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ISOLATION OF TOXIC BACTERIAL STRAINS FROM HEMOLYMPH OF LEPIDOPTERAN PEST; *Helicoverpa armigera* (HUBNER).

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ABSTRACT

The gram-negative bacterium is a *Serratia marcescens* ; it causes diseases in vertebrates, invertebrates and human being. *Enterococcus faecalis* has been isolated from *Galleria mellonella* and its toxicity to the same insect has been investigated. *Pseudomonas plecoglossicida* has been isolated from the internal organs of the diseased fish, *Plecoglossus altivelis*. Also, it has been reported to be causative agents of fish disease. Here, we isolate the above mentioned bacterial strains from single host (*H. armigera*) larvae while rearing the insect under laboratory condition. Their toxicity assays were performed and found toxic and cause insect death within 20h of treatment. Among them; *S. marcescens* found to be the most toxic strain against *H. armigera*. Also, we found that the wounded insect body are the only pave to these bacterial strains to penetrate insect body and cause the toxicity.

Keywords: *Helicoverpa armigera*, Bioassay, toxic bacteria, biochemical test.

INTRODUCTION

The cutleaf worm *Helicoverpa armigera* is an important pest of cotton crop and world-wide causes nearly loses per annum in India alone over 1000 crores, Duraimurugan and Regupathy ,(2005). Workers across the globe are working to sort out the problem of the insect pest, in which unavailability of the insect pest throughout the year is the biggest problem. *H. armigera* was first colonized in laboratory in the year of 1983 in Australia, Gunning ,(1994). Following which, the insect is reared in laboratories throughout the world. Maintaining the insect pest under laboratory conditions is a difficult task. Several infections such as, NPV viruses have been reported to cause genetic drift in the laboratory populations of *H. armigera* pest due to which lab cultures cannot be maintained for more than six generations, Ballal *et al.* (1998). Initially, we have faced difficulties in maintaining *H. armigera* pest under laboratory condition. Thus, in the present study, attempts are made to find out to investigate the cause of mortality in laboratory colonized insects. We have identified three bacteria namely, *Enterococcus faecalis*, *Serratia marcescens* and *Pseudomonas plecoglossicidai* in the diseased hemolymph of the insect. The dyanamics of the growth of the three bacteria was also studied.

MATERIALS AND METHODS

Insect and bacterial identification

The larvae of *H. armigera* were collected from the field of Indian Agriculture Research institute (IARI), New Delhi. The insect larvae were reared on chickpea semi-synthetic diet throughout the experiments. The

laboratory condition was 27 ± 2 ^{0}C with 70-80% humidity and 14:10 (L: D) photoperiod.

Heavy mortality was being observed in the larval culture, to investigate the agents responsible for the mortality, the hemolymph of the infected larva and hemolymph of healthy larva (as negative control) were serially diluted in 1x PBS from 10^{-1} to 10^{-9} and plated on the TSA plate (pH 7.0) and incubated for overnight at 37 ^{0}C. Bacterial colonies were monitored visually over the designated period of time. Three different types of colonies on the basis of colour, texture, shape, size and colony morphology were observed on plates. These colonies were re-streaked (sub-culture twice) on TSA plate to obtain pure culture for each three isolates. The single colonies were inoculated in TS broth (Tryptic Soy Broth) and cultures were stored at -80 ^{0}C as glycerol stock. Total genomic DNA from the cultured bacteria was isolated using Qiagen genomic DNA kit. 16SrRNA gene were amplified using standard PCR, Saiki *et al.* (1988) with Taq polymerase (Toyobo), 10-20 nanogram genomic DNA as template and the universal primer pair of 20F (5'-AGAGTTTGATCCTGGCTCAG-3') & 1500R (5'-GGTTACCTTGTTACGACTT-3') described by Weisburg *et al.* (1991). 16SrRNA gene were amplified using following PCR conditions; 95^{0}C for 5 minute, 37 cycles each of 95^{0}C for 30 second, 55^{0}C for 30 second and 72^{0}C for two minute followed by five minute extension at 72^{0}C. The amplified PCR product was resolved on 0.8% agarose gel and extracted from the gel using Qiagen gel extraction kit according to the manufacturer's instructions and commercially sequenced. After sequencing, all sequences were compiled using MacVector (version 7.0) software suite (Oxford Molecular Group, Oxford, UK) and compared to available database entries using BLAST analysis against NCBI GeneBank.

