THE SCREENING OF ANTIMICROBIAL ACTIONBACTERIA AND FUNGI FROM THE LARVALAND ADULT STAGES OF PACHILOPTA ARISTOLACHIA

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INTRODUCTION

Insects represent one of the most important forms of life on this planet.(pedigo,2003) insects comprise little more than two third of known species of all kinds of animals(David,1988).they are found in almost all types of environment. They have influenced human existence since it's very beginning and continue to control many of our daily activities. As contemporary to the belief that intelligence is a necessary component of evolutionary success insects are the most successful animals on earth based on their numbers (pedigo,2003)

1.1 BUTTERFLIES

Lepidoptera are the second largest order of insects and are widespread and widely recognizable in nature. Although butterfliesand moths play an important role in ecosystems as pollinators and as prey in the food chain, their caterpillars are problematic in agriculture because their main food source is live plants. Due to thelow nutrient content, indigestibility and toxicity of many plant tissues [1], herbivorous insects have developed numerous traits to overcome these dietary obstacles. While previous research has mostly focused on the counter adaptations rooted in the insect genome [2], microbial symbionts, particularly those inhabiting the gut, are becoming increasingly recognized as a significant player in insect-plant interactions [3], [4], [5], [6], [7].

Papilionids are commonly known as swallowtails , and they are large in size, black or brown in colour with varied markings. Theyare generally found in forest area's and plains . The caterpillars though sluggish and they voracious feeders and feed mainly on plants of the families S Rutacea, aristolachia, and amonacea. There are 700 species of swallowtails recorded all over the world of which 107 species arefound in India and 19 species in southern India.

1.2 NECTAR FEEDING BEHAVIOUR

Butterflies can eat anything that can dissolve in water. They mostlyfeed on nectar from flowers but also eat tree snap, dung, pollen.

They are attracted to sodium found in Salt and sweat . Butterflies are ecologically diverse group of insects showing complex foragingbehaviour during searching for food and nectar (sourakov et al. 2012) . The nectar of flower is the main source of adult nutrition(Omura and Honda ,2005) .

1.3 PHYTOCHEMICALS AND THEIRSIGNIFICANCE

The plant kingdom Is a treasure house of potential drugs and in therecent years there has been an increasing awareness about the importance of medicinal plants . All plants produce chemical substance for pollination, protection from predator and pathogen ,which are know as phytochemicals . These are produced from plants in two forms in plants – medicine and poison. They generally have biological activity in the plant host and play a role inplant growth or defense against competitors , pathogen or predators .

1.4 Pachilopta aristolachia

Pachliopta aristolochiae, the common rose,[2][3] is a swallowtail butterfly belonging to the genus Pachliopta, the roses, or red- bodied swallowtails. It is a common butterfly which is extensively distributed across south and southeast Asia

Kingdom:. Animalia
Phylum:. Arthropod
Class :. Insecta
Order:. Lepidoptera
Family:. Papilionidae
Genus:. Pachilopta
Species:. P.aristolachiae
BINOMIAL NAME
Pachilopta aristolachia

It is widely distributed in Asia including Afghanistan, Pakistan, India (including the Andaman Islands), Nepal, Sri Lanka, Myanmar, Thailand, Japan (south-western Okinawa only), Laos, Vietnam, Cambodia, Nicobar Islands, peninsular and eastern Malaysia, Brunei,Philippines (Palawan and Leyte), Indonesia, Bangladesh and Taiwan.[2][3]

Aristolochia indica (native language: Garudakkodi / Eswaramooli) isa creeper plant found in Southern India and also Sri Lanka. It Is known as 'sapsada' in Sri Lanka and is critical to the survival ofthe southern birdwing and common birdwing,[1] as well as crimson and common rose butterflies. It reaches a height of several metres on trees and cover the branches with thick foliage. It is commonly found in forest floor, rocky hillslopes. It flowers once a year to produce seeds. It can also be propagated by roots. The plant has a number of historical medicinal uses.[*vague*]

REVIEW OF LITERATURE

Bacterial symbionts inhabiting insects can significantly impact the biology of their host (Douglas, 2015). These symbionts can be distinguished as intra- and extracellular based on whether they live within insect cells, or colonize the lumen or lining of insect cavities and body surface (Dillon and Dillon, 2004; Engel and Moran, 2013; Hansen and Moran, 2014).

Symbionts are considered as primary or secondary, depending on whether they are needed by the host to survive or provide nonessential benefits (Douglas, 2015).

Obligatory symbionts are commonly harbored in specialized cells (bacteriocytes) and play important roles for nutrition in certain insect groups, particularly in phloem feeding taxa. For example, intracellular Buchnera bacteria associated with aphids provide essential amino acids and vitamins (Baumann et al., 1997; Hansen and Moran, 2014). The benefits provided by secondary symbionts, on the other hand, are often context-dependent. In aphids, forexample, secondary symbionts can provide a range of ecological benefits including resistance to pathogens and parasitoids, and heattolerance, but they can be costly under benign conditions (Oliver et al., 2010). Some Wolbachia strains living intracellularly can manipulate host reproduction to favor their own spread in the population (Werren et al., 2008; Zug and Hammerstein, 2015), whileothers can be facultative (Teixeira et al., 2008) or even become obligatory in certain species (Hosokawa et al., 2010).

