

Bacillus spp. isolated from the Mid-gut of *Anopheles arabiensis* Larvae

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ملخص البحث

أخضعت 70 عينة من محتويات المعى الوسطي ليرقات بعوض الأنوفيليس للإختبارات البكتيرية. وجد أن جنس العصوية (*Bacillus*) سائداً علي أجناس المعزولات البكتيرية الأخرى حيث سجل نسبة 81% من المجموع الكلي للمعزول من البكتيريا. وأنواع العصوية التي تم عزلها هي: عصوية خلية النحل (*B. alvei*) والعصوية القصيرة (*B. brevis*) والعصوية الشمعية (*B. cereus*) والعصوية المخثرة (*B. coagulans*) والعصوية الصلبة (*B. firmus*) وعصوية الأبواغ الجانبية (*B. laterosporus*) والعصوية البطيئة (*B. lentus*) والعصوية الحزازية (*B. licheniformis*) والعصوية الفطرية (*B. mycoides*) والعصوية البانتوثنكية (*B. pantothenicus*) والعصوية الكروية (*B. sphaericus*) والعصوية ألفة الدهن والحرارة المجموعة الأولى (*B. stearothermophilus* group I) والعصوية ألفة الدهن والحرارة المجموعة الثانية (*B. thuringiensis*) و *stearothermophilus* group II

Summary

Seventy mid-gut contents of *Anopheles arabiensis* larvae were examined bacteriologically. *Bacillus* species were the most prevalent bacteria and constituted 81% of the total number of bacteria isolated. They were *B. alvei*, *B. brevis*, *B. cereus*, *B. coagulans*, *B. firmus*, *B. laterosporus*, *B. lentus*, *B. licheniformis*, *B. mycoides*, *B. pantothenicus*, *B. sphaericus*, *B. stearothermophilus* group I, *B. stearothermophilus* group II, *B. thuringiensis*.

Introduction

Micro-organisms that infect mosquitoes and other insects are known as entomopathogens (Seigl and Novak, 1996). Many species of bacteria were isolated from a number of mosquitoes species (Chao and Wistreich, 1959; Fulton *et al.*, 1974), but a few of them are considered to be true pathogens that cause host death. Ninety-one pathogenic bacterial strains were isolated from infected mosquito larvae; of these, 40 were identified as *B. alvei* and *B. circulans* and 51 belonged to *B. sphaericus* group (Singer, 1973). In 1990, Soad isolated 34 species of bacteria from different stages of *Anopheles* spp. She found that only four *Bacillus* species were toxic to mosquito larvae and these were *B. brevis*, *B. alvei*, *B. thuringiensis* and *B. polymyxa*.

*Comments on the Arabic translation of *Bacillus* species epithets are welcomed and should be addressed to the Secretary / Sudan Journal of Veterinary Research

The objective of this study was to isolate and identify various members of the genus *Bacillus* which were present in the mid-gut of *Anopheles arabiensis*, larvae.

Materials and Methods

Collection of samples:

Larvae of *A. arabiensis* were collected from open drains and septic tanks in the cities of Khartoum, Khartoum North and Omdurman as well as vegetable farms neighbouring the River Nile. Larval collection was done by dippers and plastic dishes, and the collected larvae were transferred to the laboratory in jars containing water taken from the site of collection.

Isolation of bacteria from the mosquito larvae:

Second, third and fourth instar larvae were used. Larvae were removed from water and washed several times with tap water, sterile distilled water and sterile saline before finally transferred to a sterile slide for dissection under a dissecting microscope. The head was decapitated from the whole body using sterile blades and forceps, then the intestine of the larva was teased out. The whole contents of the intestine were inoculated onto Blood and MacConkey Agars (Oxoid). The inoculated plates were incubated at 37°C for 24hrs.

A total number of 70 intestinal contents of *A. arabiensis* larvae was collected and subjected to bacteriological examinations. Isolated bacteria were identified according to Barrow and Feltham (1993).

Results

Both Gram-positive and Gram-negative bacteria were isolated. Fifty-seven samples (81%) produced bacterial growth for *Bacillus* species. More than one species of *Bacillus* was isolated from one sample. Fourteen bacillus species were identified. They were; *B. alvei*, *B. brevis*, *B. cereus*, *B. coagulans*, *B. firmus*, *B. laterosporus*, *B. lentus*, *B. licheniformis*, *B. mycoides*, *B. pantothenicus*, *B. sphaericus*, *B. stearothermophilus* group I, *B. stearothermophilus* group II and *B. thuringiensis* (Table 1). Other isolates were *Staphylococcus* spp. *Escherichia coli*, *Klebsiella* spp. and *Proteus* spp. Colonies of *Bacillus* species were very large, rough, flat, had curled edges and produced complete haemolysis on Blood Agar. Microscopic examination of *Bacillus* isolates revealed Gram-positive short or long rods; usually occurring singly or in diplo-bacillary form. A pellicle was formed on the surface of Peptone Water (Oxoid) and Nutrient and Nitrate Broths (Oxoid) by all *Bacillus* isolates following an overnight incubation at 37°C. Some biochemical properties of *Bacillus* spp. isolated from the mid-gut of *Anopheles* mosquito larvae are shown in Table 1. All *Bacillus* isolates were motile except *B. mycoides*, catalase and oxidase positive, failed to grow in