Colony forming unit (CFU)

In order to count colony forming units (CFU) of each strain, a single colony of each three strains were inoculated in 5 ml of TSB and incubated at 37^{0}C, 200 rpm for 16 – 18 hours. Well grown cultures of each three strains were serially diluted in TSB ranging from 10^2 to 10^7. 100 µl of each dilution was spread on solid TSA plate using the glass spreader under sterilizing condition and incubated at 37^{0}C for 16 – 18 hours. The colonies in each dilution on plates were counted for CFU in each three strains.

Growth curves of bacteria

10% of the overnight grown culture of each three strains were used as a starter culture and was inoculated into 50 ml of TSB media and incubated at 37^{o}C with constant shaking for another 12 hours. The Optical density of each three strains were measured every 2 hr at A_{600nm} by using spectrophotometer (Amersham Bioscience spectrophotometer). The measured absorbency was used to study the growth rate of each three strains.

Antimicrobial sensitive assay

In order to study the susceptibility of these three isolates against antibiotics, twenty seven discs of different antibiotic types were assayed against them using Tryptic Soya Agar plates. Two different sterile antibiotic discs were used viz, octa discs along with individual discs as well. 200µl of

overnight grown culture of each strain was placed on each TSA plate; it was rotated and spread by using a sterile and non-toxic cotton swab following the manufacturer's instructions. The plates were left for 15-20 min to dry up. Discs were placed individually carefully on the agar surface with the help of sterile forceps. Finally the plates were incubated at 37^0C for overnight and the inhibition zones were measured in millimeters (mm). Each zone was considered sensitive, intermediate or resistant based on corresponding area given on the results obtained using Mueller Hinton Agar chart. All the above-mentioned procedures were done under sterilized condition to avoid any kind of contaminations.

Insect bioassay

In order to know the portal of entry of these three strains into the insect body and its toxicity to *H. armigera* pest, different types of insect bioassays were conducted viz, diet surface treatment, force feeding bioassay, injury bioassay and injection bioassay. A single colony of each strain was inoculated in 5ml TSB media and incubated for 16 -18 hr at 37^0C using 200 rpm in the incubator shaker. 100 µl of each 16 -18 hr grown culture was dissolved in 900 µl de-ionized water in eppendorf tube. For injury bioassay, 2µl containing approx. 500 bacterial cells, was injected into each larva using micro-injector (KD Scientific) with 30-gauge needle. Four replicates of five larvae each were used for each strain. For feeding bioassay, the larvae were allowed to feed on the above-mentioned fresh semi-synthetic diet. The mortality was recorded after 20, 48 and 72 hr intervals. For injury bioassays, a sterile wound was made on the cuticle of the second segment of the thorax of the larvae using a sterile surgical blade and 2 µl of the bacterial solution (that contains approx. 500 cells) was released on the open wound. Three replicates of five larvae each were used for each bacterial strain. The mortality observation was recorded 24h after of treatment.

RESULTS

In order to identify the bacterial strains responsible for heavy mortality in the *H. armigera* culture, the hemolymph of the infected larvae was streaked on TSA plates. Three different types of bacterial colonies were observed on TSA plates. This experiment was repeated several times and the same result was obtained.

Detailed bioinformatics analysis of these bacteria 16 S rRNA sequences revealed the similarity of some of the bacteria with other organism. *E. faecalis* shows 93% similarity to GenBank Accession number GQ483456, *S. marcescens* shows 97% similarity with GenBank Accession number EF035134 and *P. plecoglossicidai* shows 99% similarity with GenBank Accession number DQ140383.

The growth rate of these strains was varied. *S. marcescens* strain was the fastest grower, while the *E. faecalis* was the slowest one (Fig. 1). The OD_{600} of the overnight culture of the above-mentioned strains was 0.58 for *E. faecalis*, 2.30 for *S. marcescens* and 2.14 for *P. plecoglossicidai*. *S. marcescens* had highest number of CFU followed by *P. plecoglossicidai* and

E. faecalis, which shows the potential of *S. marcescens* as a most toxic strain causing mortality against *H. armigera* within the time frame of the investigation (Fig. 2).

Further analysis based on microbiological and biochemical test like color, shape, Gram stain, motility, catalase test, Hemolysis test, glucose test, gelatin test, L-arabinose test, tryptophan test, methyl red test, starch test, citrate test, oxidase test, xylose and voges-proskauer test were shown in Table (1).

