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Full Length Research Paper

Bacteria and fungi isolated from housefly (*Musca domestica* L.) larvae

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Housefly larvae were cultured on fresh fish and collected for the isolation and identification of microorganisms associated with them. The microbes were cultured from both the gut and body surface of the maggot on nutrient agar (for bacteria) and potato dextrose agar (for fungi) and incubated at about 37°C for 48 h before observations. A variety of microorganisms, which includes the pathogenic *Staphylococcus aureus*, *Pseudomonas aerruginosa*, *Aspergillus tamarii* and *Bacullus cereus* were found. Also, nonpathogenic microbes werer recovered including *Bacillus subtilis* and *Stroplococcus faecalis*.

Key words: Microorganism, isolation, housefly larvae.

INTRODUCTION

The housefly (*Musca domestica L.*) is known to be a vector of diseases. These flies are prevalent in items that are exposed. Contamination of drinking water, food and other dairy products with faecal remains are common features in these areas. Hence the likelihood of human excrement being transmitted by flies is great (Gangarosa and Bcisel, 1960). Housefly are the most important insect pest associated with poultry, where the accumulated organic waste and favorable environmental conditions often promote rapid development of large populations (Axtell, 1970; Howard and Wall, 1996). Large populations of *M. domestica* may reduce yields and contribute to substantial public health problems when they enter nearby human habitations (Axtell 1970; Axtell and Arends, 1990; Howard and Wall, 1996). The housefly is a carrier of germs which transmit diseases that cause havoc to man. Such disease include typhoid fever (caused by *Salmonella typhi)* (Hornick et al, 1970), cholera (caused by *Vibrio cholera)* (Gangarosa and Beisel, 1960), staphylococcal food poisoning (caused by *Staphylococcus aureus*) (Sack et al., 1971) and Shigellosis (caused by *Shigella* sp.) (Conner, 1966).

The housefly larva (maggot) and a related poultry insect pest *Alphitobius diaperinus* has been demonstrated to harbour poultry pathogens such as bacteria (Harien et al., 1970; De Las Casas et al., 1972; McAllister et al., 1994; Banjo et al., 2004), fungi (Euginio et al., 1970; De Las Casaa et al., 1972; Banjo et al., 2004), protozoa (Reyna et al., 1983) and helminthes (Elowni and Elbihari 1979). Broiler chicks and turkey poultry actively feed on these maggots and beetles at an early age and this sometimes causes nutritional problems and offer sample opportunity for pathogen transmission (Despins and Axtell, 1994, 1995). The objective of the study is to isolate and identify the microorganisms present in the housefly maggot.

MATERIALS AND METHODS

In order to culture housefly larva, fresh fishes were exposed for the infestation of houseflies so that they can feed and lay eggs on them. The fishes were placed in a shade so that direct rays from sunlight would not fall on them. This could inhibit the growth of the maggots. On the third day, a large number of developed maggots were found thriving on the fresh fishes. In order to obtain more larvae for subsequent isolation of microorganisms, more fishes were added on daily basis. The maggots were collected with sterile forceps and were placed or stored in sterile containers. The aseptic

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Table 1. Characterisation of internal and external micro-organisms isolated from the maggot on nutrient agar.

Sample No	Gram stain	Shape	Motality	Catalase	Oxidase	Campulase	Urease	Methyl Red	Voges Proskaeur	Starch hydrolysis	Oxygen relationship	Indole	Glucose	Lactose	Raffinose	Sucrose	Maltose	Xylose	Probable Identification
Internal (Gut)																			
From selenite → Nutrient agar	+	S	-	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Streptococcus aureus*
From selenite → Nutrient agar	+	S	-	+	-	-	-	-	-	+	a	-	A	AO	A	A	A	A	*Streptococcus pyogenes*
From selenite → Nutrient agar	+	R	+	-	-	-	-	-	-	+	A	-	A	AO	A	A	A	A	*Bacilius cereus*
Internal (Gut)																			
From Tryptone → Nutrient agar	-	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Pseudomonas aeruginoso*
From Tryptone → Nutrient agar	+	S	-	+	-	-	-	-	-	+	A	-	A	AO	A	A	A	A	*Streptococcus pyogenes*
From Tryptone → Nutrient agar	+	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Bacillius cereus*
From Tryptone → Nutrient agar	+	R	+	+	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Bacillius cereus*
External (Body Surface)																			
From selenite → Nutrient agar	+	S	-	-	-	-	-	-	-	+	A	-	A	A	A	A	A	A	*Streptococcus faecalis*
From selenite → Nutrient agar	+	R	+	+	-	-	-	-	-	+	a	-	A	A	A	A	A	A	*Bacillus cereus*
External (Body Surface)																			
From Tryptone → Nutrient agar	+	S	-	-	-	-	-	-	-	+	a	A	A	A	A	A	A		*Streptococcus faecalis*
From Tryptone → Nutrient agar	+	R	+	-	-	-	-	-	-	+	a	A	A	A	A	A	A		*Bacillus cereus*

