

## A STUDY OF AEROBIC BACTERIA AND FUNGI ASSOCIATED WITH *BLATTELLA GERMANICA* (LINNAEUS), *TRIBOLIUM CONFUSUM* (JACQUELIN DU VAL), AND *POEKILO CERUS PICTUS* (FABRICIUS)

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**Abstract.** Thirteen species of bacteria were isolated from surface washings, the fat body, and the alimentary tract of *Blattella germanica* (Linnaeus), and *Poecilocus pictus* (Fabricius). Five species of bacteria were isolated from surface washings and macerated adults of *Tribolium confusum* (Jacquelin du Val). Three major human pathogens were found, *Corynebacterium diptheriae gravis* was associated with *B. germanica*; *Shigella dysenteriae* was associated with *B. germanica* and *T. confusum*; and finally *Salmonella para typhi A* was associated with *B. germanica* and *P. pictus*. Only one species of fungus, *Penicillium* sp., was isolated and this was found associated with all 3 insect species.

Studies on the normal flora of insects have been undertaken at these laboratories<sup>1</sup> as part of an insect pathology research programme. Research on the normal microflora is helpful in understanding the abnormal. The present investigation reports on the aerobic bacteria and fungi occurring in apparently healthy adults of the German cockroach, *Blattella germanica* (Linnaeus), the confused flour beetle, *Tribolium confusum* (Jacquelin du Val) and the common grasshopper, *Poecilocus pictus* (Fabricius).

### Materials and Methods

For *B. germanica* and *P. pictus* bacteria and fungi were isolated separately from the integument, the fat body, the fore-gut, the mid-gut, and the hind-gut. For *T. confusum* the microflora was isolated separately from the integument, and from the whole insect. As the beetles were very small a sample of 10 were used in each experiment.

Outer washings of *B. germanica* and *P. pictus* were prepared by immersing the insects in 10 ml of sterile distilled water for 2 min. The washings were equally divided into 4 sterile petri dishes, and 20 ml of nutrient agar were added to each dish. Similar plating was carried out with Sabouraud's dextrose-agar for isolation of the fungi. The outer microflora of *T. confusum* was isolated by immersing 10 adults in 10 ml of sterile distilled water. The rest of the procedure was the same as for *B. germanica* and *P. pictus*.

To study the internal microflora of *B. germanica* and *P. pictus*, the fat body and alimentary canal were dissected out. The fat body was macerated in 10 ml of sterile distilled water. The alimentary canal was surface sterilized in absolute alcohol, and divided into fore-gut, mid-gut and hind-gut. Each portion of the gut was macerated separately with 10 ml sterile distilled water, and poured into a sterile petri dish. Finally 20 ml of nutrient agar were added to each dish. The plates were incubated at 30°C. Bacteria from isolated colonies were transferred next day to nutrient agar slants for pure culture study. Similar

plates and slants using Sabouraud's dextrose agar were run for isolating fungi.

To study the internal microflora of *T. confusum*, 10 adults were surface sterilized with alcohol, and then macerated with 10 ml of sterile distilled water. The rest of the method was as used for *B. germanica* and *P. pictus*.

The species of bacteria were identified on the basis of their morphological, cultural, and biochemical characters.<sup>2</sup> The single species of fungus was identified on the basis of its morphology, mode of sporulation, and pigmentation.<sup>3</sup>

### Results

The result on bacterial and fungal isolations from various parts of *B. germanica* is given in Table 1. Only two bacteria, *Pseudomonas aeruginosa* and *Staphylococcus citreus* were found to occur consistently in surface washings; among different parts of the gut, the largest number of bacterial species were found in the fore-gut, these were *Ps. aeruginosa*, *Alcaligenes faecalis*, *Paracolobactrum arizonae* (Providencia group), *Salmonella para typhi A* and *Staphylococcus albus*. The bacterial microflora of mid-gut and hind-gut was restricted only to three bacteria, *Staph. citreus* and *Staph. aureus* from mid-gut and *Ps. aeruginosa* from mid-gut. Again, the bacterial isolations from fat body showed six different bacteria, *Al. faecalis*, *Staph. albus*, *Corynebacterium diptheriae gravis*, *Sarcina lutea*, *Shigella dysenteriae* and *Paracolobactrum coliforme* (Hafnia group).