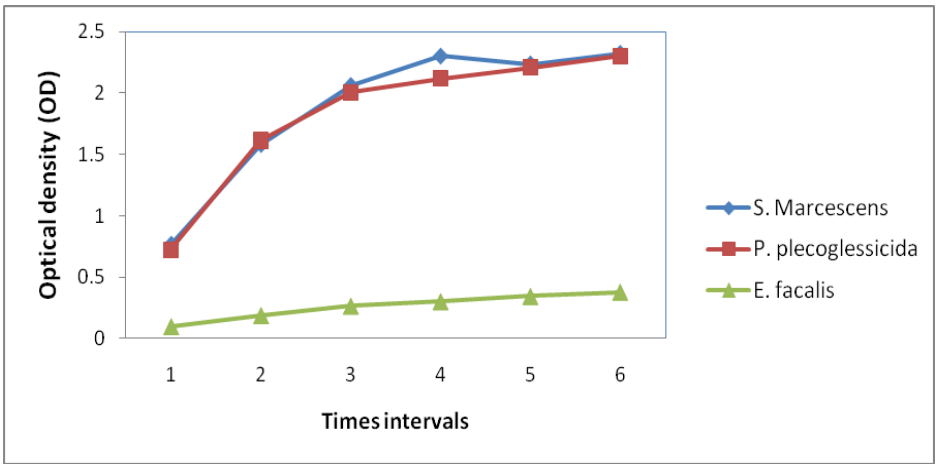

Figure 1: Growth rate of *S. marcescens*, *P. plecoglessicida*, and *E. facalis*. 10% overnight culture of each strain was inoculated in 50ml TSB media flask and again kept at 37 ^{0}C for shaking and further culturing in order to measure the optical density (OD).The optical density was reported every two hours viz, 2 (1), 4 (2), 6 (3), 8 (4), 10 (5), 12h (6).

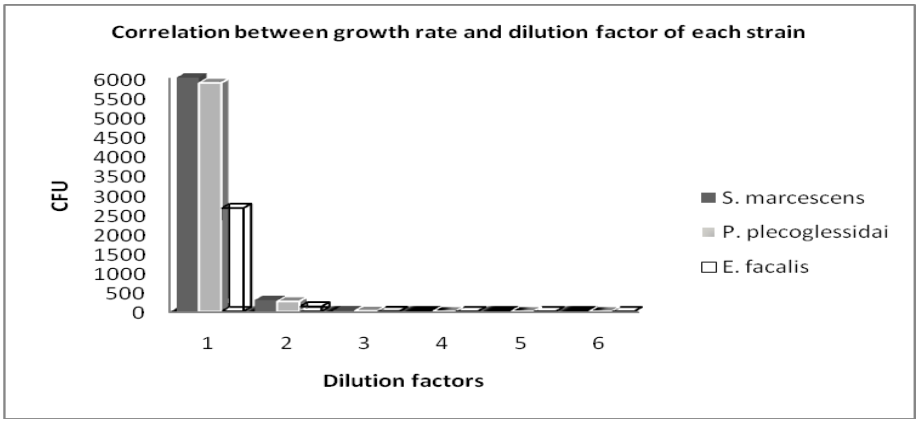

Fig. 2: Correlation between colony forming unit of *S. marcescens*, *P. plecoglessidai*, and *E. facalis* strains and their dilution factors. 1, 2, 3, 4, 5, and 6 : are reperesent 10 $^{-1}$, 10 $^{-2}$, 10 $^{-3}$, 10 $^{-4}$, 10 $^{-5}$, 10 $^{-5}$ dilution factors respectively.

Table 1: Biochemical analysis of *E. faecalis*, *S. marcescens*, and *P. plecoglossicidai* bacterial strains.

S. No.	Morphology / Biochemical Test	*Enterococcus faecalis*	*Serratia marcescens*	*Pseudomonas plecoglossicida*
1	Color	White	White at 37°C/ red at 30°C	Yellowish
2	Gram Stain	positive	negative	negative
3	Motile	Non motile	motile	motile
4	Shape	Cocci	rod	rod
5	Catalase test	-	+	+
6	Hemolysis test	+	+	β-hemolytic
7	Glucose test	+	+	+
8	Gelatin test	-	+	-
9	L-Arabinose Test	-	-	-
10	Tryptophan test	-	+	-
11	Methyl red test	+ positive for mixed acid fermentation	-	-
12	Starch test	-	-	-
13	Citrate test	-	+	+
14	Oxidase test	-	+	+
15	Xylose	-	-	-
16	Voges-proskauer test	- negative for alcohol fermentation	+	-

Twenty seven antibiotics were used to study sensitivity of each strain. The sensitivity of the different bacteria was found to vary from antibiotic to another. According to the manufacturer instruction's and zone size interpretative chart the bacterial strains were classified as sensitive, intermediate and resistant based on antibiotic type (Fig. 3). The details of the sensitivity of the bacteria are given in Table(2).