The co'position and robustness of gut bacterial communities varies extensively across the animal kingdom ranging from more than 1,000phylotypes in humans (Lozupone et al., 2012), over several hundredsin termites (Hongoh et al., 2005; Brune and Dietrich, 2015), and a fewtens in lepidopterans (Broderick et al., 2004; Robinson et al., 2010; Pinto-Tomás et al., 2011), to an almost complete absence of bacteriain aphid guts (Douglas, 1988; Grenier et al., 2006). In insects, the beststudied and most diverse gut bacterial communities are those belonging to groups feeding on wood, decaying matter, or detritus such as termites, cockroaches, crickets, and some beetles (Dillon andDillon, 2004; Engel and Moran, 2013). Gut bacterial communities often deliver metabolic benefits to their hosts by the provision of digestive enzymes and production of vitamins, thus improving nutrient uptake on deficient diets (Dillon and Dillon, 2004; Anand etal., 2010; Engel and Moran, 2013; Salem et al., 2015).

Furthermore, they can provide protection against pathogens (Dillonand Dillon, 2004) and support detoxification of pesticides or harmfulplant secondary metabolites (Kikuchi et al., 2012; van den Bosch andWelte, 2017; Xia et al., 2017).

Lepidoptera comprise the second most diverse insect order with some of the most devastating agricultural pests worldwide (Sree and Varma, 2015). Yet, clear evidence for bacterial associates playing a fundamental role in lepidopteran biology is scarce. The functional role of the gut microbiome of Lepidoptera has been challenged by a recent study reporting that caterpillars harbor no oronly few resident bacteria when compared to other insect orders (Hammer et al., 2017). The authors of this study argue that this is probably due to caterpillars being rough environments for bacterial colonization, because they possess an unusually alkaline gut with a rapid food passage of approximately two hours. In addition lepidopterans undergo a holometabolous metamorphosis which entirely re-shapes their body structures (Anand et al., 2010). In spite of this harsh environment for the gut microbiota, several studies have shown that bacteria do affect essential physiological functions in Lepidoptera, i.e., facilitation of nutrient acquisition and digestion (Pinto-Tomás et al., 2007; Indiragandhi et al., 2008; Xia et al., 2017), overcoming plant anti-herbivore defenses (Visotto et al., 2009; Xia et al., 2017), or strengthening of immune responses for protection against pathogens (Shao et al., 2017).

The high variability of the lepidopteran gut microbiome could be promoted by different drivers, which may act alone or in concert and include the environment, insect diet, insect developmental stage, and

gut physiology. Firstly, the environment where insects live affects the composition of the insects' microbiome. Insects reared in the laboratory or collected in the field show different microbial communities even if they feed on the same host plant (e.g., Staudacher et al., 2016). The habitat may thus significantly affect the bacteria associated with lepidopteran species (Staudacher et al., 2016). Secondly, diet can have a major influence on bacterial community variability. Recent studies could not (Staudacher et al., 2016) or hardly (Hammer et al., 2017) detect any resident, host insect-specific, and foodindependent bacteria in Lepidoptera. The bacterial community can therefore be expected to differ significantly between oligophagous and polyphagous species, or between herbivorous and carnivorous species. A comparative study on microbial communities associated with herbivorous and carnivorous Lycaenidae larvae, however, did not find consistent patterns in community profiles that could relate them to the diet ofthe insect (Whitaker et al., 2016). By contrast, an assessment on the influence of diet and host taxonomy on gut bacterial communities across several insect orders found that, depending on the insect taxon, either factor was significant (Colman et al., 2012). Insects feeding on decaying matter presented the richest communities, while bees and wasps had the lowest. While host taxonomy was animportant driver of bacterial communities in hymenopterans and termites, diet was important in insects feeding on lignocellulosederived components. Non-conclusive patterns of clustering among lepidopterans were found, based on a rather small number of species studied (Colman et al., 2012).

