Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (1): 122-125
© 2015 JEZS
Received: 25-12-2014
Accepted: 07-01-2015

Priyanka Saha
Post Graduate Department of Zoology, Bethune College 181, Bidhan Sarani, 700 006, India.

Rina Rani Ray
Post Graduate Department of Zoology, Bethune College 181, Bidhan Sarani, 700 006, India.

Correspondence:
Rina Rani Ray
Post Graduate Department of Zoology, Bethune College 181, Bidhan Sarani, 700 006, India.

Production of Polysaccharide degrading enzymes by the gut microbiota of *Leucinodes orbonalis* and *Bactrocera dorsalis*

Priyanka Saha and Rina Rani Ray

Abstract
Pests from brinjal and guava were collected and identified as *Leucinodes orbonalis* and *Bactrocera dorsalis* respectively. Different bacterial strains were isolated from the gut of these two pests. Morphological and biochemical features like colony characters, gram staining properties, growth rate, motility, catalase activity, fermentative activities were observed. It was tested that the gut of both pests contains bacteria that could produce enzymes that digest polysaccharides including cellulose, xylan, pectin and starch that are normally difficult to digest. It was found that there remains a positive correlation between the type of stored granules in the attacked fruit and enzyme producing efficacy of the endosymbionts of the pests.

Keywords: *Leucinodes orbonalis*, *Bactrocera dorsalis,* gut microbiota, symbiotic bacteria, digestive enzymes.

1. Introduction
Brinjal (*Solanum melongena* L.) and guava (*Psidium guajava*) are the two most commonly used vegetable and fruit in India. But each year, a large number of the fruits of these plants are attacked by pests and are destroyed. *Leucinodes orbonalis* is the most important and destructive pest of brinjal and has a countrywide distribution. The pest starts damaging the brinjal plant by boring the petioles of the leaves causing the leaves to wither and drop and the losses range from 50-70% of the production [1] and has become a major threat for brinjal cultivation. The attacked vegetables show holes on them plugged with excreta. On the other hand, *Bactrocera dorsalis* is the most serious of all fruit-flies infesting 90% of guavas. Damage is caused as they feed on guava pulp, making the fruit unacceptable for human consumption. These fruit flies are the most important pests causing enormous damage to guava [2]. Although eggplant or brinjal is known as a non-starchy food, the berry of eggplant contains swollen starch granules with lots of holocellulose (cellulose and hemicelluloses) [3], xylan [4] and two pectin fractions [5]. It was reported that the endocarp and mesocarp of guava fruit is made up of 55-60% of neutral polysaccharides, mainly cellulose, xyloglucan, xylan, arabinan, and arabinogalactans [6]. Starch content decreased with concomitant increase in alcohol-soluble sugars [7] with the advancement of ripening [8].
Although few entomopathogens were isolated from *Leucinodes orbonalis,* [9] and few cultivable bacteria could be identified from the intestinal tract of *Bactrocera dorsalis* [10, 11], but so far the literature survey is concerned, no study was done on the polysaccharide digesting bacteria present in the digestive tract of the larvae of these two pests.
The present study aims with the isolation and characterization of various amylolytic, cellulolytic, xylanolytic and pectinolytic bacteria from the gut of the larvae of brinjal and guava pests.

2. Materials and methods
2.1 Collection of pests
Brinjal and guava were collected from different localities of Kolkata, West Bengal during July to September and the larvae present inside the growing fruits were taken out aseptically. Before dissection, larvae of each of the insect samples were surface-sterilized using sterile insect saline for three times.
The pests were identified by diagnostic characters described by Whittle and Ferguson [12] and Drew & Hancock [13] respectively.

2.2 Isolation of gut microbiota
The gut of each type of larva was dissected out with sterile forceps and the content was squeezed into autoclaved insect saline to prepare a bacterial consortium.

2.3 Screening of bacteria
The bacteria were grown in nutrient agar medium (pH 7) for 24-48 hours. The selected bacterial single colonies were selected and streaked on starch agar, cellulose agar, xylan agar and pectin agar plates [14].

2.4. *In situ* detection of amylase, cellulase, xylanase and pectinase activity
The starch agar plates with bacterial colonies were flooded with iodine solution [15] whereas cellulose agar and xylan agar plates with bacterial colonies were irrigated with 0.2% congo red solution [15]. The pectin agar plates were irrigated with iodine-potassium iodide solution (1.0 g iodine, 5.0g potassium iodide and 330ml H_2O) to detect clearance zones [16]. The halo formed around each bacterial colony indicated the production of respective extracellular enzymes by the particular strain.