Among several types of bioassays done; injection and injury bioassays were the only methods that caused mortality in insects. Amongst the tested bacterial strains, bioassay results show that *S. marcescens* was the most toxic strain. It causes high mortality to the insect culture within 20h after injection followed by *P. plecoglossicidai* (Fig. 4). However, all the strains caused 100% mortality within 72h after injection. Result also showed that either single strain bioassay or a combination of all three strains caused insect death, which indicates the ability of each strain to kill the laboratory reared insect (Fig. 3).

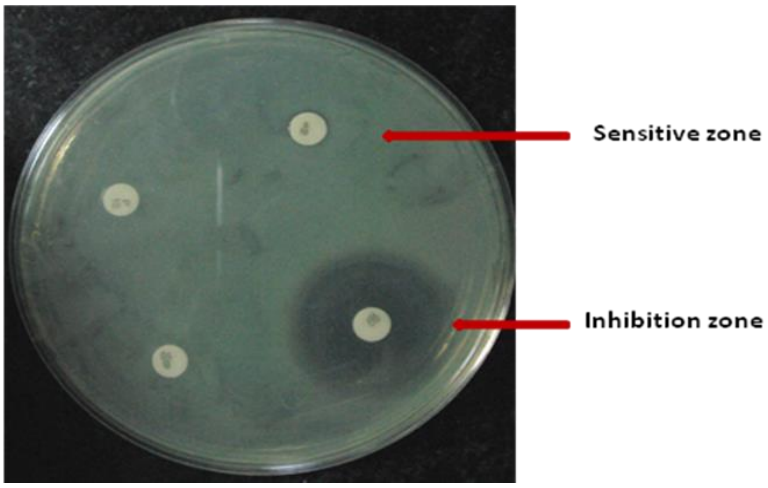

Figure 3: The inhibition zone along with the sensitive zone in TSB medial plate incubated for overnight at 37 ^{0}C.

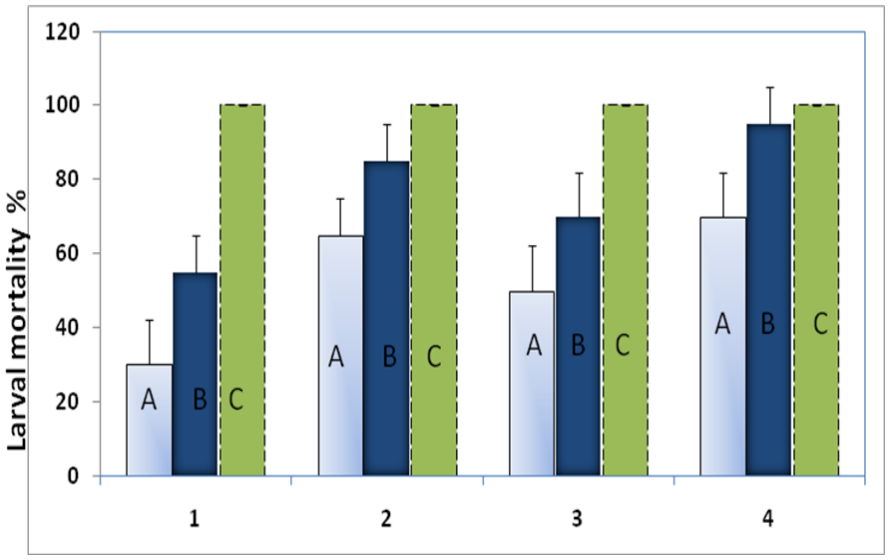

Figure 4. Mortality bioassay of the bacterial strains isolated from *H. armigera* . The mean value and Standard deviation was calculated. The observations were taken 20, 48, 72h interval after treatment. The chi-square test was carried out. Each three combined columns represent one strain at three different times intervals, viz, 20h (A), 48h (B) and 72h (C). Columns 1: represent *E. faecalis*, column 2: represent *S. marcescens* and Column 3: represent *P. plecoglossicida.* Column 4: Represent the combination between the three strains.

Table 2: Antibiotics Sensitivity test for insect microbes (Diameter of zone of inhibition (mm)). R: represents resistant, S: represents sensitive, and I: Represents intermediate to the antibiotic.