In addition to diet and environment, the developmental stage can influence the host's gut microbiota. Concordantly, instar-specific bacterial communities were detected in larvae of the moth *Spodoptera littoralis* (Chen et al., 2016). As in all other holometabolous insect orders, metamorphosis in Lepidoptera entails major morphological rearrangements and is usually accompanied by a change in diet, which can have a strong impact on gut microbiota composition. While almost all lepidopteran species feed upon plant tissue during their larval stage (with a few notable carnivorous and fungivorous exceptions), the adult stage ofmost species feeds on nectar (Strong et al., 1984). With the proviso that gut communities depend on the diet, it is not surprising that bacterial communities differ considerably between larvae and adults of the same species (Staudacher et al., 2016; Xia et al., 2017). Nevertheless, certain taxa may persist throughout the entire insect life cycle as shown for bacteria species belonging to the families Acetobacteraceae, Moraxellaceae, Enterobacteriaceae, Enterococcaceae, Streptococcaceae, and unclassified Bacteroidetes,which dominate the gut of the larval, pupal, and adult stages of the red postman (*Heliconius erato*) (Hammer et al., 2014). Some bacteria like *Enterococcus mundtii* may even survive and propagate in the digestive tract of *S. littoralis* across its life cycle, and persist up to two consecutive generations (Teh et al., 2016). Such persistence

of some bacterial symbionts across the entire development is also found in other holometabolous insects that inhabit different ecological niches during the larval and adult stage,like the emerald ash borer beetle *Agrilus planipennis* (Vasanthakumar et al., 2008), the fruitfly *Ceratitis capitata* (Lauzon et al., 2009), or the scarabaeid

In addition to helping with nutrient acquisition, resident gut bacteria can provide protection against pathogens (Dillon and Dillon, 2004; Florez et al., 2015). One way to achieve this is by outcompeting pathogens, the so-called colonization resistance (Dillon and Dillon, 2004), as found in *Homona magnanima* whose caterpillars are more susceptible to *Bacillus thuringiensis* bacteria when they are reared aseptically than when they are not (Takatsuka and Kunimi, 2000). *Enterococcus faecalis* found in the gypsy moth is known to acidify its local environment so that it can colonize alkaline niches. This probably protects the gut against pathogenic toxins that are activated in alkaline conditions, such as those from *B. thuringiensis* (Broderick et al., 2004). The gut bacterial communities in some insects produce bactericidal substances that selectively target foreign bacteria, but they do not affect autochthonous ones (Dillon and Dillon, 2004). *E. mundtii*, forinstance, is a highly abundant bacterium in the gut of *S. littoralis*, which produces an antimicrobial compound against Gram-positive pa).thogens like *Listeria*, but not against resident gut bacteria (Shaoet al., 2017).

Some common gut bacterial inhabitants may be detrimental or beneficial depending on thecommunity composition of the gut. Oneof such bacteria may be *Serratia* spp., a genus of bacteria commonly reported in lepidopterans and known to be pathogenic in many animals (Chadwick et al., 1990; Ishii et al., 2014). It would be relevant to evaluate if these bacteria provide benefits under certain conditions and whether they switch to a virulent phase when the structure of the bacterial community is altered. If the virulence of *Serratia* spp. depends on the composition of the entire insect microbial community, this would indicate a community-wide role in preventing pathogenic outbursts (Broderick et al., 2004). Therole of gut bacteria in protecting lepidopterans against pathogens should be thus studied in a community context. For instance, while aseptic rearing in some cases results in less susceptibility to *B. thuringiensis* (Takatsuka and Kunimi, 2000), for *Lymantria dispar* it was found that the midgut community is actually required for the activity of *B. thuringiensis* toxin (Broderick et al., 2006

AIM AND OBJECTIVE

AIM : This study aims to investigate antimicrobial action bacteria fromadult and larval stages of Pachilopta aristolochia with following consideration.

OBJECTIVES :

1. Isolation of Bacteria and fungi from adult and larval stage ofPachilopta aristolochia .
2. Comparison of microbial diversity of adult and larval stage ofPachilopta aristolochia.

4. MATERIALS AND METHODS

4.1 collection of samples

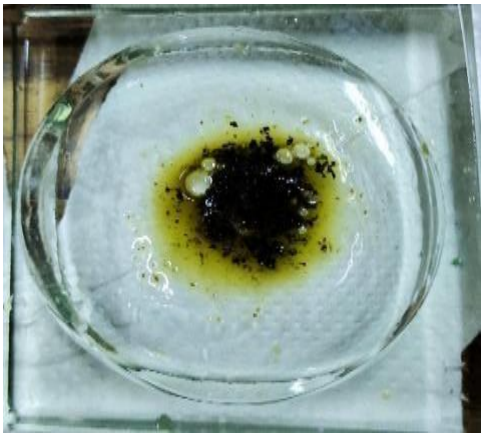
The larvae of butterfly of Pachilopta aristolachia were collected from KFRI- garden peechi, by using net and plastic container , the photographs of the sample were taken by mobile camera at the time of collection . The collected sample were kept in plastic container withlid . Remove the lid and cover the bottle with a sterile cloth to create a suitable environment for its survival.

a. Pachilopta aristolachia

b . (larva)

3.2. Dissection of larvae

- Take a drop of sterile distilled water on a sterile clean glass slide
- Use sterile forces to take termite from screw cap bottle filledwith termite mound
- Then the medium sized suitable termite is placed on a drop ofwater to a glass slide
- Take the gut region using a sterile needle
- Wash the gut with ice cold Nacl [0.1%]
- Gut is chopped with a sterile blade then the sample is transferredinto sterile Eppendorf tube
- Add about 0.3ml buffer
- Homogenize by using votex with 2 min
- Homogenized sample is placed on a centrifuge for centrifugation10000 rpm for 2 min at 35^0c
- After centrifugation take pellet and remove supernatant.