2.5 *In vitro* enzyme production
The selected bacterial strains were grown in liquid state culture in 100 ml Erlenmeyer flasks each containing 20 ml medium of Basal medium (BM) composed of (g/L): peptone, 0.9; $(NH_4)_2HPO_4$, 0.4; KCl, 0.1; $MgSO_4.7H_2O$, 0.1 (pH-7.0). For amylase, cellulase, xylanase and pectinase production the medium was supplemented with pure starch, carboxymethyl cellulose, oat, spelt Xylan and pectin respectively as sole carbon source. The strains were cultivated at 28°-30 °C for 24-36 hours.

2.6 Measurement of growth of bacterial strains
The growth of the bacterial strains were measured by turbidimetrically at 660 nm in a spectrophotometer (Shimadzu, Japan).

2.7 Assay of enzymes
The grown culture was centrifuged at 10,000 rpm for 10 minutes and the supernatant was used as the crude enzyme. The enzyme activity was detected by incubating the assay mixture (1 ml) containing equal volumes of properly diluted enzyme and 0.5-1% (w/v) substrate in 0.1 (M) phosphate buffer (pH 7) at 50° C for 10 min. The reducing sugar produced was measured spectrophotometrically at 540 nm (Shimadzu, Japan) following the dinitrosalicylic acid method [17]. The substrates used were starch (1% w/v), carboxy methyl cellulose (1% w/v), xylan (0.5% w/v) and pectin (0.5% w/v) for estimation of amylase, cellulase, xylanase and pectinase respectively. A standard curve of glucose was prepared to calculate the concentration of amylase and cellulase, whereas xylose and galacturonic acid were taken as the standards for estimating xylanase and pectinase activity respectively. The heat killed or inactivated enzymes were used as respective controls.

One unit of amylase and cellulase activity was defined as the amount of enzyme which catalyzed the liberation of 1 mmol of glucose per minute per ml under optimal conditions from starch [13] and carboxy methyl cellulose [18] respectively. One unit of xylanase activity was defined as the amount of enzyme which catalyzed the liberation of 1 mmol of xylose per minute per ml under optimal conditions from xylan [19] and One unit of pectinase activity was defined as the amount of enzyme which catalyzed the liberation of 1 mmol of galacturonic acid per minute per ml under optimal conditions from pectin [20].

2.8 The morphological characterization of the bacterial strains
The colony characters, morphological features, gram staining characteristics, motility tests, catalase activity, acid production in different sugar were done [14].

Each experiment was replicated thrice.

3. Results and Discussion
After a screening of bacterial colony, three prominent colonies, namely PSB1, PSB2 and PSB3 were isolated from gut microbiota of the caterpillar of *L. orbonalis* whereas PSG1 and PSG3 were isolated from the larval gut of *B. dorsalis*. The morphological and biochemical features showed predominance of catalase positive, non-motile, smaller rods and coccus in the gut of *L. orbonalis* and *B. dorsalis* larvae (Table 1). This observation went partially in agreement with those of other reports [11].

Table 1: Characterization of the bacterial isolates.

Bacterial Strain	Colony Character	Cell Morphology	Gram character	Motility	Catalase activity
PSB1	Round, white, convex	Rod shaped	Positive	Non motile	Negative
PSB2	Serrated, white, flat	Rod shaped	Positive	Non motile	Positive
PSB3	Round, white, raised	Rod shaped	Positive	Non motile	Negative
PSG1	Serrated, white, flat	Curved small rod	Positive	Non motile	Positive
PSG3	Round, white, raised	Cocci	Positive	Non motile	Positive

The bacterial strains could grow well in all the carbohydrate sources (Table 2), an observation similar to those of earlier reports [11] but produced less acid while fermenting cellobiose and galacturonic acid, which indicated that the intake of these sugars did not involve much proton symport.

