



Isolation and Identification of the Microflora from the Alimentary Canal and External Surface of the *Periplaneta americana*

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Abstract : Domestic cockroaches (*Periplaneta americana*) are reddish brown to black in colour and they reside at local areas like in the kitchens, bathrooms, dark areas, cracks of buildings, near the garbage, wet places, spoiled food materials and at many more similar places. They carry several types of microorganisms on their external surfaces as well as at their internal surfaces. Some of these microorganisms are dangerous for humans as they cause several life threatening diseases like salmonellosis, typhoid fever, cholera etc and some of them show potential industrial applications like antimicrobial activities, antibiotic activities. Most bacteria which are associated with the cockroaches are of the family *Enterobacteriaceae*. Most common bacterial species which are found from the cockroaches are *Serratia*, *Pseudomonas*, *Klebsiella*, *bacillus*. This study has been undertaken for isolating and identifying the bacteria from the gut and external surface of the *Periplaneta americana*. For isolating purposes several selective and differential media were prepared and for identification purposes gram staining and various biochemical tests were carried out.

IndexTerms - *Periplaneta americana*, PBS buffer, EMB media, MYP media.

I. INTRODUCTION

Cockroaches:

Cockroaches are classified as insects and they have life over 300 million years. They are known as hemimetabolous insects. They fall under the order *Blattodea*[1]. There are different varieties of cockroaches and most common includes: American, German and Oriental cockroaches. German cockroaches are small in size and are brown in colour. American cockroaches are large in size and reddish brown and black in colour. Oriental cockroaches are medium sized and are black to dark brown in color. Cockroaches feed on various food materials and also on garbage that's why they cause several minacious diseases[2]. They have distinguishing morphological makeup including flattened bodies, two pairs of wings which cover almost the full body of themselves, light brown to black in colour and they may rarely fly and move hastily. There are three stages in the life cycle of the cockroaches: egg, nymph and adult[3]. They have three major anatomical guts inside them which includes: Foregut, midgut and hindgut. These three major sections in cockroach gut have several differences in their pH, redox potential and hydrogen pressure[1]. The female cockroaches lay their eggs in the groups which are circumjacent by a leathery, bean shaped egg case or capsule known as ootheca[3]. Some female cockroaches can reserve the sperms inside them for life long after their sexual intercourse due to which these female cockroaches are able to fertilize their eggs and laid without the presence of the male cockroaches. There are diverse groups of microorganisms which exist on the cockroaches and these microorganisms are further responsible for various diseases and some microorganisms are also responsible for producing proteins and metabolites which are having different industrial applications[1].

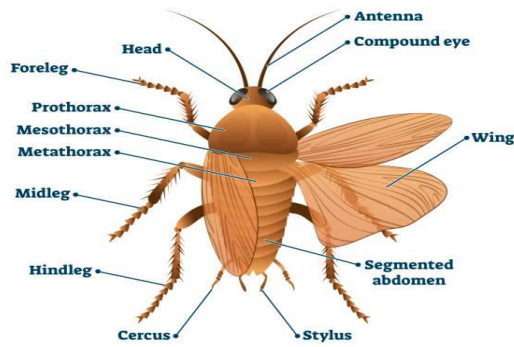
Structure of cockroaches:

Fig.1 Parts of cockroach[4]

Fig.2 *Periplaneta americana***Microorganisms associated with the cockroaches:**

Cockroaches carry a lot of microorganisms in which bacteria is most widely found and about 32 different bacteria are associated with them[2]. Most bacteria associated with cockroaches comes under the phylum *Proteobacteria* and family *Enterobacteriaceae*. Second most frequently cultivated bacterial phylum from the cockroaches is *Firmicutes* and they are most abundant in midgut(43%), then in hindgut(34%) and least in foregut(30%). Genera *Bacillus*, *Paenibacillus* and *Enterococcus* are the alkaliphilic, aerobic bacteria which are present in the midgut. *Bacillus cereus* is a filamentous form of bacteria which is attached to the gut epithelial cells. Other strains of *Bacillus* like *Bacillus anthracis* and *Bacillus thuringiensis* also reside on cockroaches and cause several diseases because they secrete lytic enzymes and toxins[1]. The bacterial species which were isolated in this paper from the gut of *Periplaneta americana* was *Klebsiella pneumoniae* and from the external surface of the *Periplaneta americana* were *Pseudomonas aeruginosa* and *Bacillus cereus*.

Applications of bacteria associated with cockroaches:

Bacteria associated with the cockroaches have distinguished and foremost factors which are having various potential industrial applications like antibiotic, antimicrobial activities. These bacteria also produce some substances like pigments which possess several important activities including antimicrobial, anti inflammatory, anti cancerous.

