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# Larvicidal effect of *Staphylococcus vitulinus* bacteria against *Spodoptera litura* Fab

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**Abstract**
*Spodoptera litura* Fab. is one of the most destructive pest of cotton. Damage caused in spite of various control methods, the discovery of new entomopathogenic bacterial species and isolates bearing insecticidal traits against novel targets is needed in the near future. In this study, isolation, characterization of *Staphylococcus vitulinus* was assessed for insecticidal activity against *Spodoptera litura*.

Bacterial isolation were performed from dead and live larvae of *Spodoptera litura*, bacterial isolates were characterized based on their morphological and biochemical characteristics. The application of concentration were conducted with five different bacterial concentrations (8.5 x $10^7$ cfu/ml, 12.2 x $10^7$ cfu/ml, 16 x $10^7$ cfu/ml, 20.2 x $10^7$ cfu/ml and 24.4 x $10^7$ cfu/ml) of the active cells and for insecticidal activity 1 ml of bacterial suspension were performed on the third instar larvae of *S. litura* with the leaves of *Ricinus communis.*

The sub lethal and median lethal dose of *Staphylococcus vitulinus* was $LD_{10}$ = 87.06 cfu/ml and LD50 = 215.5 cfu/ml at 96 hrs against third instar larvae. Result revealed that, the mortality increase with increase in concentration of bacterial populations. The highest mortality of *Spodoptera litura* is found by bacterial strain *Staphylococcus vitulinus* at 24.4 x $10^7$ cfu/ml. Stastical variance, 95% confidence limits and regression equations are presented.

**Keywords:** Larvicide, *Staphylococcus vitulinus* and *Spodoptera litura*

## Introduction
*Spodoptera litura* is serious polyphagous pest of various economically important crops such as cotton, soybean, groundnut, chilli, tobacco, caster, bhendy, jawar, maize, cabbage and pulses etc. It was found to cause 26 - 100 % yield loss in ground nut (Dhir *et al*., 1992) [4]. Loss of major crops due to insect pest varies between 10 and 30% (Ferry *et al*., 2004) [6]. India is basically an agro-based country where more than 80% of Indian population depends on agriculture. Insects are known to cause significant damage to crops and affect agricultural productivity. The body of this pest is light brown in colour while fore wings are greyish brown with white markings and hind wings with a brown border. The life cycle of *Spodoptera litura* is consisting of egg, larva, pupa and adult. The eggs on laid on leaves in clusters and the egg masses are covered with buff coloured hairs obtained from the mothers body. The caterpillars causes the damage by feeding on tender leaves, shoots and fruits at night. It is extremely destructive pest of cultivated plants.

The majority of the insect pests are controlled by using chemical insecticides that harm the environment, humans and many beneficial organisms. Thus, it is pivotal and well accepted that other control methods such as physical prevention, cultural actions and organic and environmentally friendly compounds are essential for integrated pest management programmes. The bioinsecticides are the biochemical pesticides that are naturally occurring substances that control pests by nontoxic mechanisms. Bioinsecticides are living organisms or their products (phytochemicals, microbial products) or byproducts which can be used for the management of pests that are injurious to plants. Biopesticides based on pathogenic microorganisms specific to a target pest offer an ecologically sound and effective solution to pest problems. They pose less threat to the environment and to human health. The most widely known microbial pesticides are varieties of the bacterium *Bacillus thuringienesis* or Bt which can control certain insects in various fields. In the last 50 years, microbial control of pests and plant diseases showed an amazing development associated with pronounced good results under optimized laboratory conditions. Thus we need to understand the important concepts required to produce reliable, effective, and safe entomopathogens for insect pest control.

In the presence study, we focused on the determination of bacterial species isolated and characterized based on morphological and biochemical characteristics for insecticidal acvtity against *S. litura*.

## Materials and Methods

### Insect Culture

The eggs of *S. litura* (NBAII-MP-NOC-02: *S.litura*) were purchase from National bureau of Agriculture Insect Resources Bangalore and maintained in the laboratory. The III instar larvae were used for these experiments.

