

**ISOLATION AND CHARACTERIZATION OF YEAST LIKE
SYMBIONTS ASSOCIATED WITH CIGARETTE BEETLE
(*Lasioderma serricorne*)**

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Science degree in Microbiology

By
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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This is to certify that this project report is the bonafide work of Janani.V (40300026) who carried out the project entitled "ISOLATION AND CHARACTERIZATION OF YEAST LIKE SYMBIONTS ASSOCIATED WITH CIGARETTE BEETLE (*Lasioderma serricorne*)" under my supervision from October 2022 to May 2023.

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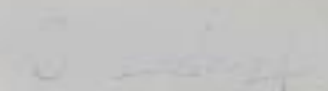
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DECLARATION

I, **LOSHINI.S (40300034)** hereby declare that the project report entitled "**ISOLATION AND CHARACTERIZATION OF YEAST LIKE SYMBIONTS ASSOCIATED WITH CIGARETTE BEETLE (*Lasioderma serricorne*)**" done by me under the guidance of **DR. M. BAVANILATHA** and **DR. S. THANGA SUJA** at **DEPARTMENT OF BIOTECHNOLOGY, SATHYABAMA UNIVERSITY** is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

Lasioderma serricorne, commonly known as cigarette beetle, is a worldwide pest of stored food commodities. The beetle prefers warmer conditions, and low temperature and humidity generally limits the pest distribution. Adults are reddish brown in color with a size of about 2 - 3 mm and a life span of 40-90 days. The beetle has four life stages (egg, larva, pupa, adult) and the larval feeding stage is the most active stage and causes most of the damage to stored products. Cigarette beetles have been reported to be closely associated with yeast symbiont; *Symbiotaphrina kochii* which is involved in detoxification of toxic phytochemicals. However, the presence of other yeast-like symbionts from cigarette beetles has not been reported yet. Beetles like red flour beetle, ambrosia beetle have been reported with a rich microbial flora associated with insect nutritional biology. The present study has been undertaken to identify, isolate and characterize yeast-like symbionts associated with cigarette beetle nutritional biology.

KEYWORDS

Cigarette beetle; Symbionts; Food source, toxic.

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LIST OF ABBREVIATIONS

S.No	Abbreviations
1	YLS - Yeast Like Symbionts
2	mm - millimetre
3	µg - microgram
4	µm - micrometre
5	ml - millilitre
6	g - gram
7	C - Celsius
8	F - Fahrenheit
9	Ph - Potential of Hydrogen
10	α - Alpha
11	β - Beta
12	SDA - Sabouraud Dextrose Agar
13	PDA - Potato Dextrose Agar
14	YEPD - Yeast Extract Peptone Dextrose Agar
15	MMN - Modified Melin Norkrans Media
16	LCB - Lacto phenol Cotton Blue
17	NCBI - National Centre for Biotechnology Information.

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CHAPTER-1

1. INTRODUCTION

Lasioderma serricorne is a troublesome warehouse pest. Also known as cigar or cigarette beetle and tobacco beetle. It is a bug that affects stored goods everywhere. It is an international pest of goods in storage. The insect like to reside in dried flowers, herbs, tobacco, and spices. They can contaminate dates, ginger, turmeric powder, chilli powder, flours, peanuts, and other foods. This insect measures around 2-3 mm, or about 1/8 inch. They have a reddish-brown colour. They are shaped like an oval, rounded ball. It thrives in dimly lit or gloomy environments. But, in bright, wide spaces, get active and move quickly. The head is often concealed by the pronotum (plate covering the first region of thorax) when the beetle is viewed from above. The elytra (wing covers) are covered with fine hair. When they disturbed, they often pull their legs, tuck their head and lay motionless. The beetle may be very comparable in look to the pharmacy beetle (*Stegobium paniceum*). But it can be distinguished by two easily identical characters: The antennae of the cigarette beetle are serrated (like the teeth on a saw) while the antennae of the drugstore beetle not, and end in a 3-segmented club. The other difference is that drugstore beetle elytra have rows of pits giving them a striated (lined) appearance while those of the cigarette beetle are smooth. Insect intestinal tracts are divided into three chambers: foregut, midgut and hindgut; differences in the nutrition of the insects are reflected in the physiology and morphology of the gut regions. The gut varies within an individual over different life stages, especially in insects "complete" (holometabolous) life cycles (having egg, larva, pupa and adult stages). Physiological differences of the gut significantly affect microbial symbiosis, such as fungi favouring the more acid or aerobic areas. In some cases, larvae and adults use different food, and these differences are reflected in modified morphology and microbial communities in a single life cycle. The relationship between Symbiotaphrina and two anobiid, the cigarette beetle (*Lasioderma serricorne*) and the drugstore beetle (*Stegobium* [= *Sitodrepa*] *paniceum*), is one of the most well-studied Coleopteran-yeast relationships. In these insects the YLS live intracellularly in enlarged cells associated with grape-bunch-like proliferations of tissues around

the midgut (Buchner et al.,1965). When the female spreads yeast on the eggshell, the larva that hatches consumes it, vertical transmission of yeasts occurs. (Buchner et al.,1965). These YLS are important in providing nutrients for the host insects and also in detoxifying plant toxins. The symbiotic yeast in the gut of the cigarette beetle is able to detoxify certain compounds by breaking them down into less harmful components. For example, the yeast has been shown to be able to degrade the toxic compound coumarin, which is found in many plants and can be harmful to insects in high concentrations. Additionally, the yeast may also play a role in the beetle's ability to digest certain types of food. In particular, the beetle is able to digest cellulose, which is a complex carbohydrate that is difficult to break down. The yeast in the gut of the beetle produces enzymes that can break down cellulose, making it easier for the beetle to extract nutrients from its food. Overall, the symbiotic yeast in the gut of the cigarette beetle appears to play an important role in both detoxification and digestion, contributing to the beetle's ability to resist certain toxins and thrive in a variety of environments.



Fig: 1.1 Cigarette beetle (Lasioderma serricorne)

1.1 LIFE CYCLE OF CIGARETTE BEETLE

Cigarette beetle has four stages of lifecycle such as:

- Egg
- Larva
- Pupa
- Adult

1.1.1 EGG

The eggs are opaque pearl white when freshly laid but become yellowish shortly before hatching. They are oval or oblong in shape. They are varying from 0.29 - 0.50mm long and 0.18-0.25mm in diameter. The average length and diameter of the egg is 0.20mm respectively each egg weight about 8.0 -9.0 µg (Eddp at el., 2019). Mature female lay up to 90 to 100 eggs. They hatch after 5 to 7 days. Number of eggs were counted using a trinocular microscope after the pre-oviposition period of three days. The period between egg laying and hatching was calculated as incubated period. The hatching percentage of the egg was calculated by (Naveen et al.,2019)

$$\text{Hatchability (\%)} = \frac{\text{Number of eggs hatched} \times 100}{\text{Total number of eggs laid}}$$

Total number of eggs laid

Crowding may cause female beetles to deposit most of their mature eggs quickly, but overcrowding will reduce the overall number of eggs laid per female. At 20°C the embryonic development of the *Lasioderma serricorne* egg takes about 10 days. The average incubation period is 7 days during the summer months.

1.1.2 LARVA

They are creamy white in colour with a yellow head and brown mouth parts. Mature larvae of the tobacco beetles are C-shaped and about 3/16' long. They have long yellowish hair (Bio protection portal). During hatching, the larvae chew its way through the chorion at the posterior margin of the papillated region.

$$\text{Larval survival} = \frac{\text{Number of larvae developed}}{\text{Total no of egg hatched}} \times 100$$

1.1.3 PUPA

Temperature and the type of food available determine how long a tobacco beetle will live. The cold bothers the babies. 4 days at 86°F-90°F and 12 days at

68°F were necessary for the stage. Humidity has little effect. Pupation lasts anything from a week to a month. This has an effect on how long the pupal stage lasts. The duration of the adult's stay in the pupal chamber from the day the cocoon was fully created to the day the cocoon was fully formed was computed. the proportion of fully developed pupations the amount of pupation was determined by (Naveena *et al.*,2019)

$$\text{Pupal survival (\%)} = \frac{\text{Number of pupae formed}}{\text{Total no. of larvae matured}} \times 100$$

1.1.4 ADULT

The adult beetles were reddish brown in colour with smooth elytra. The percentage of adult emergencies varied from 83.73 to 96.96 percent, with an average of 89.73 ± 1.27 percent (Naveena *et al.*,2019). The heat treatment of a food processing facility at 20-22 degrees Celsius. The time for 99% mortality (LT99) based on egg hatchability was shown to be 605 minutes at 46 °C, 190 minutes at 50° C, and 39 minutes at 54° C.

$$\text{Adult emergence (\%)} = \frac{\text{No. of adults emerged}}{\text{Total no. of pupae formed}} \times 100$$

1.2 FUNGI

Approximately 80000 to 120000 species of fungi have been described to date, but there are an estimated 1.5 million total species (Webster *et al.*,2007). Fungi are a diverse and widespread group of heterotrophic organisms that live as saprophytes, parasites, or as symbionts with other organisms. The vast majority of known fungi, whether parasitic or not, can survive on dead organic matter (Clayton *et al.*,1988). Fungi are eukaryotic organisms. Yeasts, moulds, and mushrooms are examples of fungi. These organisms belong to the kingdom Fungi. The organisms found in the Kingdom Fungi have a cell wall and are ubiquitous. Among living organisms, they are classified as heterotrophs. Fungi structure can be explained as follows:

Nearly all fungi have a filamentous structure except the yeast cells. They can be single-celled or multicellular in nature. Fungi are made up of long thread-like

structures known as hyphae, which are usually colourless. These hyphae combine to form a mesh-like structure known as mycelium. The hyphae frequently weave together to form dense masses that resemble tissues; these masses are known as pseudo parenchymatous. A stroma is referred to as a mass that contains multiple fructifications. Alternatively, the hyphae may group together to form a rhizomorph, which resembles a root.