3.3 serial dilution and spread plate .

- After centrifugation 1 ml pellet was taken for serial dilution
- Serial dilution was done with 1 ml Eppendorf tube
- 0.1ml of sample from dilution [10^{-1} to 10^{-6}] was transferred intoeach petri-plate containing nutrient agar for bacteria and PDA for fungi
- Incubate the nutrient agar plates for 24hours at 37^0 c
- Incubate PDA plates for 14 days at room temperature
- After incubation growth were observed in each place of NA andPDA.

3.4. Colony identification, morphologyobservation of bacteria

- After incubation each different individual colonies were spotted on NA plates and quarter streaking was performed using anotherset of plates
- Similarly, the individual colonies in the PDA plates wereinoculated on another set of PDA plates
- There after colony taken from plates and performed gramstaining

3.4.a) Gram Staining

- Loopful of culture were taken on a clean glass slide with 1drop of sterile distilled water
- Mix thoroughly and prepare smear
- Heat fixation
- Add 1 drop of crystal violet. After 1 min wash the crystalviolet under tap water
- Later add 2 to 3 drops of grams iodine for 1 min
- Wash the grams iodine under tap water after 1 min
- Add 95% ethyl alcohol [decolouriser] for 30 sec
- Wash the decolouring agent under tap water
- Air dry
- Observe it under microscope with AOX then observed withunder 100x in oil immersion
- Biochemical methods are performed for the identificationbacteria by using the colonies from NA plates

3.5. colony morphology and microscopic offungus

- Individual colonies were observed on the PDA plates afterincubation at room temperature for 7-14 days
- Similarly, the individual colony in the PDA plates wereinoculated on another set of PDA plates
- There after colonies are taken from PDA plates andperform slide culture techniques

3.5a) Slide culture

- Aseptically with a pair of forceps place a sheet of sterilefilter paper in a petri-dish
- Place a sterile U- shaped glass rod on the filter paper [rod issterilised by flaming it held of forceps]
- Pour enough sterile water [about 4 ml]
- On filter paper to completely moisten it
- With forceps place a sterile slide on the U- shaped rod
- Gently flame a scalpel to sterilize and cut a 5 mm squareblock of the medium from the place of Sabouraud's-Dextrose-Agar (SDA)
- Pick up the block of the ager by inserting the scalpel and carefully transfer this block aseptically to the centre of theslide
- Inoculate four slides of agar square with spores or mycelialfragments of the fungus to be examined
- Aseptically place a sterile cover glass on the upper surfaceof the agar cube
- Place the cover on the petri-dish and incubate at roomtemp for 48 hrs
- After 48 hrs examine the slide under low power

3.6.a) Bacteria Antibacterial property

For this identification, collect clinical samples such staphylococcusaureus, klebsiella, pseudomonas, E.coli,

- Take nutrient agar plates and inoculate the isolatedbacteria from gut and mound
- After inoculation incubate 24-48hrs at 37^0c
- After the incubation growth will appear on NA plates
- For dual culture method make a central line on a petri-plate and inoculate the clinical sample opposite to the growing bacteria from the gut and mound
- Continue the incubation at 37^0c for 7 days
- Similarly control plates are maintain to compare the growth of bacteria that is inoculated against the clinical sample
- Incubate the control plates

After incubation measure the diameter in both control and dualculture plate at 2,5&7^{th}day

Antifungal property

- Take the nutrient agar plates and inoculates the bacteriafrom gut and mound
- After the inoculation, incubate the NA plates for 24 hrs at37^0c
- After the incubation growth will appear on Na plates
- For the dual culture make a central line on petri-plate and inoculated the plant pathogenic fungi [clonostachys hyssicolae] to the growing bacteria from the gut and mound
- The Na plates is kept at room temperature
- Similarly control plates are maintain to compare thegrowth of bacteria that is inoculated against plant pathogenic fungi

[clonostachys hyssicola]

- Incubates the control plates
- After incubation measure the diameter in dual cultureplate and compare it with the control plate

3.6 b) Fungus Antibacterial property

- Take a PPA plate and inoculate the fungus from gut and mound
- After the inoculation incubate the PDA for 24-48hrs at roomtemperature
- For the duel culture make a central line on a petri-plate and inoculate the clinical sample opposite to the growing fungus fromgut and mound
- The PDA plates were kept at room temperature for 7-14 days
- Similarly control plates are compared the growth of fungus that inoculate against the clinical sample Incubate the control plates
- After incubation measure the diameter in both control and dualculture plates at 2,5&7 days