### Isolation of Bacteria

Bacterial isolation was performed on dead and living larvae separately. Healthy and dead larvae were separated based on microscopic examination, distinguishing between living larvae that showed general disease symptoms. Twenty living and dead larvae were surface sterilized with 70% ethanol and washed three times with sterile distilled water and homogenized in nutrient broth media by using a glass tissue grinder. Suspensions were diluted and 0.1ml suspension was plated on nutrient agar. Plates were incubated at 30ºC for 2-3 days. After the incubation period, the plates were examined and bacterial colonies were selected. The colonies determined were purified by subculture on the plates. (Crecchio G, *et al*., 2001) [2].

### Inoculum and culture conditions

The inoculum was prepared as following: one isolated colony was dispensed in 3 ml of Liquid Broth medium and incubated overnight at 37°C. Aliquots (0.2 ml) were used to inoculate 250 ml Erlenmeyer flasks containing 50 ml Liquid Broth medium and incubated in a rotatory shaker at 200 rpm and 37°C, From the larval extracts, 10, 25, and 50 µL were placed on nutrient agar and incubated at 30 °C for 2–3 days. The remaining mixtures were incubated at 30 °C for 3–4 h to increase the number of bacteria that had low concentrations. From these mixtures, 10, 25, and 50 µL were also placed on nutrient agar and incubated at 30 °C for 2–3 days. Isolates were distinguished based on colony color and morphology. Pure cultures of the bacterial colonies were prepared and stocked in 0.9% biological saline solution in Laboratory (Filiz, *at el* 2014) [7].

Bacterial cultures were identified according to their morphology, nutritional features, and biochemical, physiological and molecular characteristics.

### Identification of bacterial isolates

Bacterial isolates were identified by utilization of organic compounds, spore formation, Gram staining, and upon confirmation of the bacterial gram nature, same bacterial strain was identified by using bioMerieux, vitek2 compact.

### Insecticidal Bioassay

Third instar larvae of *S. litura* were used for the insecticidal assay of bacterial isolated species, *Staphylococcus vitulinus*. The concentration application experiments were conducted with bacterial isolates with various population of the active cells in colony forming unit (CFU) (Table 4). One milliliter of bacterial suspensions of different concentrations (8.5 x $10^7$ cfu/ml, 12.2 x $10^7$cfu/ml, 16 x $10^7$ cfu/ml, 20.2 x $10^7$ cfu/ml and 24.4 x $10^7$ cfu/ml) were saturated onto lettuce leaves (approximately 10 cm2) of *Ricinus communis* and placed in individual plastic boxes released 10 third instar larvae for 96 hrs of each experiment. Three replications were conducted. The percent mortality was calculated after 96h and the observed data was subjected to probit analysis (Finney 1947 [8]; Busvine 1971 [1]).

## Results

In this study, *Staphylococcus vitulinus* bacteria from the surface of dead and live larvae of *S. litura* was isolated and characterized. It was found gram positive. The colony colour of isolates was white cream and had the shape of cocci. Some morphological and biochemical characteristics of *Staphylococcus vitulinus* are summarized in Table – 2. The bacterial isolate was identified by using bioMerieux, vitek2 compact (Table -3). The isolated and identified bacteria, *Staphylococcus vitulinus* was tested against third instar larvae of *S. litura*.. The total percent mortality was observed after 96h and corrected mortality was calculated by using Abbott's formula and the results are presented. The results showed that the mortality increases with increase in concentration at all doses.

The results of the probit analysis for the estimation of $LD_{10}$, $LD_{50}$, variance, 95% confidence limits and regression equation at 96h for the mortality of third instar larvae of S. litura are presented in Table – 4. The highest mortality (60%) was found at 24.4 x $10^7$ cfu/ml at 96 hrs of third instar larvae. (Table-4 and Figure-1).

The sub lethal and median lethal dose of *Staphylococcus vitulinus* was $LD_{10}$ = 87.06 cfu/ml and $LD_{50}$ = 215.5 cfu/ml at 96 hrs against third instar larvae of *S. litura*.