II. MATERIALS AND METHODS**Isolation of microorganisms from the cockroach:**Isolation of bacteria from the alimentary canal of the *Periplaneta americana*:

The *Periplaneta americana* was dipped into the PBS buffer for 24 hours and then dissected with the help of a sterilized dissecting kit. Dissection followed by the bacteria collection from the alimentary canal of the *Periplaneta americana* with the help of a sterilized cotton swab. It was further spread on a freshly prepared selective agar media which is EMB (Eosin Methylene Blue) agar media (pH=7.2±0.2) for *Klebsiella pneumoniae*. After the spreading process of the sample, the petri plate was kept in the incubator at 37 degree celsius for the incubation process for 24 hours.

Isolation of bacteria from the external surface of the *Periplaneta americana*:

The *Periplaneta americana* was dipped into the freshly prepared PBS buffer for 24 hours so that all the microbes could pass on from its external surface to the PBS buffer. After 24 hours, with the help of the sterilized cotton swabs these microbes were spread on the selective and differential agar media viz, MacConkey agar media (pH=7.1), Cetrimide agar media (pH=7.2) and MYP (Mannitol Egg Yolk Polymyxin) agar media (pH=7.2±0.2, selective media for *Bacillus cereus*). It was followed by the incubation process at 37 degree Celsius for about 24-48 hours.

Identification of bacteria:

The most important differential stain which is used in the bacteriology is Gram staining and was discovered by Danish Bacteriologist Hans Christian Gram. This is the one and only staining process which is used to differentiate the bacteria into two groups on the basis of physical properties of their cell walls, viz Gram negative and Gram positive bacteria. Gram positive bacteria contains a thick peptidoglycan layer in its cell wall which retains crystal violet in it whereas Gram negative bacteria contains thin peptidoglycan layer[5]. Once the incubation process was completed then the bacterial strains were collected on the sterilized slides and were stained. Bacterial culture of Cetrimide media showed red colour, very small rod shaped bacteria that means Gram negative bacteria, bacterial culture on MYP media showed purple colour, small rods signifying it as gram positive bacteria and bacterial culture on EMB media showed red colour rod shaped bacteria which confirms it as Gram negative bacteria.

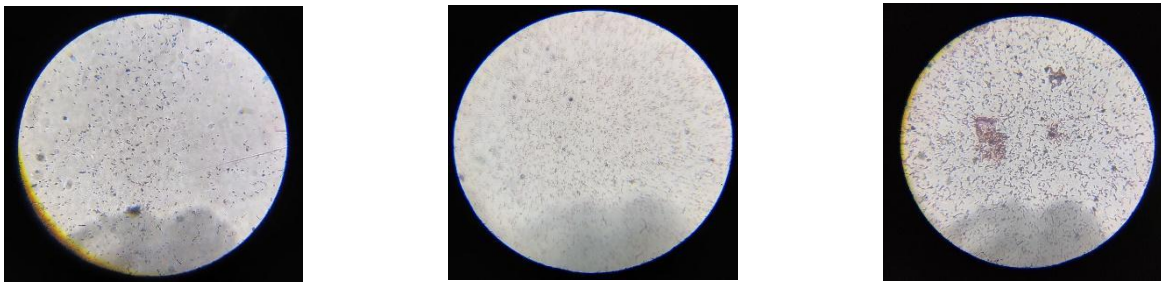


Fig.3 (i) Small rod, Gram positive (ii) Very small rod Gram negative (iii) Medium rod, Gram negative

Identification of bacteria by Biochemical Tests:

A lot of biochemical tests were performed to identify the bacteria which were collected from the alimentary canal and external surface of the *Periplaneta americana*. These tests were performed on those bacteria which were not able to distinguish on the basis of the morphological characters. These are the usual tests which were carried out to check the ability of the bacteria to produce acidic and gaseous end products, when they were presented with the individual carbohydrates or when grown on suitable culture media[6]. All the bacteria which were isolated from the alimentary canal and from the external surface of the *Periplaneta americana* showed Catalase positive test, Urease negative test and Nitrate positive test.

Biochemical Tests	<i>Pseudomonas aeruginosa</i>	<i>Bacillus cereus</i>	<i>Klebsiella pneumoniae</i>
Oxidase Test	Positive	Negative	Negative
Catalase Test	Positive	Positive	Positive
MR Test	Negative	Negative	Negative
VP Test	Negative	Positive	Positive
Urease Test	Negative	Negative	Negative
Citrate Test	Negative	Positive	Positive
Indole Test	Negative	Negative	Negative
Nitrate Test	Positive	Positive	Positive
Coagulation Test	Positive	Negative	Negative

Table 1: Results of Biochemical Tests

III. RESULTS

After performing the bacterial isolation process, three bacteria were isolated. One was isolated from the alimentary canal of the *Periplaneta americana* and the gram staining and biochemical tests were executed, the bacteria was identified as *Klebsiella pneumoniae* on EMB media. The other two were isolated from the external surface of the *Periplaneta americana*. After performing gram staining and all the biochemical tests then the bacteria was identified as the *Pseudomonas aeruginosa* and *Bacillus cereus* on Cetrinide media and MYP media respectively.

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