogenic to insects. *P. aeruginosa* was reported to be pathogenic for various species of insects (Lysenko, 1963). Asharfi *et al.,* (1965) reported that *P. aeruginosa* was pathogenic to desert locust, *Schistocerca gregaria.* It is to be noted that *P. aeruginosa* was highly pathogenic to various species of termites (Khan *et al.,* 1992). However, the present study provides the first record of attack of *P. fluorescens* on various species of termites occurring in Pakistan. Moreover, *P. fluorescence* is safer than *P. aeruginosa* so it can be recommended to be used in laboratory and field for the control of mostly all the species of termites.

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