Fungi have a cell wall composed of chitin and polysaccharides. The cell wall is made up of a protoplast that differentiates into other cell components such as the cell membrane, cytoplasm, cell organelles and nuclei. Although the fungi's cell wall structure is similar to that of plants, it differs chemically in that it is made of chitin ($C_8H_{13}O_5N$). The nucleus is clear and packed with chromatin filaments. A nuclear membrane encircles the nucleus in every cell.

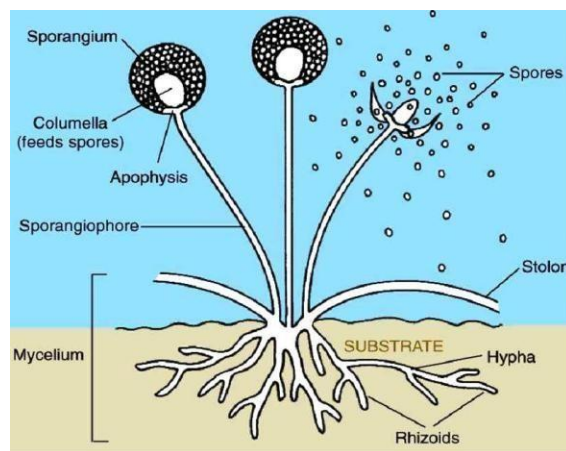


Figure 1.2 Basic Structure of Fungi (Source: Google)

1.2.1 CHARACTERISTICS OF FUNGI

Fungi are eukaryotic, non-motile and heterotrophic organisms. They can be unicellular or filamentous in nature. They lack a vascular system. Xylem and Phloem are not present. They reproduce through spores. Spores can be sexual or asexual. Sexual spores include Oospores, Zygosporangia, Ascospores, Basidiospores, and others, while asexual spores include Sporangiospores, Aplanospores, Zoospores, Conidia, and others. Sexual and asexual spores can be produced depending on the

species and conditions. Fungi exhibit the phenomenon of generational change. They go through both haploid and diploid stages. Fungi lack the chlorophyll pigments that are found in the chloroplasts of plant cells and are essential for photosynthesis because they are achlorophyllous.

To accomplish the digestion of food, fungi produce exoenzymes such as hydrolases, lyases, oxidoreductases, transferases, etc. As starch, fungi store their food. Fungi perform chitin biosynthesis. The majority of saprophytic fungi require a growth temperature of 20-30°C, whereas parasitic fungi require a temperature of (30-37) °C. When compared to bacteria, fungi grow more slowly.

Fungi can reproduce sexually and asexually. The sexual state is known as teleomorph (fruiting body), while the asexual state is known as anamorph (mold like). Asexual methods include fragmentation, somatic budding, fission, and the formation of asexual spores. Gametic copulation, gamete-gametangium copulation, gametangium copulation, somatic copulation, and spermatization are the sexual methods. A chemical compound called pheromone is produced by fungi and promotes sexual reproduction between male and female fungi cells.

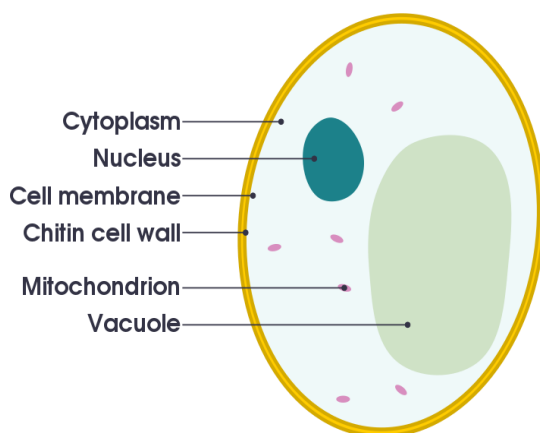
3. YEAST

Yeast is a single-celled organism from the fungi kingdom. There are about 1500 different types of yeast. The yeast fungus is a eukaryotic organism, which means that it has a nuclear membrane around its nucleus. Yeast reproduces most frequently through the budding process. Haploid cells are unable to survive. The diploid cells go through sporulation and meiosis, a sort of sexual reproduction, in these high-stress circumstances to create haploid cells.

1. MORPHOLOGY OF YEAST

They are single celled fungi. Size: generally larger than most bacteria; (1-5) um wide and (5-30) um length. Cells are egg-shaped, but some are elongated or spherical. Size and shape vary among species. Yeast cells lack flagella and other organs of locomotion. The description of the external appearance and interior makeup of yeast cells is necessary for yeast identification. Yeast undergoes vegetative reproduction. macroscopical appearance is the appearance of a yeast growth that is apparent to the unaided eye (De Becze et al.,1956). The

morphological alterations associated with ascospore production and sexual activity will be covered independently of their genetic significance. These two mating ascospores fused within the ascus to form a diploid zygote. A diploid zygote germinates within the ascus, producing a germ tube. The germ tube breaks the ascus wall and functions as diploid sprout mycelium. The sprout mycelium on budding gives rise to diploid cell which give diploid somatic cells. A unique cell wall surrounds granular cytoplasm, a large vacuole, and a nucleus in each yeast cell.



1.3 Basic Structure of Yeast (Source: Google)

1.3.2 GENERAL CHARACTERISTICS OF YEAST

Yeast are chemoorganotrophs because they can utilize inorganic chemicals as a source of energy. Yeast can be found in the intestinal flora of mammals, some insects, and even in the deep sea. Most yeast species prefer an acidic pH of 3.5 to 4 and temperatures between 20 and 28 degrees Celsius. The cellular structure of yeasts is similar to that of higher creatures like humans despite the fact that they are single-celled. As their nucleus houses their whole genetic makeup, they are regarded as eukaryotic in origin. They are also found on the skin and in the intestines of warm-blooded animals. *Candida albicans* causes common yeast infections such as vaginal yeast infections, nappy rash and mouth and throat thrush. Fermentation is an anaerobic process because it takes place in the absence of oxygen. The mouth and throat infections caused by *Candida albicans*. Yeast grows by

fermentation, which involves turning glucose into carbon dioxide and ethanol. Because fermentation occurs without the presence of oxygen, it is an anaerobic process. Due to the Crabtree effect, yeast cells prefer to proliferate through fermentation when they are grown in rich carbon sources and even in the presence of oxygen. Yeasts often procreate via asexual processes including budding and fission.

1.4 YEAST LIKE SYMBIONTS

Yeast-like endosymbionts (YLSs) of insects are frequently confined to particular hosts and are necessary for the host's survival (S O Suh et al., 2001). Nearly 100 cases of mutualisms between various yeast species and beetles were discovered in yeasts associated with insects. Even though their phylogenetic origins appear to be different, these yeasts play clear roles in nutrition and detoxification for both (Anobiid and Cerambycid beetles) (Gonzalez et al., 2014). This supports the hypothesis that endosymbiotic relationships between *Symbiotaphrina* and *Candida* species and Anobiid and Cerambycid beetles are instances of convergent evolution (Cherry et al., 1999).

1.5 DIFFERENT TYPES OF YEAST LIKE SYMBIONTS (YLS) ARE PRESENT GENERALLY IN *Lasioderma serricorne*

One of the best studied Coleopteran-yeast associations is that of *Symbiotaphrina* and two anobiid beetles, the cigarette beetle (*Lasioderma serricorne*) and the drugstore beetle (Stegobium [= Sitodrepa] paniceum). In these insects, the YLS live intracellularly in enlarged cells associated with grape-bunch-like proliferations of tissues around the midgut (Buchner et al., 1965). Yeasts are transmitted vertically when the female smears them on the eggshell, which is consumed by the hatching larva (Buchner et al., 1965). These YLS are important in providing nutrients for the host insects and also in detoxifying plant toxins.

1. SYMBIOTAPHRINA KOCHII

The symbiotic fungi *Symbiotaphrina*, which are symbiotic with cigarette and drugstore beetles. Throughout the beetle's life cycle, the fungus symbiont is both extracellular and intracellular and is found in gut-associated mycetomes in both the larval and adult stages (Martinson et al.,2020). *Symbiotaphrina* is a genus of yeast-like endosymbionts of beetles that has been linked to insect detoxification of toxic plant compounds, metabolism of fatty acids, and production of B vitamins. On the egg surface at deposition, the fungi are spread vertically. In situ studies have shown that microorganisms linked to herbivorous insects, such as the symbiotic yeast of the cigarette beetle, can produce enzymes capable of detoxification (Patrick ,Dowd et al., 1990).Initial histochemical studies indicated that the symbiont was capable of degrading aromatic esters such as ochratoxin A, 6-methoxy-2-benzoxazolinone, l-naphthyl acetate, tannic acid, and 6-methoxy-2-benzoxazolinone (Dowd et al.,1989)(Dowd et al.,1990).Additionally, the fungi are involved in using sterols and assisting with nitrogen recycling in the fat bodies of the insect hosts.

