Isolation of *Bacillus subtilis/amyloliquefaciens and Bacillus sphaericus from fruit fly larvae in the Sudan*

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Introduction

In Sudan, nearly 40 fruit fly species were recorded; the most serious ones are those attacking mango *Mangifera indica*, guava *Psidium guajava* and *Citrus spp*. In recent years, fruit production has been seriously hampered, mainly, because of the sudden and persistent outbreaks of some fruit fly species. Charles *et al*. (1996) mentioned that, the use of microorganisms as a source of biological compounds for insect pest control started after the discovery of the highly insecticidal bacteria *Bacillus thuringiensis. B. sphuericus is an aerobic bacterium that produces terminal spherical* spores. It lacks several biochemical pathways and thus cannot use sugars as metabolites. The findings of various genetic and biochemical studies indicate that this species is heterogenous. The increasing number of isolated strains (more than 150 strains) has made differentiating between toxic and atoxic strains, and between the toxic strains themselves, more difficult.

This study was conducted to investigate and identify the reason for the death of wild fruit fly larvae reared out from guava fruit collected from Khartoum, Sudan, in the laboratory.

Plate 1. Infested fruit fly larvae .

Materials and methods

Identification of the two presumptive isolates were conducted in Sudan and Poland. Dead larvae (Plate 1) were inoculated in Nutrient Agar (HIMEDIA, India) at 37C˚ for 24 hours. After incubation period, growth of the bacterial colonies and a fungal colony were observed on the surface of the plate. The fungus was identified as *Aspergillus flavus* depending on morphological characters. The bacteria were sub cultured in different culture media, such as Nutrient Agar, MacConkey Agar and Blood Agar to investigate the morphological characteristics of colonies.

According to the methods of Martin and Travers (1989), Health Protection Agency (2007) and Elyass *et al*. (2009), various available procedures were conducted to identify the isolated bacteria as (i) Smears of isolate were examined for their Gram reaction under a light microscope (ii) smears were prepared from an isolate and stained with modified Ziehl-Neelsen (M-ZN) stain which is a special stain to show the spore and (iii) ten biochemical tests were conducted on the isolate; namely, catalase production, esculin hydrolysis, citrate utilization test, anaerobic growth, production of indole, kliger iron agar (KIA test), lecithinase production, urease test, starch hydrolysis test, oxidase test and growth at 5 C˚, 37 C˚ and 40 C˚. The preliminary identification in Sudan expected the presumptive isolates is *Bacillus thuringiensis.*

More investigations were conducted in Poland using the (API 50 CHB and API 20 E) strips and contrast phase microscope.

Results

❖ The isolates bacteria from fruit fly larvae were identified as *B. subtilis/ amyloliquefaciens* and *B. sphaericus* depending on morphological characteristics, gram and spore stains, biochemical tests (table 1) and (API50CHB and API20E) strips (appendix 1).

❖ The morphological characteristics of a single colony of *B. subtilis/ amyloliquefaciens* and *B. sphaericus* were described in different cultures as:

❖ Description of *B. subtilis/ amyloliquefaciens* (i) on Nutrient agar medium which produce white to cream-coloured, flat and mucoid colonies; with a ground-glass appearance (Plate 2 left), (ii) on Blood agar media which produce large, irregular, dry colonies, opaque in colour, β haemolytic after 48 hours (Plate 4 left), (iii) on MacConkey agar media which produce large, dry, irregular, translucent and non lactose fermenting colonies (NLF). A single colony on MacConky agar culture appeared in different forms from first to third day. In the first day the colony was convex, wet and translucent; but at third day it was flat, dry and grayish to green colour. The isolate was Gram positive with sub-terminal spore in which the spore appeared colourless but in a spore stain (M-ZN) it has red colour. The isolate was capable of growth at 37C˚ and 40 C˚, but no growth at 5 C˚ until 72 hours after it was cultured.