S. No	Antibiotics Disc	Disc content (mcg)	Resistant (<mm)	Intermediate (mm)	Sensitive (>mm)	*E. facalis*	*S. marcescens*	*P. plecoglessicida*
1	Ampicilin (A)	10	13	14-17	18	6 **R**	8 **R**	0.0 **R**
2	Aztreonam (Ao)	30	15	16-21	22	6 **R**	18 **I**	10 **R**
3	Bacitracin (B)	10	8	9-12	13	8 **R**	0.0 **R**	2 **R**
4	Carbenicillin (Cb)	100	19	20-22	23	24 **S**	20 **I**	10 **R**
5	Nitrofurantoin (Nf)	300	14	15-16	17	4 **R**	0.0 **R**	0.0 **R**
6	Penicilin G (P)	10	19	20-27	28	6 **R**	4 **R**	0.0 **R**
7	Co-Trimoxazole (Co)	30	10	11-15	16	20 **S**	18 **S**	4 **R**
8	Norfloxacin (Nx)	10	12	13-16	17	24 **S**	26 **S**	24 **S**
9	Cephotaxime (Ce)	30	14	15-22	18	20 **I**	20 **I**	8 **R**
10	Chloramphenicol (C)	30	17	18-20	21	20 **I**	20 **I**	20 **I**
11	Ciprofloxacin (Cf)	5	15	16-20	21	24 **S**	28 **S**	24 **S**
12	Gentamicin (G)	10	12	13-14	15	10 **R**	6 **R**	6 **R**
13	Gatifloxacin (Gf)	5	14	15-17	18	22 **S**	20 **S**	20 **S**
14	Levofloxacin (Le)	5	15	16-18	19	20 **S**	26 **S**	22 **S**
15	Nalidixic Acid (Na)	30	13	14-18	19	20 **S**	22 **S**	4 **R**
16	Vancomycin (Va)	30	-	-	15	0.0 **R**	0.0 **R**	0.0 **R**
17	Ofloxacin (Of)	5	12	13-15	16	20 **S**	20 **S**	22 **S**
18	Novobiocin (Nv)	30	17	18-21	22	8 **R**	4 **R**	0.0 **R**
19	Nitrofurantoin (Nf)	300	14	15-16	17	0.0 **R**	4 **R**	0.0 **R**
20	Doxycycline Hydrochloride (DO)	30	12	13-15	16	10 **R**	14 **I**	10 **R**
21	Rifampicin (R)	5	16	17-19	20	2.0 **R**	0.0 **R**	0.0 **R**
22	Spectinomycin (Se)	100	14	15-17	18	6 **R**	4 **R**	6 **R**
23	Streptomycin (S)	10	11	12-14	15	4 **R**	2 **R**	2 **R**
24	Erythromycin (E)	15	13	14-22	23	10 **R**	6 **R**	10 **R**
25	Kanamycine (K)	30	13	14-17	18	6 **R**	6 **R**	6 **R**
26	Tetracycline (T)	30	14	15-18	19	12 **R**	10 **R**	12 **R**
27	Trimethoprim (Tr)	5	10	11-15	16	20 **S**	22 **S**	0.0 **R**

DISCUSSION

Several bioassays have been done as per mentioned in the material and method part. None of those bioassay experiments caused toxicity to *H. armigera* larvae except the injury and injection bioassay methods. This data suggested that bacteria penetrate into insect bodies through the wounds or the opening parts in the exoskeleton of the larval body. Also, results have been shown that all the strains used in this study caused mortality either individually or in combination. Insect death occurred within 20h after treatment and the mortality reached 100% within 72h after injection with all the strains. The growth rate varied from one strain to another. *S. marcescens* was found to have the highest growth rate followed by *P. plecoglossicidai* compared with *E. faecalis*. As a result of this, the optical density of each was

varied as well. The results show that OD and CFU colony of *S. marcescens* was higher than the other strains. The potential of these strains to kill *H. armigera* larvae is very high and may wipe out the insect culture if the sterilizing condition in the culture room are not appropriate. Our finding here proved for the first time that *H armigera* would be a suitable host for propagation the reported bacterial strains in this study and that would help in determination of such infection under laboratory condition.

It has been described before that several bacterial microbes can infect insects, human beings as well as other different invertebrate and cause different types of diseases, Aucken and Pitt (1998). Recently, it has been reported that different types of organisms belonging to four different invertebrates infected with those microbes viz, *Caenorhabditis elegans*, Shepard and Gilmore (2002); fruit fly, *Drosophila melanogaster* , Erickson *et al.*(2004); *Bombyx mori* (Kaito *et al.* (2002); *Galleria mellonella*, Choi *et al.* (2002) and Reeves *et al.* (2004). Among of these microbes, *S. marcescens*, *E. faecalis* and *P. plecoglossicida* have been found in different organisms. *S. marcescens* is a Gram-negative bacteria and it is an important microbe due to its ability to cause diseases to vertebrate, invertebrate and plant system as well, Grimont and Grimont (1978). It can infect the human being causing number of serious nosocomial infections, Coulthurst *et al.* (2006). The ability of this strain to develop the resistance to several antibiotics has shown some interest to investigate its potential as an ideal model bacterial system to screen other related pathogenic microbes and seek their mode of action into their respective hosts.