2. CANDIDA ALBICANS

Candida albicans-related yeasts were recovered from the digestive tracts of beetles from eight different families and orders of insect (Sung-Oui Suh et al.,2008). Encapsulated and diploid, also form true hyphae. The pathogen's DNA is meiosis-defective. *Candida albicans* encodes a Rme1 homolog, which is a component of the transcriptional circuitry that regulates hyphal development. Due to phenotypic flipping and bud to hypha transition, *C. albicans* displays a variety of morphological morphologies. Chlamydospores, which can grow on the pseudo hyphae, can persist in unfavourable environments such dry or hot seasons. Spores are a technique for fungi to reproduce both asexually and sexually as well as a means of surviving in unfavourable environments. Fungi typically reproduce sexually to increase genetic diversity within the colony and improve survivability in novel environments.

3. ASPERGILLUS

The genus *Aspergillus* represents a diverse group of fungi that are among the most abundant fungi in the world(Krijgsheld et al .,2013). It consists of a few hundred opportunistic mould species that are found in a variety of climates (Mousavi et al.,2016). *Aspergillus versicolor* is an insect-associated fungus. This fungus lives

inside the invertebrate host's body. They could provide nutrition and antibiotics for the host insect (Tian-xio et al.,2022). *Aspergillus* species secrete a large number of secondary metabolites known as mycotoxins into their environment. Some of the most significant known mycotoxins produced by *Aspergillus* include aflatoxin, gliotoxin, and ochratoxin A (B Mousavi, et al.,2016). This fungus' metabolites could be a valuable resource for the development of anti-inflammatory drugs (Tian-xio et al.,2022)

1.5.4 *PENICILLIUM*

Penicillium is well known and one of the most common fungi occurring in a diverse range of habitats, from soil to vegetation to air, indoor environments and various food products *Penicillium* is a varied genus found all over the world, and its species serve key roles as organic material decomposers and cause harmful rots in the food business, producing a wide spectrum of mycotoxins. It has a global distribution and a significant economic impact on human life. Its primary role in nature is organic materials, where organisms cause destructive rots on food crops as pre- and postharvest infections. Every species in the *Penicillium* subgenus *Penicillium* produced an extrolite profile. The 3-9 extrolite biosynthetic families into which these extrolites could be arranged. (Chung et al., 2004). The long, septate, multinucleated, and heavily branching filamentous structures called hyphae make up the mycelium. Conidia are formed by specialized cells called phialides, which are present in the group and give the fungus a brush-like look. The means of transmission of symbionts with fungus-growing insects are directly related to the stability of symbioses. Transmission modes of non-social fungus-farming insects must be studied further than those of social fungus-farming insect.

1.6 BENEFITS OF YEAST-INSECT ASSOCIATIONS

After establishing the existence of an association, the next step must be to identify the nature of the relationship, with a focus on the effects on both participants. The majority of the time, the association is neutral (neither of the two participants benefits or suffers from it), but it can also be mutualistic (positive for both), commensal (positive for one, neutral for the other), amensal (negative for one,

neutral for the other), or parasitic (negative for one, positive for the other) (Starmer et al., 2011)

Yeasts play an important role in attracting insects to suitable food sources before they form an association with the insect. Insects' initial attraction to food is usually based on olfactory stimuli. These yeasts have been shown to provide nitrogen and vitamins to their hosts. They have been demonstrated to give their hosts vitamins and nitrogen, to break down the disaccharide cellobiose, and to produce trypsin, phosphatase, lipase, and α - and β -glucosidase, which may aid the host in breaking down the food and eliminating a variety of substances (Stefanini et al., 2018). Studies using the intracellularly derived yeasts of the genus *Symbiotaphrina* of two anobiid beetles (*L. serricorne* and *S. paniceum*) have provided some of the best information on the function of symbionts. It has been shown that these yeasts give their hosts vitamins, sterols, and nitrogen. Nutritional studies using prescribed diets, however, revealed that the symbionts could not provide all of the B vitamins required for *L. serricorne*'s optimum growth. Other studies have demonstrated that the yeasts provide essential amino acids. In the absence of tryptophan, survival was comparable to that of a casein diet; no survivors were found among the symbiont-free insects, indicating that tryptophan was provided by the symbionts (Fernando E. Vega Patrick F. Dowd et al., 2005).

CHAPTER - 2

2.1 LITERATURE SURVEY

Dowd *et al.*, 1989 The article "In Situ Production of Hydrolytic Detoxifying Enzymes by Symbiotic Yeasts in the Cigarette Beetle (Coleoptera: Anobiidae)" by Patrick F. Dowd discusses the role of symbiotic yeasts in the detoxification of harmful compounds in the cigarette beetle. The study found that symbiotic yeasts in the beetle's gut produce hydrolytic enzymes that are capable of breaking down harmful compounds such as nicotine and other alkaloids found in tobacco. The enzymes produced by the yeasts enable the beetle to feed on and survive on tobacco, which would otherwise be toxic to the insect. The research highlights the importance of microbial symbionts in the digestive systems of insects and their ability to produce enzymes that aid in the detoxification of harmful compounds. The findings may have implications for the development of new strategies for pest control that target the symbiotic microbes in insect digestive systems.

Dowd *et al.*, 1990 The article by Patrick F. Dowd and Samuel K. Shen discusses the contribution of symbiotic yeast to toxin resistance in cigarette beetles (*Lasioderma serricorne*). The authors found that the yeast present in the gut of the beetles was able to detoxify certain plant allelochemicals, including phenolics and flavonoids, which are known to be toxic to insects. The authors conducted experiments in which they exposed beetles to different concentrations of a phenolic compound called p-coumaric acid, and found that the beetles with the symbiotic yeast in their guts were more resistant to the toxin than those without the yeast. They also found that the yeast was able to degrade the toxin, producing a less toxic compound in the process. The authors suggest that the symbiotic yeast may play an important role in the ability of cigarette beetles to feed on plants containing allelochemicals. This finding may have implications for the development of more effective pest control strategies, as well as for our understanding of the ecological interactions between plants, insects, and microorganisms.

Shen et al., 1991 These fungi create enzymes that degrade the poisons, allowing the insect to absorb the plant material safely. These enzymes may find value in a variety of industries, including agriculture and medicine, where they can be utilised to detoxify toxic substances. Additionally, studying these symbiotic relationships can provide insights into the evolution of detoxification mechanisms and the coevolution of insects and fungi. Overall, insect fungal symbionts have the potential to be a valuable source of detoxifying enzymes with numerous applications in various fields.

Naveena et al., 2019 The article investigates the suitability of different hosts for the mass multiplication of the cigarette beetle, a common pest of stored tobacco products. The authors conducted experiments to determine the growth and development of the beetle on various host materials, including tobacco, maize, wheat, and chickpea. The results showed that tobacco was the most suitable host for the beetle, followed by maize and wheat, while chickpea was the least suitable. The authors suggest that the findings can be useful for the development of effective management strategies for the cigarette beetle in stored tobacco products.

Moura et al., 2017 The authors conducted experiments to study the infestation level of *L. serricorne* on black sesame and found that the insect was capable of infesting the seeds and causing significant damage. The authors also studied the life cycle of *L. serricorne* on black sesame and found that the insect could complete its life cycle successfully on this host. The findings of this research could be useful for developing effective pest management strategies for black sesame cultivation.

Vishali et al., 2022 In this study, the researchers examined the development, reproduction, and survival of cigarette beetles in dry chillies under laboratory conditions. They found that the beetles were able to complete their development from egg to adult in approximately 33 days at a constant temperature of 30°C and 75% relative humidity. The researchers also observed that female cigarette beetles laid an average of 57 eggs over their lifespan, and that the eggs hatched in

approximately 4 days. The larvae of the beetle fed on the dry chillies, and underwent three instars before pupating. The pupal stage lasted for approximately 7 days before the adult beetles emerged. In addition to studying the development and reproduction of the cigarette beetle, the researchers also investigated the effect of different temperatures and humidity levels on the survival of the beetles in dry chillies. They found that the beetles had a higher survival rate at lower temperatures and higher humidity levels.

Edde, et al. 2019 According to Edde, *L. serricorne* has a worldwide distribution and is commonly found in warm, humid environments. The beetle's life cycle includes four stages: egg, larva, pupa, and adult. The female beetle lays her eggs in a suitable food source, and the larvae feed on the food until they pupate and emerge as adults. The entire life cycle can be completed in as little as 30 days under ideal conditions. Edde also discusses the ecology of *L. serricorne*, noting that the beetle is attracted to food sources with high moisture content and temperatures between 20-35°C. The beetle is capable of infesting a wide range of products, including dried fruits, nuts, and pet food.

Sung-Oui Suh et al., 2001 The authors describe how they discovered that some of these fungi also contain yeast-like endosymbionts, which are believed to be derived from a separate yeast lineage. They suggest that the presence of these endosymbionts may help the fungi to survive in certain environments and to infect their insect hosts more effectively. Overall, the article provides new insights into the complex relationships between insects and their endosymbionts, and highlights the importance of studying these relationships in order to better understand the ecology and evolution of both the hosts and the symbionts.

