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## ISOLATION OF BACTERIA FROM *CALLOSBRUCHUS MACULATUS*, COLEOPTERAN PEST OF STORED PRODUCTS

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### ABSTRACT

The bacteria isolates from *Callosobruchus maculatus*, a Coleopteran pest of stored Cowpea seeds were studied by standard microbiological procedures. All the isolated bacteria were non spore forming cocci. The bacteria associated with *C. maculatus* were *Staphylococcus* sp., *Micrococcus* sp., *Peptostreptococcus* sp., *Ruminococcus* sp., *Veilonella* sp. and *Megasphaera* sp.. The presence of anaerobic forms like *Peptostreptococcus* sp. and *Ruminococcus* sp. in the pulse beetle were also recorded which may be due to the fact that the pest lives inside the grains.

**Key words :** *Callosobruchus maculatus*, Bacteria, Stored products.

### INTRODUCTION

The cowpea weevil *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is a serious pest of peas, beans and pulses (Pingale 1976). A mutual relationship exists between insects and microorganisms which occur either extracellularly in the gut lumen or intracellularly in special cells called mycetocytes found in the haemolymph or in the fat body (Wigglesworth 1993; Chapman 1983). The function attributed to the microbes include assistance in normal digestion, assistance in digestion of specialized food like cellulose and supply essential nutrients like aminoacids, vitamins etc. Microbes associated with insects of specialized feeding habits (blood, silk, wood etc.) are dealt within a special section of Bergey's manual Vol. I which includes some stored product pests also (Krieg & John Holt 1984). The aim of present study was to isolate normal microbial flora of stored product pest, *Callosobruchus maculatus* pest of cowpea seeds.

### MATERIALS AND METHODS

Adults of *Callosobruchus maculatus* were collected from stored cowpea seeds. The collected insects were directly homogenized in sterile distilled water. The homogenate was serially diluted and pour plated in the nutrient agar medium. From these primary culture, the isolated bacterial colonies were

sub cultured till pure cultures were obtained. Pure cultures were stored on nutrient agar slants as a stock. Gross morphological observations of cell such as margin, shape, elevation on solid agar media and growth characteristics in broth were observed and recorded (Michael *et al* 1998). The isolated bacterial colonies were subjected to staining process both Gram positive and negative (Aneja 1993) and spore forming (Cappuccino & Sherman 1992). There were also subjected to various biochemical tests and identified the bacteria to the generic level.

## RESULTS AND DISCUSSION

Six morphologically distinct bacterial colonies were obtained from *Callosobruchus maculatus* (Table 1). Cultural characteristics of isolated bacterial colonies on nutrient broth (Table 2) indicated that colonies were aerobic (A & D), facultative anaerobic (D) and anaerobic forms (B, C, E & F). Both Gram negative and Gram positive bacteria were found in *C. maculatus*. However, all these were non-spore formers (Table 3). The results of biochemical tests (Table 4) revealed that the six bacterial genera namely Staphylococcus sp.(A), *Veilonella* sp.(B), *Megasphaera* sp.(C), *Micrococcus* sp.(D), *Peptostreptococcus* sp.(E) and *Ruminococcus* sp.(F).

Table 1 Cultural characteristics of isolated bacterial colonies on nutrient agar plates from the sample of *C. maculatus*

Colonies Isolated	Shape	Margin	Elevation
A	Round	Entire	Convex
B	Round	Entire	Convex
C	Round	Entire	Convex
D	Punctiform	Entire	Convex
E	Punctiform	Entire	Convex
F	Irregular	Undulate	Umbonate

Table 2 Cultural characteristics of isolated bacterial colonies on nutrient broth from the sample of *C. maculatus*

Colonies Isolated	Aerobic	Facultative Anaerobic	Anaerobic
A	+	-	-
B	-	-	+
C	-	-	+
D	+	+	-
E	-	-	+
F	-	-	+

Table 3 Grams' and spore staining of isolated bacteria from the sample of *C. maculatus*

Colonies Isolated	Grams' stain	Shape	Spore stain
A	+	Cocci	-
B		Cocci	-
C		Cocci	-
D	+	Cocci	-
E	+	Cocci	-
F	+	Cocci	-

Table 4 The Biochemical tests of isolated bacteria

S. No.	Name of biochemical test	Name of the isolated bacteria					
		A	B	C	D	E	F
1	Catalase test	+	+	-	+	+	+
2	Glucose fermentation	-	-	-	-	+	+
3	Utilization of Dextrose	+	+	+	+	+	+
4	Hydrolysis of starch	-	+	+	+	-	-
5	Hydrolysis of casein	-	-	-	+	+	+
6	Citrate utilization	+	+	+	+	+	-
7	Urease test	-	-	+	+	+	+
8	Methyl red test	-	-	-	-	-	-
9	Voges-proskauer test	-	-	-	-	-	-
10	Acid from mannitol	+	-	-	+	+	+
11	Endo agar test	-	-	-	-	-	-

Analysis of the bacterial flora of a coleopteran pest of stored products revealed that there exists a diversity in the insect-bacteria association in the insects. This diversity could be related to the nature of the food supplied and is probably to the broad environmental condition. *Veilonella* sp. and *Megasphaera* sp. had been reported from gut of rodents. As rodents are common in storage godowns, the bacteria might had been released through their faeces into the stored products, and from there entered into insects feeding on the same diet (Cruickshank 1965).

*Micrococcus* sp., *Peptostreptococcus* sp. and *Ruminococcus* had been recorded from the rumen chamber of ruminants (Krieg & John Holt 1984) which too freed on the entire seeds. Thus, Cowpea weevil, *Callosobruchus maculatus* associated several bacterial species in the present study, which can contaminate, damage and spoil stored peas and pulses and also can cause health problems in consumers (Burros *et al* 1964). The bacterial species present in the insect gut or any other part plays an important role in the feeding habits of host and they also supply certain essential nutrients, aminoacids etc; to the host by its symbiotic relationship (Cruickshank *et al* 1975).

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