E. faecalis bacterium strain has been theorized to play a role in bacterial synergy, Montravers *et al.* (1994) and Montravers *et al.* (1997). Among several types of enterococcal strains, *E. faecalis* is the most infectious strain causing wide varieties of diseases to the human being and other organisms, Jett *et al.* (1994). It has been reported to cause severe virulence infections like cytolysin, Sannomiya *et al.* (1994) extracellular proteases (gelatinase and serine protease), Engelbert *et al.* (2004) and to aggregation substance, Galli *et al.* (1989). The *Enterococci* species have been documented as common pathogens present in the hospitals and found to infect bloodstream, urinary tract and biliary tract as well as burn wounds and periodontal tissues, Park *et al.* (2007).

Pseudomonas is considered as a huge and broadly varied bacterial group, Palleroni *et al.* (1973). Till date, several debates related its taxonomical status has been pushed forward, Tamaoka *et al.* (1987); Willems *et al.* (1989, 1990); Yabuuchi *et al.* (1990, 1992, 1995). Initially, its classification status has been principally done based on their phenotypic characterizations, Stanier *et al.* (1966) and the amino acid sequence homology, Palleroni *et al.* (1973). Earlier, *Pseudomonas* species have been subdivided into five groups on the basis of rRNA and DNA similarity, Palleroni *et al.* (1973). It has been described that there is similarity between the infectious models of *Pseudomonas aeruginosa* in different organisms like mice and *G. mellonell*a. So that it would be easy to fish out the mode of action of this microbe that subsequently will help us to understand the process of transferring such organism to the human being, Jander *et al.* (2002).

Recently, *E. faecalis has* been isolated from the hemolymph of dead *G. mellonella* larvae and GelE protein has been purified as an insecticidal toxin from *E. faecalis* culture media. Also, the same group studied the virulence effect of some proteases of *P. aeruginosa*, on the host immune response, Azghani *et al.* (1993) and Rumbaugh *et al.* (1999).

As a result of previous studies, it has been assumed that GelE may perform as an important factor in the pathogenesis of enterococcal infection in human blood. Accordingly, it has been concluded that *E. faecalis* GelE may affect the balance system and inhibits the immune reactions in the host, Park *et al.* (2007).

The main finding in the current study was to isolate *E. facalis, S. marcescens* and *P. plecoglossicida* from *H. armigera* larvae for the first time while rearing the insect culture in the laboratory. According to this investigation and previous studies, we would propose clear correlations between the above three strains and their respective environment. Also, our study would help and support the researchers in identification the bacterial infections that may cause insect death to *H. armigera* larvae while maintaining its culture under laboratory condition.

REFERENCES

Aucken, H. M., Pitt, T. L. (1998). Antibiotic resistance and putative virulence factors of *Serratia marcescens* with respect to O and K serotypes. J Med Microbiol. (47), 1105–1113.

Azghani, A. O., L. D. Gray, A. R. Johnson. (1993). A bacterial protease perturbs the paracel*lular barrier funct*ion of transporting epithelial monolayers in culture. Infect. Immun**.** (61), 2681–2686.

Ballal, C. R., Singh, S. P., Joshi, S., Rao, N. S. .(1998). Multicellular tray for rearing the l*arvae of Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) Entomon. (23), 307-312.

Choi, J. Y., C. D. Sifri, B. C. Goumnerov, L. G. Rahme, F. M. Ausubel, S. B. Calderwood. **(**2002). Identification of v*irulence genes in a p*athogenic strain of *Pseudomonas aeruginosa* by representational difference analysis. J. Bacteriol. (184), 952–961.

Coulthurst, S. J., Lilley, K. S., Salmond, G. P. C. (2006). Genetic and proteomic analysis of the role of luxS in the enteric phytopathogen, Erwinia carotovora. Mol Plant Pathol. (7), 31–46.

Duraimurugan P., Regupathy A. (2005). Synthetic Pyrethroid Resistance in Field Strains of *Helicoverpa armigera* (Hubner)(Lepidoptera: Noctuidae) in Tamil Nadu, South India, American Journal of Applied Sciences. (7), 1146-1149.