Jones et al.,1999 The article describes the results of a study that investigated the origins of yeast-like endotoxins found in two groups of beetles: anobiid beetles and cerambycid beetles. Endocytobionts are microorganisms that live inside the cells of other organisms. The study also found that the endocytobionts from the anobiid

beetles were more closely related to other fungal species than the endocytobionts from the cerambycid beetles. This suggests that the endocytobionts in anobiid beetles may have been acquired more recently in evolutionary history. Overall, the study provides insights into the evolution of endocytobionts in beetles and sheds light on the diversity of these microorganisms.

Kevin G. Jones et al., 1996 The study by Kevin G. Jones and Meredith Blackwell utilized ribosomal DNA sequence analysis to examine the placement of the yeast-like genus *Symbiotaphrina* within filamentous ascomycetes. The researchers found that the genetic analysis placed *Symbiotaphrina* within this group, rather than with other yeasts as previously thought. This finding has important implications for our understanding of the evolution and diversity of fungi, as well as for the classification and identification of different fungal species. It also highlights the power of molecular techniques such as DNA sequencing in providing new insights into the relationships between different organisms.

Stefanini et al., 2017 The author explains that yeast can be found in the guts of many different types of insects, and the interactions between yeast and insects can be beneficial, neutral, or harmful. The article provides examples of each type of interaction and discusses how these interactions may impact both the insect and the yeast. Additionally, the author discusses the potential for studying these interactions to better understand the ecology and evolution of both yeast and insects. Overall, the article highlights the importance of considering the microbial communities associated with insects in order to fully understand their biology and ecology.

Moura et al., 2017 The authors conducted experiments to study the infestation level of *L. serricorne* on black sesame and found that the insect was capable of infesting the seeds and causing significant damage. The authors also studied the life cycle of *L. serricorne* on black sesame and found that the insect could complete its life cycle successfully on this host. The findings of this research could be useful for developing effective pest management strategies for black sesame cultivation.

Berasategui et al., 2015 The literature suggests that insect symbionts have a wide range of potential applications in biotechnology, including pest control, bioremediation, bioprospecting, and biotechnology. Insect symbionts represent a largely untapped source of novel bioactive molecules. Bioprospecting, the process of discovering and developing new drugs and other biologically active compounds from natural sources, could lead to the discovery of new antibiotics, antifungals, and anticancer drugs, among other things. However, further research is needed to fully explore and exploit the potential of these symbiotic relationships.

Starmer et al., 2011 The author provides an overview of the ecology of yeasts, including their distribution, diversity, and interactions with other organisms. It discusses the different types of habitats in which yeasts can be found, such as soil, water, and plants, and the factors that influence their growth and survival, such as temperature, pH, and nutrient availability. The author also examines the relationships between yeasts and other organisms, including bacteria, fungi, plants, and animals, and their roles in various ecological processes, such as nutrient cycling and decomposition. It also discusses the importance of yeasts in various industries, such as food and beverage production, biotechnology, and medicine.

Milne et al., 1958 In terms of control strategies, there are several approaches that can be effective against the cigarette beetle. These include physical procedures like trapping and vacuuming, as well as chemical approaches like fumigation and insecticide treatment. The best strategy will depend on the unique situation and the intensity of the infection. One potential new method for controlling the cigarette beetle is the use of biological control agents, such as parasitic wasps or nematodes, which can target the beetle at different stages of its life cycle. This approach has shown promise in some studies, but further research is needed to fully evaluate its effectiveness. Overall, controlling the cigarette beetle can be difficult, but a variety of approaches, such as correct storage and cleanliness practices, can reduce the likelihood of infestation.

Retief et al., 1988 The author discusses the impact of the cigarette beetle on herbarium collections. The cigarette beetle is a little brown beetle that feeds on dried plant material such as tobacco, spices, and herbarium specimens. The article highlights the importance of identifying and controlling this pest to prevent damage to valuable collections. The authors discuss the life cycle and habits of the cigarette beetle, including its preference for warm, humid environments. They note that the beetle can quickly infest an entire collection, causing damage to specimens and making them unsuitable for research or display. The article also discusses various methods for controlling cigarette beetle infestations, including fumigation and freezing. The authors emphasise the importance of prevention in reducing cigarette beetle infestations, including proper herbarium specimen storage and handling. They also emphasise the importance of ongoing monitoring and inspection to discover infestations early and prevent further spread. The article's conclusion emphasises the value of cooperation and communication among herbarium employees and other experts in order to successfully manage pest infestations and save priceless collections

Li et al., 2020 The study "Antioxidant coumarin and pyrone derivatives from the insect-associated fungus *Aspergillus Versicolor*" by Tian-Xiao Li et al. describes the isolation and identification of several new coumarin and pyrone derivatives from a fungus known as *Aspergillus Versicolor*, which is associated with insects. The researchers used a combination of chromatographic techniques, such as silica gel column chromatography and preparative HPLC, to isolate and purify the compounds from the fungal extract. The structures of the compounds were elucidated using various spectroscopic techniques, such as 1D and 2D NMR, MS, and UV. The study found that the isolated compounds exhibited significant antioxidant activity, as determined by DPPH and ABTS radical scavenging assays. This suggests that the compounds may have potential applications in the food and pharmaceutical industries as natural antioxidants.

Shen *et al.*, 1989 The article reports on a study of the yeast-like symbiont found in the cigarette beetle (*Lasioderma serricorne* F.). Specifically, the authors investigate the induction of esterase (enzymes that break down esters) in cultures of this symbiont in response to exposure to xenobiotics (foreign substances, such as pesticides). The article is significant because it sheds light on the ability of the yeast-like symbiont to respond to environmental stressors, and may have implications for the development of pest control strategies.

Noda *et al.*, 1996 The article discusses the phylogenetic position of yeast-like endosymbionts found in anobiid beetles. The authors, Hiroaki Noda and Kentaro Kodama, use molecular techniques to study the genetic makeup of the endosymbionts and compare it to other known organisms. They conclude that the endosymbionts belong to a unique clade of fungi, distinct from other known fungal groups. The study provides important insights into the evolution of these endosymbionts and their relationship to their host organisms. It also highlights the potential for using molecular techniques to better understand the genetic diversity of microorganisms, including those that live in close association with other organisms. Overall, the article contributes to our understanding of the microbial world and the important role that microorganisms play in the ecology and evolution of other organisms.

Sung-Oui Suh *et al.*, 2007 The authors of this study used culture-based methods to isolate yeasts from beetles and other insects collected from plant habitats in Louisiana. They then used molecular methods to identify the yeasts and compare them to known *Candida* species. The study identified seven novel *Candida* species that are closely related to *C. albicans*, suggesting that there may be a complex of related species that are associated with insects and plants. Overall, the study contributes to our understanding of the diversity and ecology of yeasts associated with insects and highlights the potential for further research into the roles that these yeasts may play in insect-plant interactions and biotechnology.

Karen Reiner *et al.*,2012 The article provides a step-by-step protocol for performing carbohydrate fermentation tests in the laboratory to identify bacteria based on their ability to ferment specific carbohydrates. The materials required for the protocol include carbohydrate fermentation test tubes, an inoculating loop, an incubator set to 37°C, and Durham tubes. The protocol involves preparing the carbohydrate fermentation test tubes, inoculating them with the test organism, incubating them at 37°C for 24-48 hours, observing them for gas production and a change in colour of the medium, interpreting the results based on the presence or absence of gas and colour change, recording the results, and disposing of the test tubes and contaminated materials. The article emphasizes the importance of using sterile technique, following laboratory safety protocols, and properly identifying the test organism based on the results.

CHAPTER - 3

AIM

This study aims to isolate and characterize the yeast like symbionts in cigarette beetle.

OBJECTIVES

- To isolate yeast like symbionts in cigarette beetle.
- To identify the yeast like symbionts.
- To characterize yeast like symbionts associated with cigarette beetle nutritional biology.

CHAPTER – 4

MATERIALS AND METHODS

1. Cigarette beetle culture

Adults of cigarette beetle along with the infested wheat flour were collected from International Research Centre, Sathyabama Institute of science and technology, Chennai, Tamil Nadu.

1. *Maintenance of stock culture*

The collected insects were maintained in the Laboratory in different types of flours such as wheat flour, rice flour, turmeric powder, gram flour to enrich the nutrient supplements for cigarette beetle.

The beakers' surfaces were sterilized with ethanol and these were sterilized under UV light for 30 mins to kill microbes. Hundred grams of powdered feed were transferred to each beaker. The larvae of insects were released into each beaker (500 ml) containing culture material (feed) and the beakers were covered with silver foil sheet and secured with rubber bands. Holes were made in the silver foil for air circulation. They were maintained at room temperature. The beakers were observed every day until egg hatch, and days required for egg development was recorded. The larval developmental period was counted from the day of emergence of the first instar to the day on which the larva constructed the protective case to initiate pupation, and was recorded by observing the beaker every other day. The pupal period was counted from the time the larva constructed a protective case, or when detectable changes occurred in larval to pupal morphology to the emergence of the adult. Observations were made under a stereo zoom trinocular microscope by emptying the contents of the breaker onto a piece of black paper. The total developmental period was calculated by the summation of egg, larval and pupal period.