Table 2: Biochemical Characterization of the isolated strains

Bacterial Strain	Growth rate				Sugar fermentation pattern			
	Starch	Cellulose	Xylan	Pectin	Glucose	Cellobiose	Xylan	Galacturonic acid
PSB1	++++	++++	++++	++++	++	+	+++	+
PSB2	+++++	++++	+++++	++++	+++	+	+++	++
PSB3	++++	+++	+++	+++	+++	+	+++	++
PSG1	+++++	++++	+++++	+++++	+	+	++++	++
PSG3	++++	++++	++++	++++	++	++	++++	++

O.D at 660 nm: +++++: > 0.9, ++++: 0.8-0.9, +++**:** 0.6 - 0.7, ++: 0.4-0.5, + : < 0.3

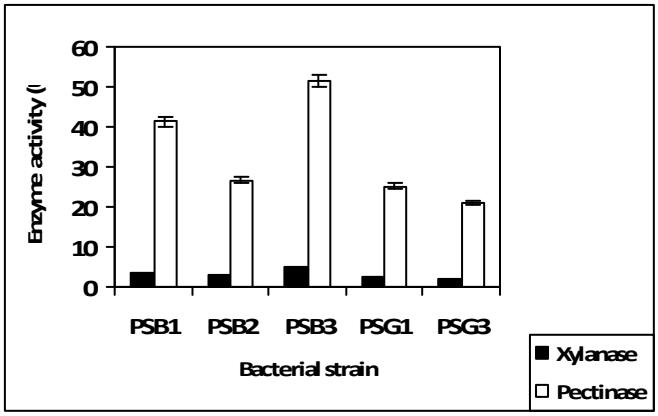

Fig 1: Production of extracellular xylanase and pectinase by various bacterial strains

The bacterial strains could produce quite a high amount of pectinase (Fig 1), as the pulp of both the target fruits contains pectin residues [5, 7, 21].

The bacteria from the brinjal pest were a better producer of pectinase than the bacterial isolates from guava pest, though the xylanase producing efficacy was lower than that of pectinase producing ability.

On the other hand, the amylase and cellulase synthesizing efficacy of the strains were much lower than that of pectinase and xylanase (Fig 2), which indicated that the relative amount of pectin and hemicellulose residues exceed the amount of starch and cellulose granules present in brinjal [4] and guava [8] pulp.

As the immature forms of the pests are voracious eater and remain within the pulp of the attacked vegetable or fruit, they must have produced an adequate level of enzymes, helping them to digest the stored food.

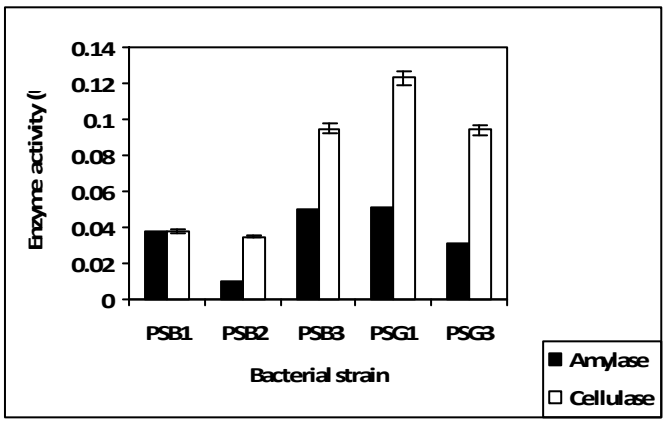

Fig 2: Production of extracellular amylase and cellulase by various bacterial strains.

4. Conclusion

The polysaccharides present in the food that are normally difficult to digest, are digested by endosymbiotic bacteria [20, 22]. Enzymatic assays for estimation of amylase, xylanase, cellulase and pectinase production by the isolated bacterial strains clearly indicated the positive correlation between their productivity and the food habit of the pest. However, these bacterial isolates demonstrate their unique property of secreting economically important enzymes, which could be further utilized in commercial production of these enzymes.

5. Acknowledgement

The authors are grateful to Dr. L. K. Ghosh, former Deputy Director, Zoological Survey of India for identifying the pests.