❖ Description of *B. sphaericus::* (i) on Nutrient agar medium which produce white to cream-coloured, irregular and moist colonies; with a ground-glass appearance and 3 – 4mm in size (Plate 2 right), (ii) on Blood agar media which produce grey round colonies and non haemolytic after 48 hours (Plate 4 right), (iii) on MacConkey agar media which produce large, dry, irregular, translucent and non lactose fermenting colonies (NLF) with 1 – 2 mm in size. The isolate was Gram positive with terminal spore with separated bacilli (Plate 5) in which the spore appeared colourless but in the spore stain (M-ZN) appeared to have red colour. Also, the isolate was capable of growth at 37C˚ and 40 C˚, but no growth at 5 C˚ until 72 hours after it was cultured.

Plate 2. Growth isolates on a nutrient agar culture, Left: *B. subtilis/ amyloliquefaciens* , right: *B. sphaericus*

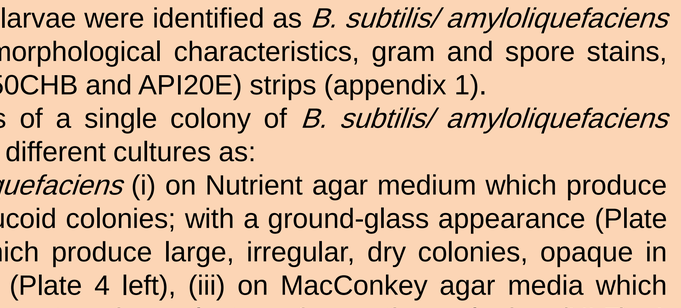


Plate 3. Egg yolk precipitation zone :Left: *B.subtilis/amyloliquefaciens* , right: *B. sphaericus*.

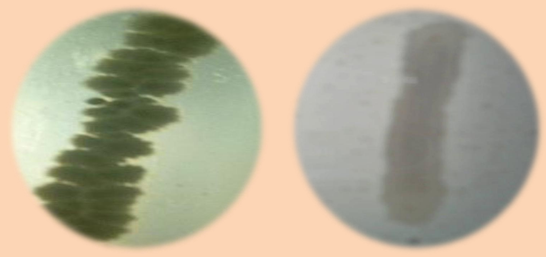


Plate 4. Haemolysis in blood agar.: Left: *B. subtilis/amyloliquefaciens*, right: *B. sphaericus*

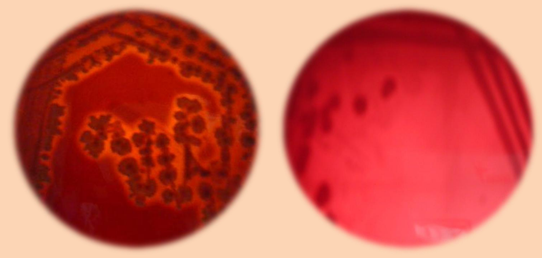
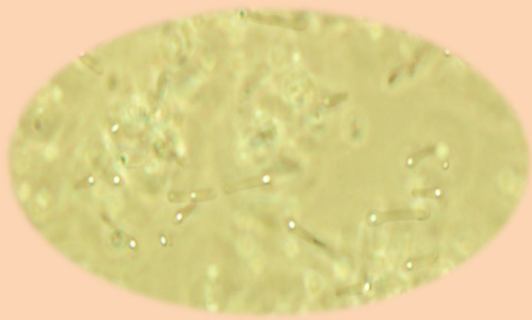


Table. 1. Identification of bacteria isolates.

Biochemical tests	B. subtilis/amyleliquefaciens	B. sphaericus
Catalase production	+	+
Esculin hydrolysis	+	-
Starch hydrolysis	+	
Anaerobic growth	+	-
Lecithinase production *	+	+
Urease test	+	+ (strong)
Citrate utilization	-	-
Indol production	-	-
Oxidase	-	
Kliger iron agar	-	-

*** Plate 3.**

Plate 5. *B. sphaericus* spores under contrast phase microscope.



Conclusions

❖ The isolations of these bacterial species from fruit fly larvae are consider a first detection in Sudan. * Plate 3.

❖ Further investigations on the isolate of *B. sphaericus* are going in Poland .and California to determine the strain and the toxic effects on insects.

Literature cited

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عزل نوعين من البكتيريا من يرقات ذبابة الفاكهة في السودان

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