2. Isolation of Yeast Like Symbionts

1. Sample Extraction

Cigarette beetle adults were surface sterilized in ethanol for 1 min and insect gut were dissected under dissection microscope with sterile conditions. Mycetome tissues (which contain the symbiont yeast) were removed from the gut region of the cigarette beetle. The isolated gut region is further smashed with the help of homogenizer. And sample were mixed with 1ml of distilled water and made into serial diluted. The serially diluted sample were poured in culture media such as nutrient agar, sabouraud dextrose agar, yeast extract peptone dextrose agar, potato dextrose agar, melin modified norkrans media by spread plate technique.

3. Media composition

The isolated yeast like symbionts from cigarette beetle was observed for its suitable growth in different media composition such as SDA (Sabouraud Dextrose Agar), PDA (Potato Dextrose Agar) and YEPD (Yeast Extract Peptone Dextrose), MMN (Modified Melin Norkrans media). The sample was poured in different mediums and observed the growth pattern.

1. *Nutrient agar media*

It is a general-purpose agar, or more specifically, a nutritional medium that supports the growth of a wide variety of non-selective organisms and is mostly used for growing microorganisms. Its capacity to support a wide range of bacterial and fungal growth makes it the most often used form of agar. Mix the components such as Beef extract - 0.3 g, Peptone - 0.5 g, Sodium chloride -0.8 g, Agar -1.5 g. Suspend it in a 100ml of distilled water. Bring the mixture to heat making sure you constantly stir the mixture to totally dissolve all its components. Once the mixture is fully dissolved, the next step is to an autoclave for 15 minutes at 121°Celsius. Once autoclave is complete, let the medium rest. The purpose is to cool it down and not solidify.

2. *Sabouraud dextrose agar media*

SDA is a selective medium for fungal culture and primarily used for the isolation of Dermatophytes, Yeasts and various other Pathogenic and Non- pathogenic fungi as well as it also supports the growth of *Nocardia* spp. and other filamentous bacteria. Moreover, the slightly acidic pH of this SDA medium inhibits the growth of most of the bacteria and permits the growth of almost all the fungi with various modifications applicable. Mix the ingredients such as dextrose - 4g, peptone -1g, agar -1.5g. Suspend it in a 100ml of distilled water. Bring the mixture to heat making sure you constantly stir the mixture to totally dissolve all its components. Once the mixture is fully dissolved, the next step is to an autoclave for 15 minutes at 121° Celsius. Once autoclave is complete, let the medium rest. The purpose is to cool it down and not solidify.

3. *Potato dextrose agar media*

Potato Dextrose Agar (PDA) is used for the cultivation of fungi. Potato Dextrose Agar (PDA) is a general-purpose medium for yeasts and moulds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products and testing cosmetics. PDA can be used for growing clinically significant yeast and moulds. The nutritionally rich base (potato infusion) encourages mould sporulation and pigment production in some dermatophytes. Suspend potato extract -0.4g, dextrose -2g in a 100ml of distilled water and boil to dissolve. Autoclave 15 min at 121°C.

4. *Yeast extract peptone dextrose media*

Yeast extract peptone dextrose (YPD or YEPD) Growth Agar is used for the maintenance and propagation of yeasts including *S. cerevisiae* in various molecular microbiology procedures. YPD functions as a complete medium for yeast growth. YEPD consists of yeast extract, peptone, and glucose or dextrose. Yeasts grow well on a minimal medium containing only dextrose and salts. The addition of protein and yeast cell extract hydrolysates allows faster growth so that during exponential or log-phase growth. Yeast extract supplies B-complex vitamins and it contains all the amino acids necessary for growth. Peptone acts as the source of nitrogen, vitamins, and minerals. Dextrose serves as the carbon source. This medium supports the

vigorous growth of wild type as well as mutant strains of all kinds of budding yeast. Heat the agar medium with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave the broth media at 121°C for 15 minutes. Cool to 45-50° C.

4.3.5 Modified melin norkrans media

MMN medium(Modified Melin-Norkrans medium) is a type of agar growth medium, used to grow cultures of mycorrhizal fungi, such as *Boletus edulis* and *Tricholoma matsutake*. It was first described by DH. Marx in the influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. *I. Antagonism* of mycorrhizal fungi to root pathogenic fungi and soil bacteria in 1969. The acidic pH (5.6) of MMN agar inhibits bacterial growth. 10 g/L glucose, 3 g/L malt extract, 0.25 g/L (NH₄)₂HPO₄, 0.025 g/L NaCl, 0.5 g/L KH₂PO₄, 0.05 g/L CaCl₂, 0.15 g/L MgSO₄·7 H₂O, 0.012g/L FeCl₃· 6 H₂O, 0.003 g/L thiamine, 15 g/L agar suspend these ingredients with 1000ml of distilled water. Once the mixture is fully dissolved, the next step is to autoclave for 15 minutes at 121° Celsius. Once autoclave is complete, let the medium rest. The purpose is to cool it down and not solidify.

4. Plating technique

Prepare the culture media and add antibacterial reagent like ampicillin. Mix it thoroughly. Ampicillin used as an inhibitor for bacterial contamination. Then pour the media into sterile Petri plates and allow solidify. After solidification of media pour 0.1ml sample. Spread the sample by using l-rod. Incubate at 28°C and 37°C for two to three days and observed the mycelial growth.

5. Lacto phenol cotton blue staining

Lacto phenol cotton blue is a stain that is used to examine fungal elements following either a tape preparation or a scraping. This stain contains phenol, which will kill the organisms, lactic acid which preserves fungal structures, and cotton blue which stains the chitin found in the fungal cell walls. The microscopic fungal

morphology will be used to identify the mould. A loop full of smear culture was taken and streaked in a clean glass slide and heated slightly over the flame. The smear was covered with a thin film of LCB for 1 minutes and placed a cover slip and observed under 40x in the microscope. The culture retaining the long septate hyphae and conidiophores. Microscopic morphology of the filamentous fungus is critical in the identification of moulds since biochemical and molecular methods are not readily available for the identification of moulds. Hyaline fungi will pick up the blue color of the lactophenol, while the dematiaceous fungi will stain brown due to the pigment produced by the mould.

6. Biochemical assay

Biochemical testing necessitates the determination of different parameters, and the identification of the main biological chemical compounds, by using molecular and biochemical tools. Biochemical tests are among the most important methods for microbial identification. Routine biochemical tests include tests for carbohydrate fermentation test and germ tube test.

1. Fermentation test

The carbohydrate fermentation test is used to determine whether or not bacteria and fungi can ferment a specific carbohydrate. Carbohydrate fermentation patterns are useful in differentiating among bacterial and fungal groups or species. It tests for the presence of acid and/or gas produced from carbohydrate fermentation. Prepare the Phenol red carbohydrate broth (Trypticase or protease peptone - 10g, NaCl -5g, Beef extract -1g, Phenol red -0.018g, carbohydrate -10g include glucose, sucrose, galactose, lactose, maltose, fructose, dextrose, xylose). Prepare broth medium by mixing all ingredients in 1,000 ml of distilled and deionized water; heat gently to dissolve. Use a single carbohydrate for each batch of medium prepared. Fill 13- by 100-mm test tubes with 4 to 5 ml of phenol red carbohydrate broth. Insert an inverted fermentation tube or Durham tube to detect gas production. Sterilize media by autoclaving for 15 minutes at 116 to 118°C. The sterilization process will also drive the broth into the inverted fermentation tube or Durham tube. When using arabinose, lactose, maltose, salicin, sucrose, trehalose, or xylose, autoclave at 121°C for only 3 minutes as these carbohydrates are subject to breakdown by

autoclaving. Broth medium will be a light red color. The final pH should be 7.4 ± 0.2 . Cool all media before use. Store prepared media at 4 to 10°C. Refrigerated media has a shelf life of approximately 6 to 8 weeks. Run controls to check for possible breakdown of carbohydrates. Inoculate loop of culture on media. After 24 hours observe the colour changes and bubble formation.

4.6.2 Germ tube test:

The Germ Tube Test procedure is used for the presumptive identification of *Candida* species. The culture of *Candida* species is treated with normal human pooled serum or sheep or rabbit or fetal calf serum and incubated at 37°C for 2-4 hours. A loop of culture streaked in the sheep serum. Then incubated at 37°C for 3 hours. After incubation 0.5ml of serum smear in the glass slide. Then placed a coverslip and observed under microscope.

4.7 Sequencing analysis:

Followed by the biochemical assays, the isolates were given for sequencing analysis to identify the organism by molecular technique and to confirm the species level information of the isolate. DNA was isolated and amplified with 18s specific universal primer. NS1 (GTAGTCATATGCTTGTCTC) and NS4 (CTTCCGTCAATTCCTTTAAG) 18s universal primers were used as forward and reverse primer. The amplified products were further checked for its quality and subjected to sanger sequencing.

The obtained sequences were undergone for quality checking and eliminated the not so good sequences by analysing the chromatogram on bio edit sequence analysis tool. Forward and reverse sequences were obtained and reverse sequences selected and changed as reverse compliment to the forward sequence. CAP contig assembly program was used to overlap the forward and reverse sequence to produce contig sequence which further taken for NCBI BLAST. The contig was given as query sequence and search for the similar sequence and to infer functional and evolutionary relationships between the similar sequences.