Key
+ = Positive
- = Negative
S = Sphere
R = Rod
A = Acid
G = Gas
A = aerobic

Table 2. Fungal identification isolated from the maggots on potato dextrose agar.

Sample	Fungi isolated	Description of the isolates
External (body surface). From tryptone to potato dextrose agar	*Alteruaria* sp. *Fusarium oxysporum* *Cladosporium* sp.	***Alternaria* sp:** Black to grey black with moderately abundant and dense aerial mycelium. The genus is distinguished by chains of dark, tad pole-shaped spaces with walls in two directions. ***Fusarium oxysporum*:** The texture is floccose and whitish-stream in colour, Chlarmydospores are abundant and usually single on hyphae. The reverse is pale to bluish-violet in color. ***Cladosporium* sp:** The growth is very slow and remain relatively small, seldom exceeding a diameter of 1-2cm. There is little or no aerial mycelium the surface of the colonies is olive green to olive brown and powdery with spores
Internal (gut). From tryptone to potato dextrose agar	*Fausarium oxysporum* *Aspergillus tamari* *Penicillum axalicum*	***Aspergillus tamari*:** The colony is rusty brown when viewed and creamish brown at reverse. The stipe is long and rough. The head is partly globular. The cenidia is thick and strongly roughened orange-yellow. ***Penicillum axalicum*:** The texture is volutimous, Sponilation very heavy. The obverse is grayish green while the reverse is pale yellow. The stipes is long and smooth. The penicillin is systematically biverticilae, Metulse closely appressed, phialides acerose, cellula very short. The conidia is ellipsoidal, large, smooth, pale green.

procedures were carried out so as to minimize contamination from bacteria not associated with the collected maggots.

Culture media

The following media were used in culturing the micro-organisms; nutrient agar, potato dextrose agar, selenite broth, tryptone broth, and blood agar. To prepare the blood agar, 10 g of agar powder was dissolved in 1 L of distilled water and boiled. It was sterilized at a temperature of 121°C for 15 min. 7% of sterile defibricated blood was prepared by the addition of 1.5 ml of human blood to 10 ml agar was added and allowed to cool at 45°C.

Isolation of external microbes

Two maggots were allowed to move freely on the solidified agar media for 5 min so that they can deposit the microbes on them on the agar media.

Two maggots were placed in a beaker containing 10 ml sterile water and thoroughly mixed together by shaking the beaker in order to ensure even distribution of the particles on the maggots. 0.5 ml of the suspension was pipetted into molten agar media. The preparation was gently mixed together.

Isolation of internal microbes

The maggots were first surface sterilized by placing them in 70% ethanol and then rinsing in sterile water. A sterile blade was then used to dissect the maggots thereby revealing the gut. A maculating loop was flamed and allowed to cool and then it was used to obtain exudates of the gut. The obtained exudates were streaked on the solidified agar media.

Culture procedures

The selenite and tryptone broth were used to culture both external and internal microbes. Then 0.5 ml of the body surface suspension was pipetted and transferred into prepared selenite and tryptone in test tubes. To culture the microbes from the gut, 0.5 ml solution of already dissected 2 maggots in 10 ml in distilled water was pipetted and poured into the already prepared selenite and tryptone broth. The test tubes are then plugged with non-absorbent cotton swab, and then properly sealed with aluminum foil. They were placed in an incubator under a temperature of 37°C for 48 h. After incubation period, the rest tubes were checked for microbial growth.