Antifungal property

- Take a PDA plates and inoculate the fungus from gut andmound
- After the inoculation incubate the PDA for 24-48hrs atroom temperature
- For the dual culture make a central line on a petri-plateand the plant pathogenic fungi [clonstachys hyssicolae]opposite to the growing fungus from gut and mound
- The PDA plates were kept at room temperature for 7-14days
- Similarly control plates are compared the growth of fungus that inoculate against the plant pathogenic fungi [clonstachys hyssicolae]
- Incubate the control plates

After incubation measure the diameter in both control anddual culture plates at 2,5, &7 days

3.7 Biochemical test for identification of bacteria

1. *methylene red test*

Principle

- Used to determine the ability of organism to produce stable acid as the end product of glucose fermentation
- If the large amount of acid that include formic acid acetic acid and lactic acid succinic acid from glucose fermentationthe colour of both will remain red after the addiction of methyl red indicator (positive)
- The organism do not produce acid, the both medium willchanges to yellow indicating a negative test

Procedure

- Pure culture organism is inoculated in the MRVP broth
- Incubate at 37^0c 4 hrs
- Add 5 to 6 drop of methyl red indicator
- Observe the colour change of broth medium

2. Voges-Proskauer testPrinciple

The VP test is used to determine if an organism produce acetylmethyl carbinol is converted to diacetyl in the presence of ∞ naphthol, strong alkaline [45% KOH] and atmospheric oxygen

Procedure

- Inoculate MRVP broth with a pure culture of organism
- Incubate at 35-37^{0}c for a min of 48 hrs in ambient air
- Add 6 drops of VP reagent [∞ Naphthol] and 2 drops VP reagent

II [40% KOH]

- Observe for the colour change in the both medium

3. Citrate testPrinciple

- Citrate agar is used to test an organism's ability to utilize citrate

as a source of energy

- The medium contains citrate as the sole carbon source andinorganic ammonium salt [as] the sole source of nitrogen
- Bacteria that can grow on this medium produce an enzymecitratepermease capable of converting citrate to pyruvate
- When the bacteria metabolize citrate the ammonium salts arebroken down to ammonia which increases alkalinity
- The shift in PH turns the bromothymol blue indicator in themedium from green to blue above PH 7.6

Procedure

- Sterile the slant back and forth with a light inoculum pickedfrom the centre of a well – isolated colony
- Incubate aerobically at 35 to 37 ^{0}c for up to 4-7 days
- Observe a colour change from green to blue along the slant

4. Urease testPrinciple

- The urease test identifies those organisms that are capable of

hydrolysing area to produce ammonia and carbon dioxide

- It is primarily used to distinguish urease – positive protea fromother Enterobacteriaceae

Procedure

- Sterile the surface of a urea ager slant with a portion of a well -isolated colony
- Leave cap on loosely
- Incubate the tube at 35-37^{0}c in ambient air for 48 hrs to 7 days
- Examine for the development of a pink colour for a long as 7days

5. catalytic testPrinciple

- The enzyme catalase mediates the break down of hydrogenperoxide into oxygen and water
- The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide andrapid elaboration of oxygen bubbles occurs
- The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hrs old2H2o2 >2H2o+o2[gas

bubbles] catalase procedure [slid method]

- Use a loop or sterile wooden stick to transfer a small amountof colony growth in the surface of a clean dry glass slide
- Place a drop of 3% H_2O in the glass slide
- Observe for the evolution of oxygen bubbles

6. oxidase testPrinciple

- The oxidase test is used to determine if an organism possess thecytochrome C oxidase enzyme
- The test is used to differentiate pseudomonas from relatedspecies
- The test can also be performed by flooding the culture plate withoxidase reagent but is not recommended because the reagent kills the bacteria
- The test is spot method based on colour change and useful in theinitial characterisation of gram -negative micro-organism
- It is used to differentiate oxidase positive micro -organisms suchas Aeromonas SP. Pseudomonas SPP and Halophila SP. From theoxidase negative Enterobacteriaceae

Procedure

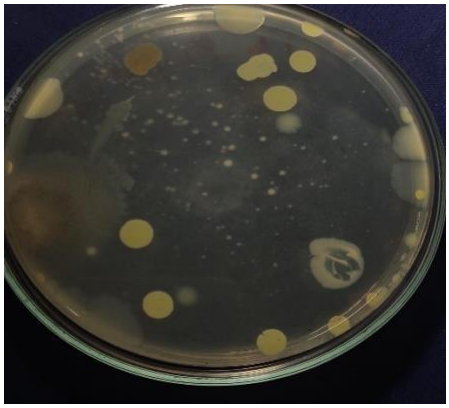
- Take a commercially available oxidase disc containing thereagent
- Pick the isolated colony to be tested and rub it in the disc Observe for colour change within 10 seconds