CHAPTER -5

RESULTS AND DISCUSSION

5.1 Cigarette beetle growth in different types of flour

Adult insects were maintained in the Laboratory in different composition of flours such as wheat flour, rice flour, turmeric powder; gram flour to enrich the nutrient supplements for cigarette beetle. The developmental period of cigarette beetle varied significantly among various feed (Table 5.1). Wheat flour recorded the shortest developmental period which was followed by turmeric powder, rice flour and Bengal gram flour. Shortest developmental period of *L. serricorne* in wheat flour may be because of the balanced and essential nutrients present in the wheat flour.

Table 5.1 Cigarette beetle growth in different types of flour

S.No	TYPES OF FLOUR	GROWTH
1	Wheat Flour	+++
2	Rice Flour	+
3	Turmeric Powder	++
4	Gram Flour	+

Note: + Slight growth, ++ Moderate Growth, +++ Good growth

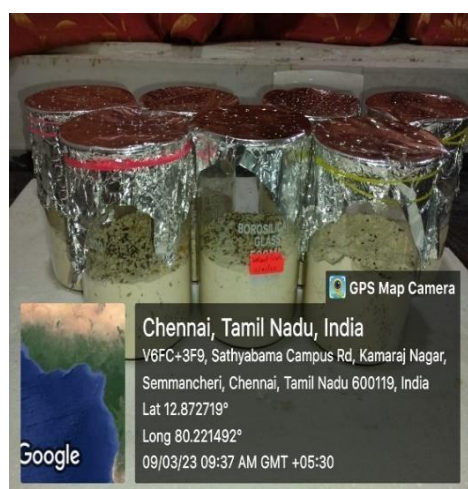


Fig :5.1 Wheat flour



Fig: 5.2 Rice flour



Fig: 5.3 Turmeric powder



Fig: 5.4 Bengal gram flour

5.2 GROWTH OF CIGARETTE BEETLE

Egg

The eggs are opaque pearl white when freshly laid but become yellowish shortly before hatching. They are oval or oblong in shape. They are varying from 0.29 - 0.50mm long and 0.18-0.25mm in diameter. Egg observed under microscope 5-7 days show in (fig: 5.5).

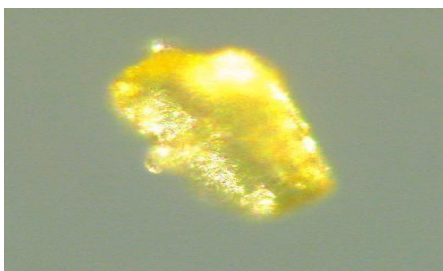


Fig: 5.5 Cigarette beetle egg (microscopic view)

Larva

They are creamy white in colour with a yellow head and brown mouth parts. Mature larvae of the tobacco beetles are C-shaped and about 3/16' long. They have long yellowish hair (Bio protection portal). Larvae observed under microscope 40-42 days show in (fig:5.6)



Fig: 5.6 Larvae of cigarette beetle(Microscopic view)

Pupa

The duration of the adult's stay in the pupal chamber from the day the cocoon was fully created to the day the cocoon was fully formed was computed. Pupa observed under microscope 1-4 weeks show in (fig : 5.7)

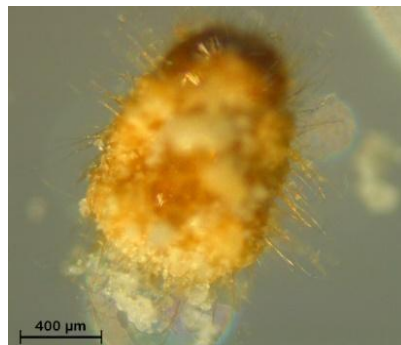


Fig : 5.7 Pupa of cigarette beetle(Microscopic view)

Adult

The adult beetles were reddish brown in colour with smooth elytra. Adult observed under microscope 1-4 weeks show in (fig : 5.8)



Fig : 5.8 Adult of cigarette beetle (Microscopic view)

5.3 Growth Characterization

The isolated yeast-like symbionts from the cigarette beetle were tested for their ability to grow in a variety of media, including SDA (Sabouraud Dextrose Agar), PDA (Potato Dextrose Agar), YEPD (Yeast Extract Peptone Dextrose), and MMN (Modified Melin Norkrans medium). The sample's growth pattern was studied after being put onto various media (Table: 5.2). In YEPD and MMN, the sample grows faster than in SDA and PDA. The optimum temperature is 28°C.

Table 5.2 Growth Characterization

CHARACTERISTICS	GROWTH	OPTIMUM GROWTH
Different Media		
Nutrient agar	+	
SDA	+	
PDA	+	YEPD&MMN
YEPD	+++	
MMN	++	

Temperature:		
28 °C	+++	28°C
37°C	+	
No of days		
1	+	
2	++	3
3	+++	

Note: +++ good growth, ++ Moderate growth, + slight growth

5.4 Morphological characterization

The morphological and cultural characteristics of the strain were observed by naked eyes after incubated on YEPD for 3 days. At the early growth stage, the colony of the strain was white and grew mycelium. In the maturing stage, the colony of the strain was displayed smoky green in the center and white around with spore on the surface.

Table: 5.3 Morphological Characterizations

S.NO	CHARATERISTICS	OBSERVATION
1	Colour of the colony at early stage	White opaque
2	Colour of the colony at mature stage	Smoky green
3	Pigmentation	Black
4	Hyphae	Long septate hyphae
5	Spore	Conidiospore
6	Spore surface	Slightly smooth and roughend surface

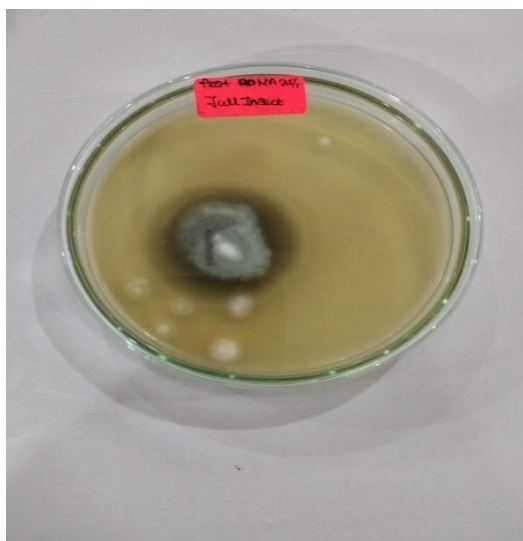


Fig : 5.9 Colony morphology at early stage



Fig : 5.10 Colony Morphology at mature stage

5.5 Fermentation Test:

A loop full of *Aspergillus* culture from a well sporulated mycelium was streaked in the Phenol red carbohydrate broth (Trypticase or protease peptone - 10g, NaCl - 5g, Beef extract -1g, Phenol red -0.018g, carbohydrate -10g include glucose, sucrose, galactose, lactose, maltose, fructose, dextrose, xylose) containing Durham

tubes. Then incubated at 28°C for 24 hours. In this test yeast like symbiont can able to ferment monosaccharides. But, it cannot able to ferment disaccharides.

Table 5.3 Fermentation test result

FERMENTATION TEST	
SUGARS	RESULTS
Sucrose	Negative
Maltose	Negative
Fructose	Positive
Dextrose	Positive
xylose	Negative
Glucose	Positive
Galactose	Positive
Lactose	Positive

NOTE: (+) positive (Media colour changes into yellow and bubble formation occurs)

(-) Negative (No colour changes and bubble formation not occur)