Among the various estimate of regression based probit analysis, the $\chi 2$ values for the regression coefficients showed homogeneity to the data.

**Table 1:** *Staphylococcus* species used in this study and their host.

| Species | Host | Source |
|---|---|---|
| Staphylococcus | *Spodoptera litura* | Larvae |

**Table 2:** Morphological characteristics of *Staphylococcus vitulinus.*

| Isolate | Colony Color | Shape of Colony | Shape of Bacteria | Gram stain | Spore stain | Source | Growth in Nutrient Broth |
|---|---|---|---|---|---|---|---|
| S-1 | White | Round | Cocci | + | - | Dead larvae | Turbid |

**Table 3:** The biochemical characteristics of *Staphylococcus Vitulinus* isolate from s. litura larvae based on conventional and vitek 2 compact Biomerix identification system.

| Well | Test | Mnemonic | Result |
|---|---|---|---|
| 2 | D-Amygdalin | AMY | - |
| 4 | Phosphatidylinositol Phospholipase C | PIPLC | - |
| 5 | D-Xylose | dXYL | - |
| 8 | Arginine Dihydrolase 1 | ADH1 | - |
| 9 | Beta Galactosidase | BGAL | - |
| 11 | Alpha Glucocidase | AGLU | - |
| 13 | Ala-Phe-Pro Arylamidase | APPA | - |
| 14 | Cyclodextrain | CDEX | - |
| 15 | L-Aspartate Arylamidase | AspA | - |
| 16 | Beta Galactopyranosidase | BGAR | - |

| | | | |
|---|---|---|---|
| 17 | Alpha-Mannosidase | AMAN | - |
| 19 | Phophatase | PHOS | - |
| 20 | Leucine Arylamidase | LeuA | - |
| 23 | L- Proline Arylamidase | ProA | - |
| 24 | Beta Glucuronidase | BGURr | - |
| 25 | Alpha-Galactocidase | AGAL | - |
| 26 | L- Pyrrolidonyl- Arylamidase | PyrA | - |
| 27 | Beta-Glucuronidase | BGUR | - |
| 28 | Alanine Arylamidase | AlaA | - |
| 29 | Tyrosine Arylamidase | TyrA | - |
| 30 | D-Sorbitol | Dsor | - |
| 31 | Urase | URE | - |
| 32 | Polymixin B Resistance | POLYB | - |
| 37 | G-Galactose | Dgal | - |
| 38 | D-Ribose | dRIB | - |
| 39 | L-Lactatealkalization | ILATk | - |
| 42 | Lactose | LAC | - |
| 44 | N-Acetyl-Dglucosamine | NAG | - |
| 45 | D-Maltose | dMAL | - |
| 46 | Bacitracin Resistance | BACI | - |
| 47 | Novobiocine Resistance | NOVO | - |
| 50 | Growth In 6.5% Nacl | NC6.5 | - |
| 52 | D-Mannitol | dMAN | - |
| 53 | D-Mannose | dMAN | - |
| 54 | Methyl-B-D-Glucopyranoside | MBdG | - |
| 56 | Pullulan | PUL | - |
| 57 | D-Raffinose | Draf | - |
| 58 | 0/129 Resistance (Comp.Vibrio.) | 0129R | - |
| 59 | Salicin | SAL | - |
| 60 | Saccharose/Sucrose | SAC | - |
| 62 | D-Trehalose | dTRE | - |
| 63 | Arginine Dihydorolase 2 | ADH2s | - |
| 64 | Optochin Resistance | OPTO | - |

**Table 4:** Effect of *Staphylococcus vitulinus* bacteria isolated from larvae of *Spodoptera litura* at various population on larval mortality

| S. No | Bacterial population cfu/ml | No. of insects exposed | Percent mortality at 96 hrs |
|---|---|---|---|
| 1 | 85 | 10 | 10 |
| 2 | 122 | 10 | 20 |
| 3 | 160 | 10 | 30 |
| 4 | 202 | 10 | 40 |
| 5 | 244 | 10 | 60 |