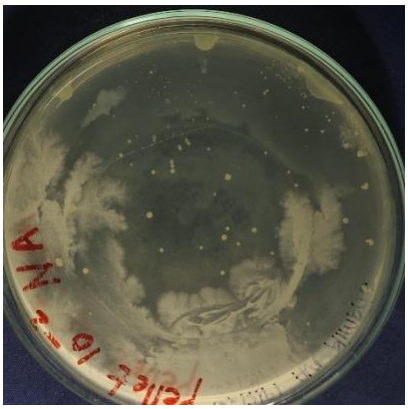
RESULT AND DISCUSSION

Isolation And Identification of Bacteria fromlarva

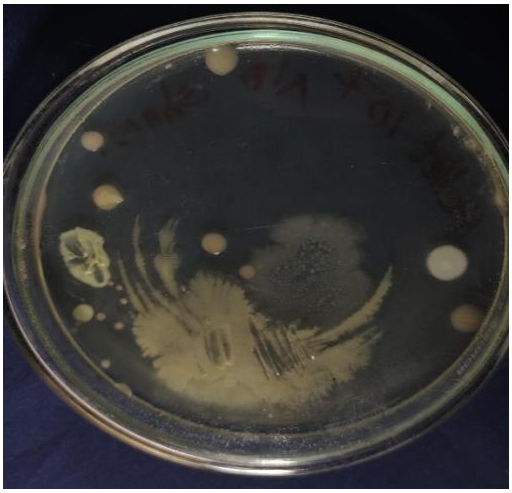
Three bacteria are isolated from larval gut. The bacterial isolates were obtained by dilution plate method at different dilution 10^{-3},10^{-4} and 10^{-5} plating on NA medium [fig 1].

Fig 1.





B.



C.

Morphology And Microscopic Observation

Morphologically all the bacterial isolates exhibited typically colonization characters. These bacteria produced white colonies andsome were yellow, orange colour colonies on nutrient agar medium within 2 to 3 days of incubation.

Fig.2

A.

B.

C.

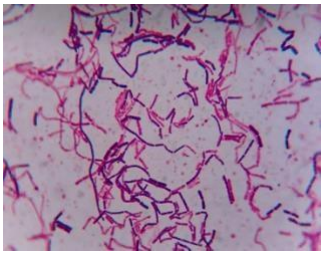
Table 1.1 colony morphology

Test	A	B	C
Size	Medium	Small	Large
Shape	Round	Round	
Margin	Entire	Entire	Irregular
Elevation	Raised	Raised	Flat
Surface tension	Smooth	Smooth	Glisten
Optical character	Translucent	Translucent	Opaque
Pigmentation	White	Yellow	Creamy

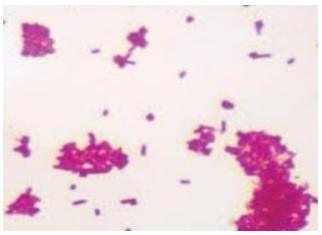
Table 1.2 gram staining

Bacterial Isolates	Cell shape	Gram reaction
A	Cocci	Negative
B	Cocci	Negative
C	Rod	Positive

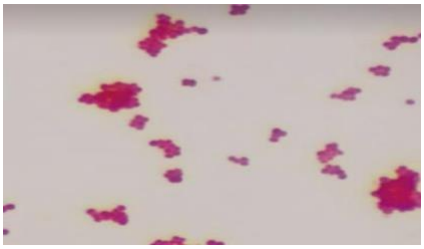
Microscopic examinationFIG.3



A.



B.



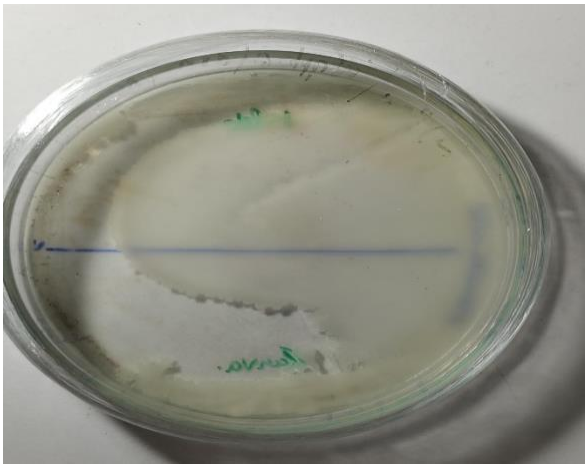
C.