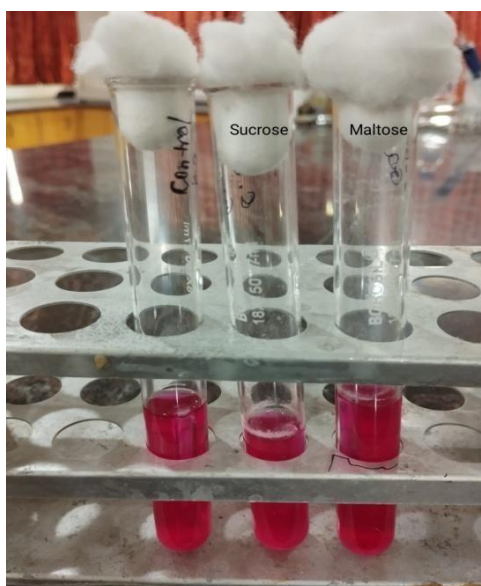


Fig:5.11 Sucrose and maltose fermentation

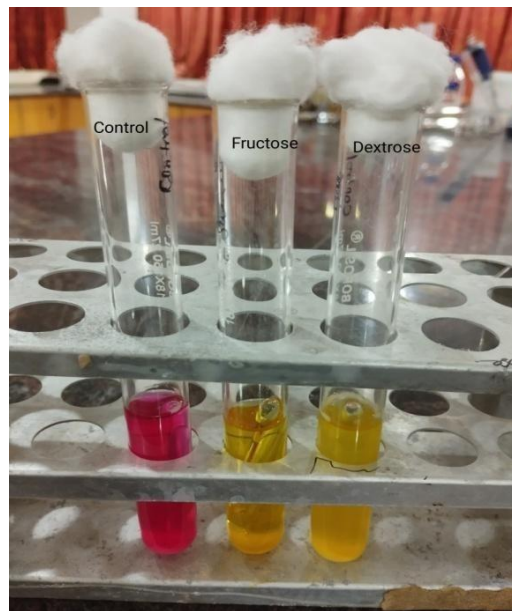


Fig:5.12 Fructose and dextrose fermentation

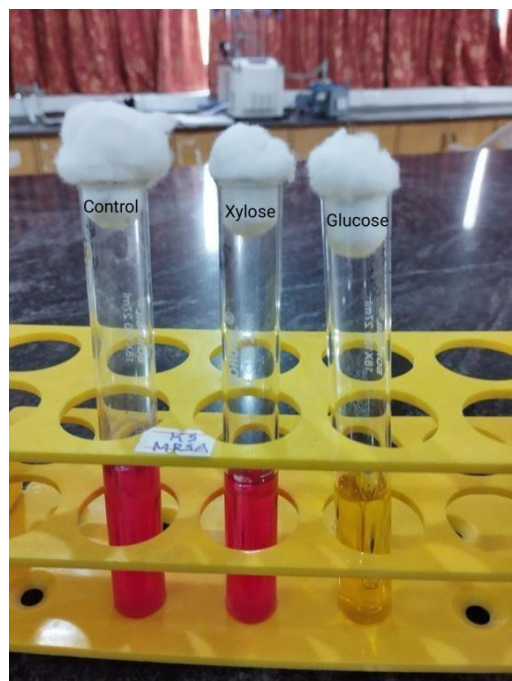


Fig:5.13 Glucose and xylose fermentation

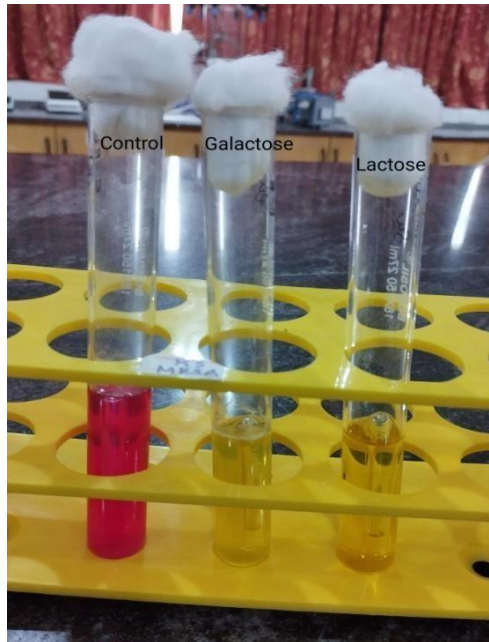


Fig:5.14 Galactose and lactose fermentation

6. Germ tube test

A loop of culture streaked in the sheep serum. Then incubated at 37°C for 3 hours. After incubation 0.5ml of serum smear in the glass slide. Then placed a coverslip and observed under microscope. Germ tube not occurs it indicate negative result.

7. Lacto phenol Cotton Blue Staining:

A loop full of smear culture was taken and straked in a clean glass slide and heated slightly over the flame. The smear was covered with a thin film of LCB for 1 minutes and placed a cover slip and observed under 40x in the microscope. The culture retaining the long septet hyphae and conidiophores.

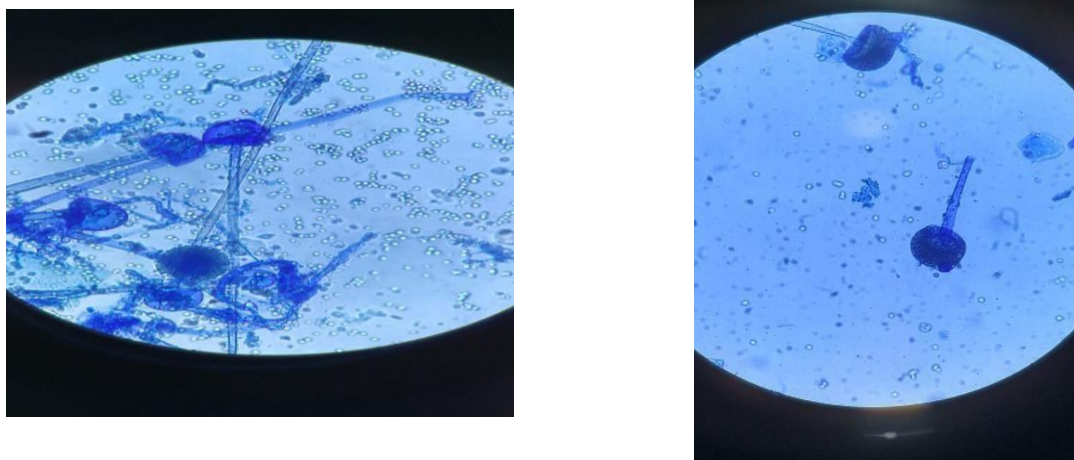


Fig: 5.15 Microscopic images of Aspergillus

5.8 Sequencing analysis

The organism cultured from the gut region of cigarette beetle subjected for 18S rRNA sequencing to confirm its species level information and its evolutionary relationship with the nearby organisms. Chromatogram shows the sequences obtained from the sequencing and its peak intensity infer with the respective nucleotide. Each nucleotide denoted in different colours (A- green; T- red; G- black and C- Blue) The peak intensity and sharpness will improve the reliability of the particular nucleotide. Based on this, good nucleotide reads were selected and imported to bio edit tool. Contig was prepared and the contig length was 1152 base pairs. The sequence BLAST with the nucleotide sequence database and the organism similar with our query has been listed. The highest query coverage was 96% for uncultured fungus with percentage identity of 96.39 % and aspergillus puulaauensis with the percentage identity of 96.30 %. Totally 100 similarity hits were listed out and most of the similar organism was aspergillus sps. Alignment of the sequences were analysed with query and result organism. An unrooted phylogenetic was developed with the results obtained from NCBI BLAST. Our query observed to be a sister taxon of aspergillus sp. Thus, the phylogenetic analysis revealed that the query sequence comes under Aspergillus sps.

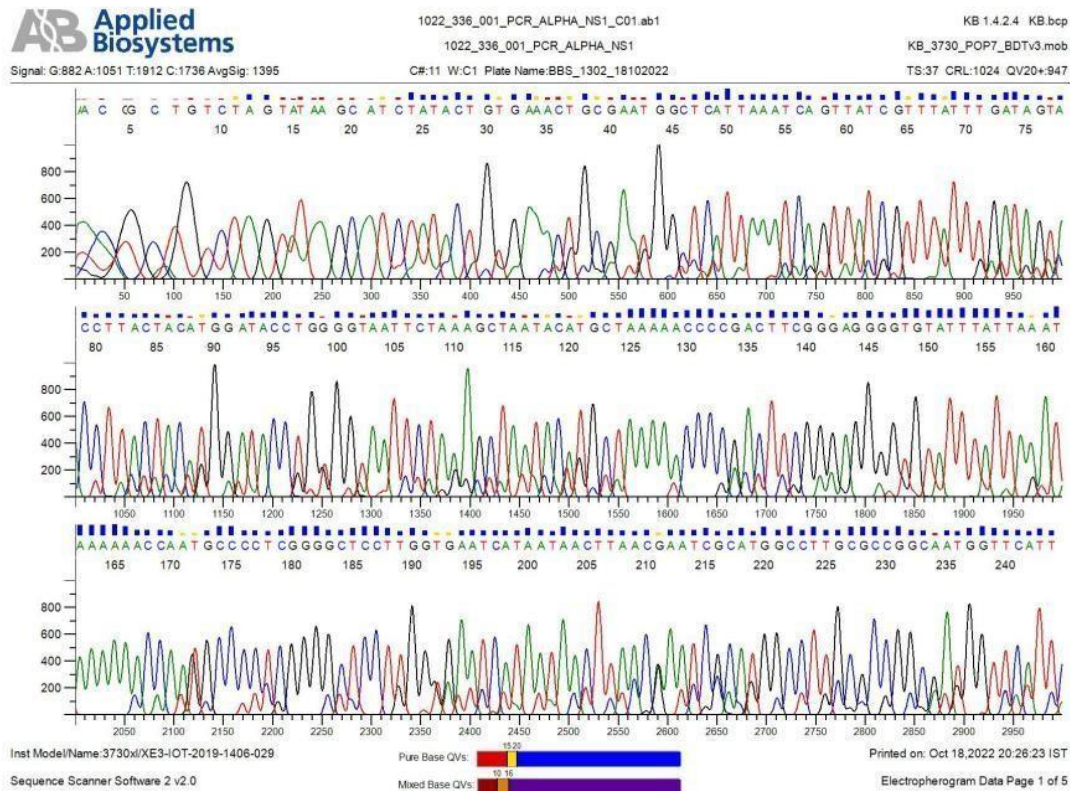


Fig 5.16: Chromatogram Data

Contig:

>Contig-0

TTGTTATGGTGCATATATTGCTGTTCTCAAAGAATTAAGCATGCATGTTCTTAGGTATTAAGCCATTCTATACTG
 TGAAACTGCGAATGGCTCATTAAATCAGTTATTCGTTTTATTTGATAGTACCTTACTACATGGATTACCTGGGG
 TAATTCCTAAAGCTTAATACATGCTTAAAAACCCCGACTTCGGGAGGGGATGTTATTTTATTAATAAAAAAAC
 AATGCCCTTCGGGGCTCCTTTGGTGAATCATAATTAACCTTTAACGAATCGCATGGCCTTTGCGCCGGCAATGG
 TTCATTCAAATTTCTGCCCTATCAACTTTCAATGGTAGGGATAGTGGCCTACCATGGTGGCAACGGGTAACG
 GGAATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGC
 GCAAATTACCAATCCCGACACGGGGAGGTAGTGACAATAAATACTGATACGGGGCTCTTTGGGTCTCGTAA
 TTGGAATGAGTACAATCTAAATCCCTTAACGAGGAACAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGT
 AATTCAGCTCCAATAGCGTATATTAAGTTGTTGCAAGTTAAAAAGCTCGTAGTTGAACCTTGGGTCTGGCTG
 GCCGGTCCGCCTACCGCGAGTACTGGTCCGGCTGGACCTTTCTTCTGGGGAATCCCATGGCCTTCACTGGC
 TGTGGGTGGAACAGGACTTTTACTGTGAAAAAATTAGAGTGTTCAAAGCAGGCCTTTGCTCGAATACACTAG
 CATGGAATAATAGAATAGGACGTGCGGTTCTATTTTGTGGTTTCTAGGACCCCGTAATGATTAATAGGGAT
 AGTCGGGGGCGTCAGTATTCAGCTGTGAGAGGTGAAATCTTGGATTTGCTGAAGACTAACTACTGCGAAAG
 CATTGCGCAAGGATGTTTTATTAAATCAGGGAACAAAAGTTAGGGGATCGAAAACGATCAAATACCGTCGTAG
 TCTTAACCATAAACTATGCCGACTAGGGATCGGGGCGGCTTTCTATGATGACCCGCTCGGCACCTTACAAAAA
 ATCAAAGTTTTTGGGTTCTGGGGAGAATTTGCCATAGCGAAAAATTTCTCACGTG

Fig: 5.17 Contig Data

Descriptions	Graphic Summary	Alignments	Taxonomy						
Sequences producing significant alignments									
Download Select columns Show 100 ?									
select all 100 sequences selected									
GenBank Graphics Distance tree of results MSA Viewer									
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
✓	Uncultured fungus clone nco38g07c1 18S ribosomal RNA gene, partial sequence	uncultured fungus	1801	1801	96%	0.0	96.39%	1802	KC670680.1
✓	Aspergillus puulaauensis 18S ribosomal RNA (APUU_r60001S).rRNA	Aspergillus puul...	1796	1796	96%	0.0	96.30%	1797	XR_005976930.1
✓	Uncultured fungus clone nco40c10c1 18S ribosomal RNA gene, partial sequence	uncultured fungus	1796	1796	96%	0.0	96.30%	1764	KC670824.1
✓	Aspergillus puulaauensis MK2 DNA, chromosome 6, nearly complete sequence	Aspergillus puul...	1796	7184	96%	0.0	96.30%	3585120	AP024448.1
✓	Aspergillus versicolor NRRL 238 18S rRNA gene, partial sequence, from TYPE material	Aspergillus vers...	1790	1790	96%	0.0	96.21%	1771	NG_067623.1
✓	Aspergillus versicolor gene for 18S rRNA, partial sequence	Aspergillus vers...	1786	1786	94%	0.0	96.52%	1733	AB008411.1
✓	Aspergillus sp. Y31-2 18S ribosomal RNA gene, partial sequence	Aspergillus sp...	1781	1781	94%	0.0	96.43%	1732	KP872504.1
✓	Uncultured marine eukaryote clone UI12H09 small subunit ribosomal RNA gene, partial sequence	uncultured mari...	1777	1777	94%	0.0	96.50%	1558	EU446322.1
✓	Aspergillus sydowii strain DAOM 213727 18S ribosomal RNA (SSU) gene, partial sequence	Aspergillus syd...	1775	1775	93%	0.0	96.75%	1057	JN938975.1
✓	Aspergillus sp. LFJ1403 18S ribosomal RNA gene, partial sequence	Aspergillus sp...	1773	1773	92%	0.0	97.00%	1563	KM925044.1
✓	Aspergillus versicolor strain PSFNRO-2 18S ribosomal RNA gene, partial sequence	Aspergillus vers...	1773	1773	92%	0.0	97.00%	1587	HQ393875.1
✓	Aspergillus versicolor strain HDJZ-ZWM-16 18S ribosomal RNA gene, partial sequence	Aspergillus vers...	1773	1773	92%	0.0	97.00%	1699	GU227343.1
✓	Aspergillus terreus isolate F38C small subunit ribosomal RNA gene, partial sequence	Aspergillus terre...	1772	1772	92%	0.0	97.08%	1656	MT192569.1
✓	Aspergillus sp. strain 3G small subunit ribosomal RNA gene, partial sequence	Aspergillus sp...	1772	1772	93%	0.0	96.66%	1686	MH071383.1
✓	Aspergillus sp. Y19-2 18S ribosomal RNA gene, partial sequence	Aspergillus sp...	1772	1772	92%	0.0	97.08%	1679	KP872521.1
✓	Aspergillus sp. isolate CISHDT-CLF16 small subunit ribosomal RNA gene, partial sequence	Aspergillus sp...	1772	1772	92%	0.0	97.08%	1645	MT762356.1
✓	Aspergillus sp. isolate 50 small subunit ribosomal RNA gene, partial sequence	Aspergillus sp...	1770	1770	91%	0.0	97.16%	1654	MT649543.1

Fig: 5.18 NCBI Result

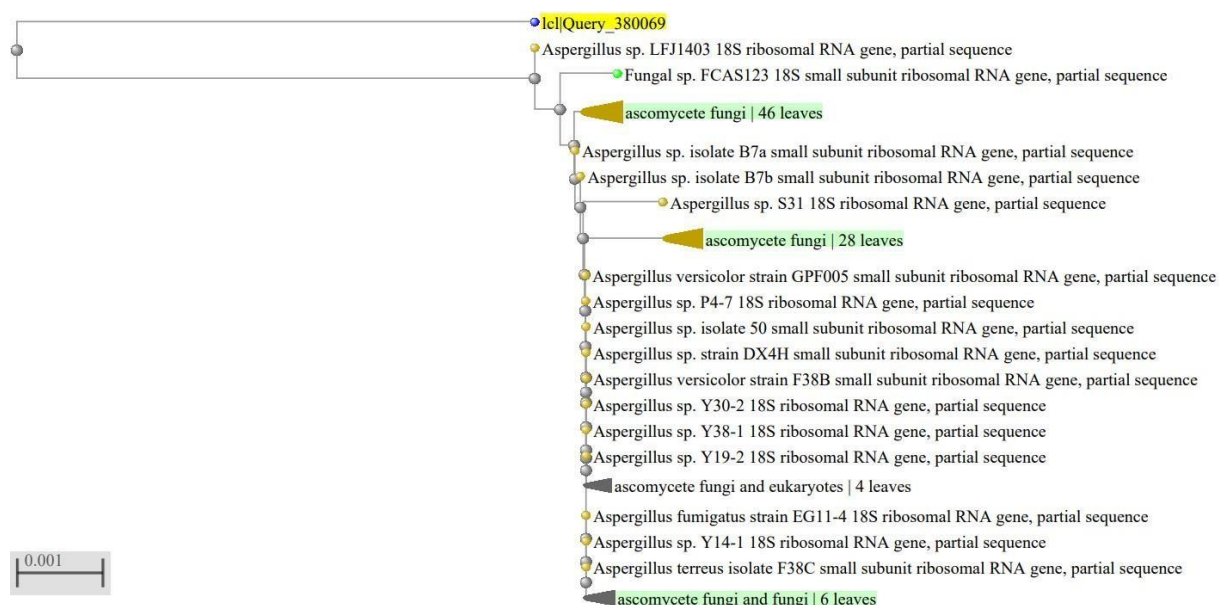


Fig 5.19: Phylogenetic tree

CHAPTER -6

6.1 SUMMARY AND CONCLUSION

Yeast-like endosymbionts (YLSs) of insects are frequently confined to particular hosts and are necessary for the host's survival. Nearly 100 cases of mutualisms between various yeast species and beetles were discovered in yeasts associated with insects. Even though their phylogenetic origins appear to be different, these yeasts play clear roles in nutrition and detoxification.

The developmental period of cigarette beetle varied significantly among various feed. Wheat flour recorded the shortest developmental period which was followed by turmeric powder, rice flour and Bengal gram flour. Shortest developmental period of *L. serricorne* in wheat flour may be because of the balanced and essential nutrients present in the wheat flour.

The isolated yeast-like symbionts from the cigarette beetle were tested for their ability to grow in a variety of media, including SDA (Sabouraud Dextrose Agar), PDA (Potato Dextrose Agar), YEPD (Yeast Extract Peptone Dextrose), and MMN (Modified Melin Norkrans medium). The sample's growth pattern was studied after being put onto various media (Table: 5.2). In YEPD and MMN, the sample grows faster than in SDA and PDA. The optimum temperature is 28°C.

The organism cultured from the gut region of cigarette beetle subjected for 18S rRNA sequencing to confirm its species level information and its evolutionary relationship with the nearby organisms. The results obtained in chromatogram and prominent peaks were noticed. FastA format for the same were obtained and the contig file was uploaded in NCBI website for BLAST. The blasted data showed significant species *Aspergillus puulaauensis* and an evolutionary tree was made to determine closely related species and also determined the correct species in data.

These data shows yeast like species are present in the gut of the insect and specifically *Aspergillus puulaauensis*.

Further more studies needs to be done in the organism to know the role and function of the organism and its nature which helps in finding the growth and development of *Lasioderma serricorne*.

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