In order to identify the microorganisms cultured on broth, they were sub-cultured on already prepared nutrient agar and potato dextrose agar. The Petri-dishes containing the media were placed in an incubator under a temperature of about 37°C for 48 h (for bacteria) and 4-5 days (for fungi). After the incubation period, the isolates were observed and identified. Only microbes from tryptone broth was sub-cultured on potato dextrose agar and this is because selenite broth is a selective medium for enteric bacterial and so will not encourage the growth of fungi. Another inoculation was carried our using tryptone and selenite broth but was sub-cultured on blood agar for identification.

Identification of isolates

To identify the bacteria growth on the agar media, tests were carried out to determine their biochemical and morphological characteristics. Gram staining was carried out according as described by Baker (1967). Motility test was according to the technique described by Humphries (1974). Starch hydrolysis and urease production was according to technique of Harrigan and McCance (1976). Methyl Red Voges-Proskaeur (MRVP), oxidase and catalase test were according to Olutiola (1991), while indole test was according to the specification of Cruickshank et al. (1965) To identification of fungi was done according to Beech et al (1968).

RESULTS AND DISCUSSION

The objective of the study is to isolate and identify the micro - organisms that can be found on the housefly

Table 3. Biochemical and morphological characteristics of bacteria isolated from the maggots on blood agar.

Source of bacteria	Gram stain	Shape	Modility	Catalase	Coagulase	Oxidase	Methy red	Voges Proskaeur	Starch hydrolysis	Indole	Glucose	Lactose	Sucrose	Raffinose	Probable Identification
(External (Body Surface)															
From tryptone broth to blood agar	+	C	-	+	-	-	-	-	-	-	A	-	A	-	*Streptococcus* sp.
From Selenite broth to blood agar	+	C	-	+	-	-	-	-	-	-	A	-	A	-	*Streptococcus* sp.
Internal (Gut)															
From tryptone broth to blood agar	+	C	+	+	-	-	-	-	-	-	A	A	A	-	*Micrococcus* sp.
From Selenite broth to blood agar	+	C	-	+	-	+	-	-	-	-	A	-	A	-	*Streptococcus* sp.

\+ = Positive.
\- = Negative.
C = Coccoid.
A = Acid.

maggot. (Table 1). The essence is to determine if these microbes are pathogenic or non-pathogenic. It is a common practice of peasant farmers to feed livestock (birds) and fishes with maggots. It is therefore necessary to determine the type of microorganisms that can be found around the maggot. And if these microorganisms are pathogenic, it would be desirable to avoid the risk of disease on man and his livestock consequent form feeding directly or indirectly on the maggot that are not reared aseptically.

It was observed that the bacteria isolates recovered were mostly Gram positive bacteria. Only *P. aeruginosa* was Gram negative. The bacteria recovered were found to be mainly rod-shaped bacteria (bacilli) and sphere-shaped bacteria (cocci) and they are all aerobic. They all show acidic response towards glucose, lactose, raffinose, sucrose, maltose and xylose test. Most of these microbes are pathogenic except *B. subtilis. P. aeruginosa*, *B. cereus*, *S. faecalis* and *S. anareus* are pathogens. *S. feacalis* is an opportunistic pathogenic. The fungi recovered were *Alternaria sp.*, *Penicillum oxalicum* and *Aspergillus tamari. A. tamarii* is a pathogenic fungus that is liable to produce mycotoxin (Table 2).

The use of maggots as feed supplement for livestock and fishes is a common practice by local farmers. The recovery of pathogenic micro-organism as microflora of the maggot shows the practice may poses some risk. Microbes such as *S. aureus, P. aeruginosa, Aspergillus sp. S. faecalis and B. cereus* are liable can cause various infectious diseases in livestock. *S. aureus* is capable of causing toxigenic food poisoning and some other infectious diseases which would result in diarrhea (Ako-nai et al, 1991; Nawigen and Koenig, 1981). Those diseases could be associated with livestock that have ingested the organisms.

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