**Table 5:** $LD_{10}$, $LD_{50}$ values with variance, 95% confidence limits and probit analysis parameters for larvae of *Spodoptera litura* after 96h of treatment.

| Isolated Bacterial strain | $LD_{10}$ CFU | $LD_{50}$ CFU | Variance | 95% CL | | Regression equations | $\chi^2$ (degree of freedom) |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| *Staphylococcus vitulinus* | 87.06 | 215.5 | 0.003857 | 2.2118 | 2.4552 | Y= 3.2350x - 2.5955 | 0.2275 (2) |

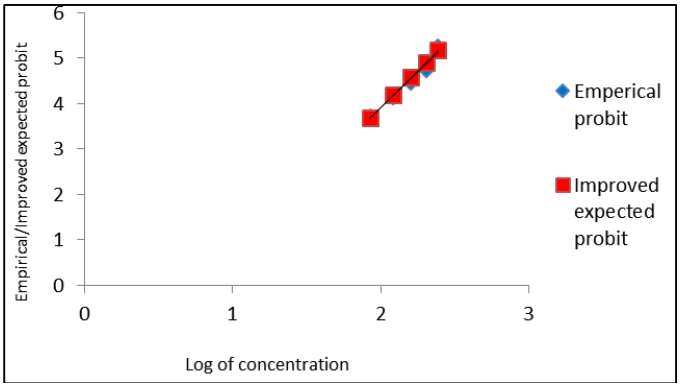


**Fig 1:** Regression and provisional lines for *Spodoptera litura* exposed to bacterial strain isolate after 96h

## Discussion

*Spodoptera litura* is one of severe agricultural pest. Entomopathogenic bacteria are of great importance in the control of insect pests. In the presented study we isolated bacteria from the surface of dead and living larvae of *Spodoptera litura*. The isolated bacteria were purified, cultured and identified. We also tested their insecticidal potential of isolated bacteria, against *S. lirua*. The isolated bacteria, *Staphylococcus vitulinus* was identified from the sample. These bacterial species are the first report from *S. litura*.

The members of the genera *Staphylococcus* were isolated most commonly from the pest insect. These genera include entomopathogenic bacterial species isolated from insects (Yaman *et al*. 1999 [16], 2000 [17], 2002 [18], 2005 [19], 2010 [20]; Kuzina *et al*. 2001 [10]; Darriet and Hougard 2002 [3]; Ertürk *et al*. 2008 [5]; Manimegalai and Shanmugam 2013) [11]. The members of *Staphylococcus*, *S. aureus* and *S. sciuri* were isolated from the pest's population in Samsun. Different *Staphylococcus* species have been isolated from insects (Yaman *et al*. 2002 [18]; Nagaraju *et al*. 2012 [12]; Katı and Katı 2013[9]; Manimegalai and Shanmugam 2013) [11]. Nagaraju *et al*. (2012) [12] isolated *S. aureus* from termites and Manimegalai and Shanmugam (2013) [11] from mulberry silkworm, *Bombyx mori*. Katı and Katı (2013) [9] isolated S. sciuri from *Xylosandrus germanus* (Blandford) (Coleoptera:

Curculionidae). Podgwaite *et al*. (2013) [13] found that *S. sciuri* is the most common isolate associated with adults of the Asian long horned beetle (Coleoptera: Cerambycidae). Both *Staphylococcus* species were isolated from *C. aurata* for the first time.

In this study, we showed that the isolated bacteria can infect larval development stages of S. litura. This could be a very promising advantage in the biocontrol of this pest because it may not be necessary to consider targeting the most susceptible stage of the pest during field application. Bioassay experiments showed that the isolated bacteria S. vitulinus have insecticidal potential and $LD_{10}$ and $LD_{50}$ against the larval instars of S. litura are 87.06 CFU and 215.5 CFU respectively. There are a few studies on using living microorganisms for the control of harmful insects (Sidor and Jodal 1986 [14]; Vriesen and Keller 1994 [15]; Ziemnicka 2007 [21]). The study needs further investigation to find out active ingredients responsible for insecticidal properties against *Spodoptera litura.*

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