Engelbert, M., E. Mylonakis, F. M. Ausubel, S. B. Calderwood, M. S. Gilmore. **(**2004). Contribution of gelatinase, serine protease, and *fsr* to the pathogenesis of *Enterococcus faecalis* endophthalmitis. Infect. Immun. (72), 3628–3633.

Erickson, D. L., J. L. Lines, E. C. Pesci, V. Venturi, D. G. Storey. **(**2004). *Pseudomonas aeruginosa relA* contributes to virulence in *Drosophila melanogaster*. Infect. Immun. (72), 5638–5645.

Galli, D., R. Wirth, G. Wanner. (1989). Identification of aggregation substances of *Enterococcus faecalis* cells after induction by sex pheromones. An immunological and ultrastructural investigation. Arch. Microbiol. (151), 486–490.

Grimont, P. A., Grimont, F. (1978). The genus Serratia. Annu Rev Microbiol (32), 221–248.

Gunning R. V. (1994). Pyrethroid Resistance Increasing in Unsprayed Populations of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) in New South Wales 1987-90, J. Aust. ent. SOC. (33), 381-383.

Jander, G., L. G. Rahme, F. M. Ausubel. **(**2000). Positive correlation between virulence of *Pseudomonas aeruginosa* mutants in mice and insects. J. Bacteriol. (182), 3843–3845.

Jett, B. D., M. M. Huycke, M. S. Gilmore. **(**1994). Virulence of enterococci. Clin. Microbiol. Rev. (**7),** 462–478.

Kaito, C., N. Akimitsu, H. Watanabe, K. Sekimizu. **(**2002). Silkworm larvae as an animal model of bacterial infection pathogenic to humans. Microb. Pathog. (32), 183–190.

Montravers, P., A. Andremont, L. Massias, C. Carbon. **(**1994). Investigation of the potential role of *Enterococcus faecalis* in the pathophysiology of experimental peritonitis. J. Infect. Dis. (169), 821–830.

Montravers, P., J. Mohler, L. Saint Julien, C. Carbon. **(**1997). Evidence of the proinflammatory role of *Enterococcus faecalis* in polymicrobial peritonitis in rats. Infect. Immun. (65), 144–149.

Palleroni, N. J., Kunisawa, R., Contopoulou, R., Doudoroff, M. (1973)**.** Nucleic acid homologies in the genus *Pseudomonas*. Int J Syst Bacteriol. (23), 333-339.

Reeves, E. P., C. G. Messina, S. Doyle, K. Kavanagh. **(**2004). Correlation between gliotoxin production and virulence of *Aspergillus fumigatus* in *Galleria mellonella*. Mycopathologia. (158), 73–79.

Rumbaugh, K. P., J. A. Griswold, A. N. Hamood. (1999). Contribution of the regulatory gene *lasR* to the pathogenesis of *Pseudomonas aeruginosa* infection of burned mice. J. Burn Care Rehabil. (20), 42–49.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R.,Horn, G. T., Mullis, K. *B.* & Erlich, H. A. (1988). *Primer-directedenzymat*ic ampli®cation of DNA with a thermostable DNA polymerase. Science 239, 487±491.

Sannomiya, P., R. A. Craig, D. B. Clewell, A. Suzuki, M. ujino, G. O. Till, and W. A. Segers, P., Vancanneyt, M., Pot, B., Torck, U., Hoste, B., Dewettinck, D., Falsen, E., Kerste*rs, K.,* Vos, P. D. (1994). Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Bu$ sing, Do$ ll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., respectively. Int J Syst Bacteriol. (44), 499-510.

Shepard, B. D., M. S. Gilmore**. (**2002). Differential expression of virulence-related genes in *Enterococcus faecalis* in response to biological cues in serum and urine. Infect. Immun. (70), 4344–4352.

Park S. Y., Kyoung M. K., Joon H. L., Sook J. S., In Hee L. (2007). Extracellular gelatinase of *Enterococcus faecalis* destroys a defense system in insect hemolymph and human serum, infection and immunity. (75), 1861–1869.

Stanier, R. Y., Palleroni, N. J., Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. J Gen Microbiol. (43), 159-271.

Tamaoka, J., Ha, D. M., Komagata, K. (1987). Reclassification of *Pseudomonas acidovorans* den Dooren de Jong 1926 and *Pseudomonas testosteroni* Marcus and Talalay 1956 as *Comamonas acidovorans* comb. nov. and *Comamonas testosteroni* comb. nov., with an Emended Description of the Genus Comamonas. Int J Syst Bacteriol. (37), 52-59.