6. References

1. Asing SM, Ahamad MNU, Azad BU, Sikdar D. Characterization of α-amylase of eggplant frui and shoot borer *Leucinodes orbonalis* larvae. The Chittagong Univ J B Sci 2007; 2(1 &2):137-150.
2. Narayan ES, Batra HN. Fruit flies and their Control. Indian Council of Agricultural Research Fruit flies and their Control. Indian Council of agricultural Research, New Delhi 1960, 68.
3. Copur Y, Ozyurek Q, Tozluoglu A, Kutuk S. Enzymatic digestibility of tomato, pepper and eggplant stalks mixture. Bioresources 2012; 7(3):3252-3261.
4. Sone Y, Fujikawa Y. Estimation of xyloglucan in vegetables by enzyme-linked immunosorbent assay. J Nutr Sci Vitaminol (Tokyo) 1993; 39(6):597-606.
5. Esteban RM, Lopez-Andreu FJ, Martin-Cabrejas MA, Molla E. Pectin changes during the development and ripening of eggplant fruits. Food Chem 1993; 46:289–292.
6. Lim TK. Edible Medicinal and Non-Medicinal Plants, Fruits, Springer, 2012, 2.
7. Jain N, Dhawan K, Malhotra S, Singh R. Biochemistry of fruit ripening of guava (Psidium guajava L.): compositional and enzymatic changes. Plant Foods Hum Nutr 2003; 58(4):309-15.
8. Jain N, Dhawan K, Malhotra SP, Siddiqui, S Singh R. Compositional and enzymatic changes in guava (*Psidium guajava* L.) fruits during ripening Acta Physiologiae Plantarum 2001; 23:357-362.
9. Yasodha P, Natarajan N. Pathogenecity of naturally isolated entomopathogens in *Leucinodes orbonalis* and cross infectivity on *Helicoverpa armigera*. Madras Agric J 2009; 96:200-207.
10. Wang H, Jin L, Peng T, Zhang H, Chen Q, Hua Y. Identification of cultivable bacteria in the intestinal tract of Bactrocera dorsalis from three different populations and determination of their attractive potential. Pest Manag Sci 2014; 70(1):80-7.
11. Pramanik MK, Al-Mahim A, Khan M, Miah AB. Isolation and identification of mid gut bacterial community of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae) Res. J. Microbiol 2014; 1:9.
12. Whittle K, Ferguson DC. Egg plant fruit borer, *Leucinodes orbonalis* Guenée. Pests not known to occur in the United States or of limited distribution, U. S. Department of Agriculture, APHIS 1987; 85:1-9.
13. Drew RAI, Hancock DL. The Bactrocera dorsalis complex of fruit flies in Asia. Bulletin of Entomological Research: Supplement Series. Supplement No. 2. CAB International, Wallingford, UK, 1994.
14. Kumar R, Chattopadhyay A, Maitra S , Paul S, Banerjee PK. Isolation and Biochemical Characterizations of Mid Gut Microbiota of Culex (*Culex quinquefasciatus*) Mosquitoes in Some Urban Sub Urban & Rural Areas of West Bengal 2013; 1(3):67-78.
15. Ray RR. Production of α-amylase and xylanase by an alkalophilic strain of *Penicillium griseoroseum* RR 99. Acta Microbiol. Polon 2001; 50:305-309.
16. Fernandes-Salomão TM, Amorim ACR, Chaves-Alves VM, Coelho JLC, Silva DO, Araújo EF. Isolation of

pectinase hyperproducing mutants of *Penicillium expansum*. Rev. Microbiol. 1996; 27:15-18.
17. Bernfeld P. Amylases α and β, Method Enzymol 1955; 1:149-158**.**
18. Karmakar M, Ray RR. Extracellular Endoglucanase Production by Rhizopus oryzae in Solid and Liquid State Fermentation of Agro Wastes. Asian J. Biotechnol 2010**;** 2(1):27-36.
19. Kundu A. Ray RR. Effect of salicin on induction and carbon catabolite repression of endoxylanase synthesis in *Penicillium janthinellum* MTCC 10889. Chemical Papers 2014; 68(4):451-456
20. Dey A, Karmakar M, Ray RR. Extra cellular thermostable polygalacturonase from *Bacillus* sp AD1. Der Pharmacia Lettre 2011; 3(2):358-367.
21. De Abreu JR, dos Santos CD, de Abreu CMP, Correa AD, Lima LC, de O. Sugar fractionation and pectin content during the ripening of guava cv. Pedro Sato. Ciênc. Tecnol. Aliment, Campinas 2012; 32(1)156-162.
22. Anand AAP, Vennison SJ, Sankar SG, Prabhu DIG, Vasan PT, Raghuraman T *et al.* Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. J Insect Sci 2010; 10:107.