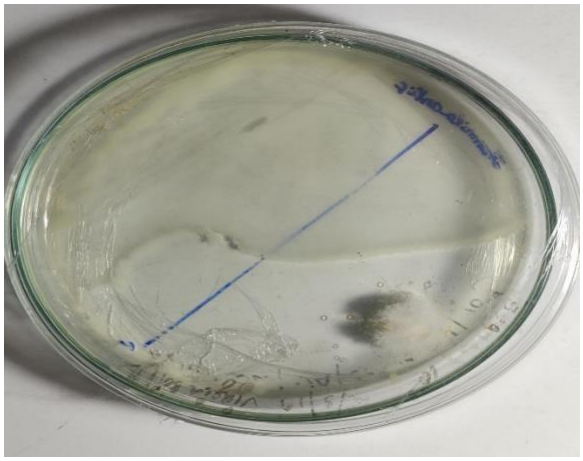
Determination of antibacterial and antifungalproperty of larvae gut bacteria

the three Bacteria were dual cultured to check if they have any antibacterial and antifungal property. As a result, the bacteria showing antibacterial property against clinical sample such as klebs, psuedo and staph. After 2to 3 days of incubation only samples C Shows the antibacterial and antifungal property the rest were eliminated.

Fig.4

a.dual culture plate with clinical sample kleb.





b. Dual culture plates with fungal pathogen.

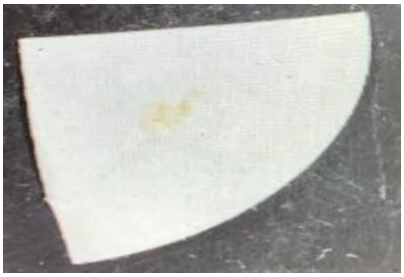
Table 5: Biochemical tests for sample c

Isolated strain	Samplec
MR	-ve
VP	+ve
Citrate	+ve
Urease	+ve
Catalase	+ve
Oxidase	-ve
Indole	-ve

Fig6.

a. MRVP biochemical test slants for sample c

b. Catalase + ve



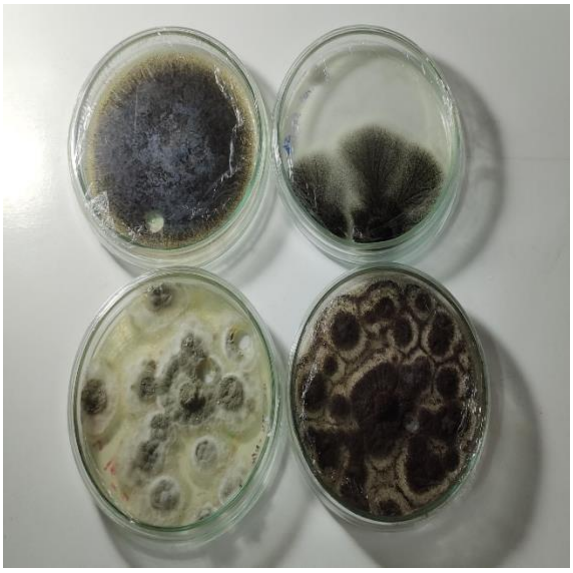
C. Oxidase – ve

Isolation and Identification of Fungus fromlarvae

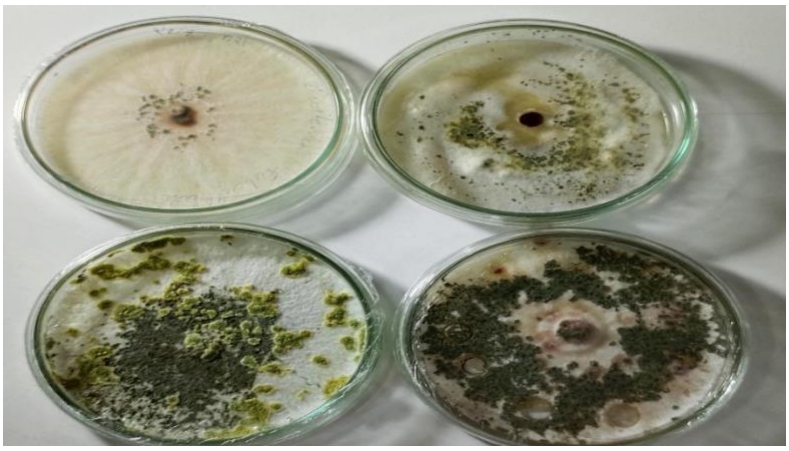
3 type fungi are isolated from the larval gut, the fungus isolates were obtained by dilution plate method at different dilution 10^{-2} ,10^{-3} , 10^{-4} and 10^{-5} plated on PDA plates.[fig 6]

Fig 6.

A type variety .



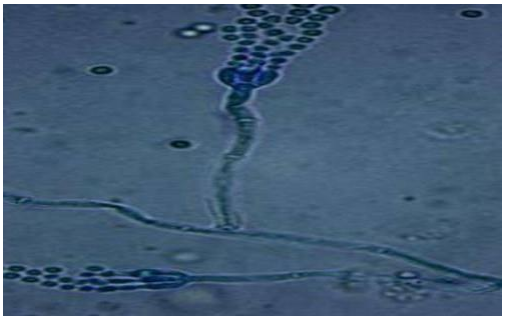
B type variety.