In surface washings of flour beetle, only one bacterium *Al. faecalis* was found to occur while isolations from surface sterilized whole insect gave four different bacteria, *Klebsiella pneumoniae*, *Staph. dysenteriae*, *Staph. aureus* and *Staph. albus* (Table 2).

The microflora isolated from *P. pictus* consisted of *Staph. aureus* and *S. para typhi A* which were isolated from fat body. No bacteria were isolated from surface washings or any part of the gut in several experiments conducted with this insect (Table 3).



TABLE 1. THE MICROFLORA OF *Blattella germanica*.

Species	Source of isolate				
	Integument	Fore-gut	Mid-gut	Hind-gut	Fat-body
<b>Bacteria</b>					
<i>Pseudomonas aeruginosa</i>	+	+	—	+	—
<i>Alcaligenes faecalis</i>	—	+	—	—	+
<i>Paracolobactrum arizonae</i> (Providence group)	—	+	—	—	—
<i>Salmonella para typhi A.</i>	—	+	—	—	—
<i>Staphylococcus albus</i>	—	+	—	—	+
<i>Staphylococcus citreus</i>	+	—	+	—	—
<i>Staphylococcus aureus</i>	—	—	+	—	—
<i>Corynebacterium diphtheriae gravis</i>	—	—	—	—	+
<i>Sarcina lutea</i>	—	—	—	—	+
<i>Shigella dysenteriae</i>	—	—	—	—	+
<i>Paracolobactrum coliforme</i> (Hafnia group)	—	—	—	—	+
<b>Fungi</b>					
<i>Penicillium sp.</i>	+	+	+	+	+

Key: + present; —absent.

TABLE 2. THE MICROFLORA OF *Tribolium confusum*.

Species	Source of isolate	
	Integument	Whole insect
<b>Bacteria</b>		
<i>Alcaligenes faecalis</i>	+	—
<i>Klebsiella pneumoniae</i>	—	+
<i>Shigella dysenteriae</i>	—	+
<i>Staphylococcus aureus</i>	—	+
<i>Staphylococcus albus</i>	—	+
<b>Fungi</b>		
<i>Penicillium sp.</i>	+	+

Key: + present; —absent.

From all parts of all the three insects one fungal species, *Penicillium sp.* was isolated. This species of *Penicillium* produced bluish-green velvety growth with the reverse side, a pale yellow. The conidiphores were about 300  $\mu$  long by 2.5  $\mu$  in dia. The conidia were elliptical which became globose in old cultures. These measured about 3–4  $\mu$  long, and were palegreen. The characters of this species are similar to *P. chrysogenum*, described already.

### Discussion

The aerobic bacterial flora of *B. germanica* was the most diversified of all the three species studied for this purpose. None of the bacteria found associated with male adults of *B. germanica* as reported by Steinhaus,<sup>4</sup> were found in our investigations. It is, however, interesting to note that *Ps. aeruginosa*, a potential pathogen of grasshoppers occurs as part of normal microflora of integument, fore-gut and hind-gut of *B. germanica*.

TABLE 3. THE MICROFLORA OF *Poeciloceris pictus*.

Species	Source of isolate				
	Integument	Fore-gut	Mid-gut	Hind-gut	Fat-body
<b>Bacteria</b>					
<i>Staphylococcus aureus</i>	—	—	—	—	+
<i>Salmonella para typhi A.</i>	—	—	—	—	+
<b>Fungi</b>					
<i>Penicillium sp.</i>	+	+	+	+	+

The fore-gut of *B. germanica* harbours five different types of bacteria but these are diminished to two in the mid-gut and to only one in the hind-gut which suggests strong enzymatic activity in latter parts of the gut. Steinhaus<sup>5</sup> has reported *Corynebacterium lipoptenae* from house fly, *Lipoptena depressa* (Say); in our investigations *C. diphtheriae gravis* occurred as part of normal flora of *B. germanica*. *Sh. dysenteriae* and *S. para typhi A* were also found associated with *B. germanica*. *C. diphtheriae gravis*, *Sh. dysenteriae* and *S. para typhi A* are major human pathogens; their occurrence as normal flora of *B. germanica* is of particular significance in public health and epidemiological studies. *Sh. dysenteriae* and *S. para typhi A* have also been found associated with *T. confusum* and *P. pictus* respectively.

Repeated experiments to isolate fungi associated with the three insect species gave only a culture of one species of *Penicillium* which probably occurred in the environments of these insects.

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