Weisbur*g,* W. G., Barns, M. S., Pelletier, A. D., Lane, J. D. (1991). 16S ribosomal DNA amplication for phylogenetic study. J Bacteriol. *(*173), 697-703.

Willems, A., Busse, J., Goor, M., 8 other authors (1989). *Hydrogenophaga*, a new genus of hydrogen-oxidizing bacteria that includes *Hydrogenophaga ¯ava* comb. nov. (formerly *Pseudomonas ¯ava*), *Hydrogenophaga palleronii* (formerly *Pseudomonas palleronii*), *Hydrogenophaga pseudo¯ava* (formerly *Pseudomonas pseudo¯ava* and `*Pseudomonas carboxy¯ava*'), and *Hydrogenophaga taeniospiralis* (formerly *Pseudomonas taeniospiralis*). Int J Syst Bacteriol. (39), 319-333.

Willems, A., Falsen, E., Pot, B., Jantzen, E., Hoste, B., Vandamme, P., Gillis, M., Kersters, K. Ley, J. D. (1990). *Acidovorax*, a new genus for *Pseudomonas facilis*, *Pseudomonas delafeldii*, E. Falsen (EF) group 13, EF group 16, and several clinical isolates, with the species *Acidovorax facilis* comb. nov., *Acidovorax delafeldii* comb. nov., and *Acidovorax temperans* sp. nov. Int J Syst Bacteriol. (40), 384-398.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H. Nishiuchi, Y. (1995). Transfer of two *Burkholderia a*nd an *Alcaligenes s*pecies to *Ralstonia g*en. nov.: proposal of *Ralstonia pickettii (*Ralston, Palleroni and Doudoroff 1973) comb. nov., *alstonia solanacearum (*Smith 1896) comb. nov. and *Ralstonia eutropha (*Davis 1969) comb. nov. Microbiol Immunol. (39), 897-904.

Yabuuchi, E., Kosako, Y., Oyaizu, H., Yano, I., Hotta, H., Hashimoto, Y., Ezaki, T. Arakawa, M. (1992). Proposal of *Burkholderia* gen. nov. and transfer of seven species of the genus *Pseudomonas* homology group II to the new genus, with the type species *Burkholderia cepacia* (Palleroni and Holmes 1981) comb. nov. Microbiol Immunol. (36), 1251-1275.

Yabuuchi, E., Yano, I., Oyaizu, H., Hashimoto, Y., Ezaki, T. Yamamoto, H. (1990). Proposals of *Sphingomonas paucimobilis* gen. nov. and comb. nov., *Sphingomonas parapaucimobilis* sp. nov., *Sphingomonas yanoikuyae* sp. nov., *Sphingomonas adhaesiva* sp. nov., *Sphingomonas capsulata* comb. nov., and two genospecies of the genus *Sphingomonas*. Microbiol Immunol. (34), 99-119.

عزل سلالات بكترية سامة من هيموليمف حشرة دودة اللوز الأمريكية

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في هذه الدراسة تم عزل ثلاث سلالات بكتيرية مختلفة لأول مرة من هيموليمف حشرة دودة اللوز الأمريكية أثناء تربيتها في المعمل لعدة أجيال متتالية .. وباستخدام تكنولوجيا الـ PCR والتتابع النيكلوتيدى لكلا منهما تم التأكد من أن هذه السلالات هي كالأتي: Serratia *marcescens, Enterococcus faecalis*, *Pseudomonas plecoglossicida*

وقد أجريت تجارب التقييم الحيوي لهذه السلالات ضد دودة اللوز الأمريكية وأثبتت التجارب أن هذه السلالات الثلاثة له القدرة على قتل الحشرة خلال ٢٠ ساعة من المعاملة وتلاحظ أن السلالة *Serratia marcescens* هي أكثر السلالات سمية لهذه الحشرة و أثبتتا الدراسة أن الجروح الموجودة على سطح جسم الحشرة ربما هي من أهم أسباب إصابة الحشرة تلك الأنواع البكتيرية ونظرا للاصا بات البكتيرية والمتكررة من آن لآخر عند تربية دودة اللوز الأمريكية في صورة تعدادات حشرية داخل المعمل ، تعتبر هذه الدراسة من الدراسات الهامة التي اختصت بتعريف تلك السلالات البكترية وتحديد المشكلة حيث أن هذه المشكلة كانت من أهم المشاكل التي تعيق تربية الحشرة في المعمل بطريقة توسعية.

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