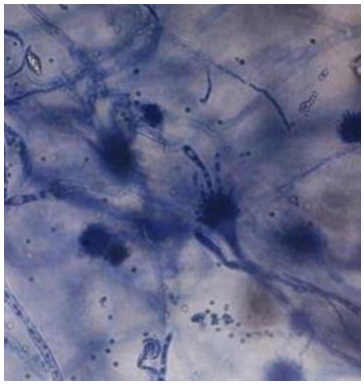
C type variety.

Microscopic Observation of Fungus

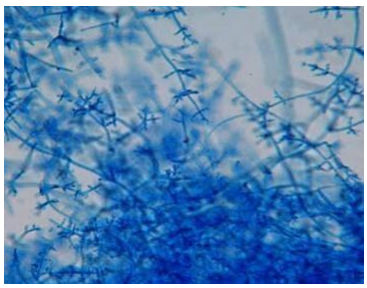
By using slide culture technique, microscopically all the isolates exhibit typical spores or mycelial growth. Most of the isolates have well developed spores on PDA plates within 6 to 7 days of incubation.[fig 7.]



A



B



C

Determination of antibacterial andantifungal property of gut fungus

By using dual culture techniques to check if they have any antibacterial and antifungal property. As a result [fungus Aand C] shows antifungal property against known plant pathogenic fungus. [clonostachys byssicola]

Type. C

Type. A

CONCLUSION

In many insect taxa, coevolution between hosts and their beneficial symbionts has been shown to broaden the ecological niches that can becolonized by the host. In Lepidoptera, such host–symbiont coevolution has not been demonstrated, because most studies have found little evidence of a core bacterial community with functional relevance in thisorder. However, the acquisition and transfer of some persistent bacterial members has been reported in several species, in spite of the harsh physiological conditions of the lepidopteran larval gut and the change of ecological niches between juvenile and adult stage. The high variability of the lepidopteran gut microbiome implies on the one hand that Lepidopterans do not rely on a fixed beneficial microbiota that is present in each generation. On the other hand, such variability may alsoimply the chance of harboring a very dynamic microbiome that allows their hosts to adapt to changing conditions including changes in abiotic conditions, food resources, and risk of natural enemy attack.

The factors leading to the evolutionary success of the highly diverse lepidopteran taxon are still unclear. According to Hammer et al. (2017), independence of microbes may have resulted in high diversification rates and lead to an extraordinary diversity and abundance of Lepidoptera. A lack of a vertically transmitted core microbiome that dictates host plant use, in combination with an ancient horizontal transfer of genes originating from bacteria (Wybouw et al., 2014) could be thus possible reasons for their success. The latter explanation has been shown to precede the diversification of phasmids (stick and leaf insects) (Shelomi et al., 2016a). While microbial symbiosis can provide novel ecological functions, Hammer and co-workers argue that dependence on symbionts might increase extinction risks because insects are constraint in their diet breadth and less able to switch to new food plants. It is therefore possible that independence of symbiosis might have facilitated switching to different host plants and promote diversification. In fact, the most species-rich superfamily of Lepidoptera, Noctuoidea, consists of many polyphagous species, among them numerous agricultural pests (Mitchell et al., 2006). Each host plant switch confronts a lepidopteran individual with a novel environment andnovel microbiome present on the host plant, which may lead to the symbiont community of caterpillars being often dominated by leaf- associated bacteria that are taken up from the host plant (Hammer et al., 2017). More studies are needed to understand the relationship between plant and lepidopteran microbiomes and their role in host plant shifts, and diversification. These studies should also consider plant-induced defenses and explore how bacteria that originate from caterpillar frass or salivary regurgitants affect plant physiology as found in beetles by Chung et al. (2013).

The microbiome of moths and butterflies may not only be shaped by their interactions with plants, but also by interactions with antagonists like pathogens, predators, and parasites. Defensive symbioses are known in many

animal taxa (Florez et al., 2015), and it is likely that theyalso exist in Lepidoptera. Defensive symbionts are often facultative, andunder laboratory conditions they are likely to get lost because the pressure imposed by natural enemies is lacking. Detection of defensive symbionts in natural lepidopteran populations is therefore a challenge for future research.

Studies on individuals from the field are of great significance in order todistinguish between ecologically important bacteria colonizing lepidopterans in their natural habitats and bacteria that are the productof laboratory rearing conditions (compare: Hammer et al., 2014, 2017; Staudacher et al., 2016). There is a strong selection pressure when animals are reared under laboratory conditions. This can result in the loss of traits, including relationships with facultative symbiotic bacteria,which may have an effect that is only beneficial in natural populations.

In a nutshell, to gain a deeper understanding of the mechanisms by which Lepidoptera-associated microbes affect host traits, ecological, microbiological, and molecular approaches are needed. This knowledge will provide fundamental insights into host–microbe interactions in oneof the most speciose animal groups on the planet, and may ultimately lead to a better control of important agricultural pests.

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