Table 1: Some biochemical characters of *Bacillus* species isolated from the mid-gut of *A. arabiensis* Larvae

| Test | <i>Bacillus</i> species | | | | | | | | | | | | | |
|---------------------|-------------------------|------------------------|---------------------------------|------------------|--------------------|------------------|-------------------------|-------------------------|----------------------|-----------------|--------------------------------|-------------------------|---------------------|------------------|
| | <i>B. brevis</i> | <i>B. laterosporus</i> | <i>B. stearothermophilus</i> II | <i>B. cereus</i> | <i>B. mycoides</i> | <i>B. firmus</i> | <i>B. pantothenicus</i> | <i>B. licheniformis</i> | <i>B. sphaericus</i> | <i>B. alvei</i> | <i>B. stearothermophilus</i> I | <i>B. thuringiensis</i> | <i>B. coagulans</i> | <i>B. lentus</i> |
| Glucose | - | + | + | + | + | + | + | + | d | + | + | + | + | + |
| Galactose | - | - | + | - | - | - | - | + | - | + | - | -* | d | d |
| Mannose | - | - | + | d | - | - | d | + | d | d | + | -* | + | + |
| Raffinose | - | - | + | - | - | - | - | - | - | + | - | - | + | - |
| Salicin | - | + | + | + | - | - | d | d | - | d | + | d | d | d |
| Xylose | - | + | - | - | - | - | - | - | d | -* | - | - | * | - |
| VP | + | + | - | + | - | - | -* | + | - | d | -* | -* | - | d |
| Citrate utilization | - | - | - | - | - | - | - | d | d | -* | - | + | d | d |
| Nitrate reduction | + | + | + | + | + | + | + | d | d | + | + | + | + | + |
| Starch hydrolysis | + | + | + | + | + | + | + | d | - | + | + | d | d | d |
| Gelatin hydrolysis | - | - | + | - | + | + | d | d | - | + | d | d | d | d |
| No. isolates tested | 1 | 1 | 1 | 2 | 2 | 2 | 4 | 2 | 3 | 4 | 6 | 7 | 10 | 12 |

+ = All isolates tested positive; - = all negative; d = different reactions by different isolates; +* = only one negative isolate; -* = only one positive isolate; VP= Voges Proskauer

10% NaCl, formed acid from glucose, except *B. brevis* and *B. sphaericus* and hydrolyzed starch except *B. sphaericus*. The ability to ferment other sugars varied from one species to another. Moreover, variation of test reaction within the isolates of the same species did occur (Table 1).

Discussion

Anopheles mosquito larvae harbour a complex mixture of organisms or normal flora in their mid-gut, but a few of these organisms seem to be pathogens which cause death of these larvae. All isolates of *Bacillus* species

isolated had more or less typical cultural characteristics and biochemical properties (Claus and Berkeley, 1986; Barrow and Feltham, 1993).

Although *B. thuringiensis*, *B. larvae*, *B. lentimorbus*, *B. poplliae* and *B. sphaericus* are the only species, regarded as insect pathogens (Claus and Berkeley, 1986), several other *Bacillus* species are included in this study. These are *B. mycoides*, *B. brevis*, *B. alvei*, *B. lentus*, *B. cereus*, *B. coagulans*, *B. firmus*, *B. staerothermophilus* group 1, *B. laterosporus*, *B. pantothenicus*, *B. staerothermophilus* group II and *B. licheniformis*; whether they are pathogenic or not awaits further investigation. Our results are similar to those reported by Soad (1990) who isolated 15 *Bacillus* species from different stages of *Anopheles* and *Culex* mosquitoes larvae. But her list did not include *B. mycoides*, *B. laterosporus* and *B. pantothenicus* which were isolated in the present study. On the other hand, *B. subtilis*, *B. megaterium*, *B. polymyxa* and *B. lumnus* which have previously been isolated (Soad 1990) were not encountered in the present study. Previous isolation of *B. alvei*, *B. brevis* and *B. circulans* from the mid-gut of *Anopheles* mosquito larvae was also reported (Singer, 1973).

It may be concluded, however, that many *Bacillus* species are present in the mid-gut of *A. arabiensis* larvae as normal microflora or pathogens.

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