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Optimization of Growth Conditions for *Serratia marcescens* (CBCC 47) for Enhanced Insecticidal Activity against Diamond Back Moth (*Plutella xylostella)*

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Diamond Back Moth *(Plutella xylostella),* is one of the most serious pests on cruciferous crops. The insecticidal activities of the metabolites produced by *Serratia marcescens* (CBCC 47) grown in different fermentation media were determined by testing the crude extract against diamond back moth(*Plutella xylostella*). The larvicidal activities of the isolates varied when grown in different medium and growth conditions. When grown in Soy-NaCl-yeast extract-glucose medium, the extract produced was more toxic, indicating an enhanced production of the metabolites. Toxicity assays showed that the toxins produced from the novel growth media were more effective in killing larvae of DBM (Diamond Back Moth) as compared to that produced from NB (Nutrient broth) medium. These observations suggest that soy-NaCl-yeast extract-glucose medium can serve as a cheap source of medium for the production of non-hazardous, biodegradable and economically viable biocide formulations based on the metabolites of the isolated *Serratia marcescens.*

Key Words: Biocide Formulation, Biodegradable, Diamond Back Moth, Non-Hazardous, Secondary Metabolites, *Serratia marcescens.*

Diamond Back Moth(*Plutella xylostella)*, DBM is one of the serious pests, infecting cruciferous crops like cabbage, broccoli, cauliflower etc. The moth has a short life cycle (14 days at 25°C), is highly fecund and capable of migrating long distances. It is one of the most important pests of cruciferous crops in the world and will usually only feed on plants that produce glucosinolates. The larvae damage leaves, buds, flowers and seed-buds of cultivated cruciferous plants. Although the larvae are small, they can be very numerous and cause complete removal of foliage tissue except for the leaf veins. This damages the young seedlings and can disrupt head formation in cabbage, broccoli and cauliflower. The presence of the larvae in florets can result in complete rejection of the produce. The diamondback moth is considered a pest in areas that do not experience very cold winters, as these help to kill off overwintering moths.

Increasing awareness of the environmental risk associated with the exclusive use of chemicals has given an urgent need of an environmental friendly as well as cost effective mode of pest control that are completely biodegradable for disease free environment[1]. Chemical as well as biological agents are prevalent for the killing of DBM. But compared to chemical pesticides, biological insect control agents have a

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greater preference. Not only are biological agents potent but they are also non hazardous to the environment, completely biodegradable, with no adverse effect on the host and are totally residue free[2]

Serratia marcescens is Gram negative rod shaped bacteria that belongs to the family Enterobacteriaceae producing prodigiosin[3], has received attentions due to its potential to produce extracellular proteins like nuclease, phospholipase, haemolysin, siderophore, chitinase, protease and lipase. Secondary metabolites like carbapenem and prodigiosin produced by *Serratia* will be of much use to come out with a biocide[4]. It is reported to be one of the successful organisms employed for degrading chitin, a structural component of exoskeleton of insects. Chitin inhibition activity is one of the strategies that can be employed in pest control[5]. These bacteria grow well on standard media and produce a red to dark pink pigment that aids in identification. One or more proteinaceous factors with insecticidal activities in the locust pathogen *Serratia marcescens* culture filtrates are found to cause the death of scarab beetle[6] .

The production of secondary metabolites with antibiotic activities is temporarily related with sporulation, when the cells are particularly sensitive to competitors and require special protection when a nutrient runs out. To a normal basal media, when any special or different component is added or substituted, it brings about an enhanced secondary metabolite production. Along with the components of a medium, alterations in temperature, pH and incubation period induce changes in growth and physiology of the microorganism, which leads to high metabolite production. Hence, the study was undertaken to come out with a cost effective commercial fermentation media for the growth and metabolites production from *Serratia* in correlation to its larvicidal activity on DBM.

MATERIALS AND METHODS

Isolation

In our study, we isolated the *Serratia* from dead DBM larvae. It was obtained from a pinkish mass in the gut region of the dead DBM larvae, which was transferred to sterile water aseptically[7]. It was kept under shaking condition for a stipulated time period and then subjected to the process of isolation. On streaking onto MEA(Malt extract agar), it appeared as mucoid red colonies bearing the typical colony morphology of *Serratia marcescens.*

Insect bioassay

The second instar larvae (lab culture) of DBM was used for bioassay of the culture filtrates and solvent extracts in all the cases. Fresh mulberry leaf was taken, washed with water and blot dried and used for the assay. The mulberry leaf was dipped in the culture free extract/solvent extracts (100 dilution with water) blot dried and about 10 larvae were released and observed for 72 hrs, against suitable controls.

Influence of media components

In order to enhance the efficacy in the insecticidal activity of *Serratia*, various media optimization categories and growth conditions were taken under consideration. Various medium were tested with varying components and their varying quantities for optimized fermentation conditions for the maximum growth and efficacy of *Serratia*. The commercial Nutrient broth medium served as a standard reference medium. Three replicates of 100 ml each with 3% of 24 hours grown culture was incubated in all the cases. The medium that were used under testing were composed of the following :-

a) Yeastextract(0.3%),NaCl (0.5%), Peptone (0.5%), glucose(2%)
b) Yeast extract(0.3%),NaCl (0.5%),Soy flour (0.5%), glucose(2%)
c) Yeast extract(0.3%),NaCl (0.5%),Soy hydrolysate (0.5%),glucose(2%)
d) Malt extract (0.3%), Yeast extract (0.3%), Glucose (2%), Peptone(0.5%)
e) Malt extract(0.3%), Yeast extract(0.3%),Glucose (2%), Soy flour(0.5%)
f) Malt extract(0.3%), Yeast extract (0.3%), Glucose(2%), Soy hydrolysate(0.5%)
g) Nutrient broth

Influence of the culturing parameters

The medium that showed maximum efficacy in the mortality of the insect, was tested for its better insecticidal property by incubating it at different incubation periods of 3-7 days. After the incubation for the above mentioned time period, the culture filtrate of each was assayed on DBM larvae.

The isolated *Serratia* culture was incubated in a range of temperature of 26-34°C, with the increment of 2°C. The best suited temperature was found out based on the mortality rendered on the DBM larvae by the culture filtrate incubated at the mentioned temperatures.

In order to check the influence of initial media pH on growth and larvicidal activity of *Serratia*, it was grown in the selected media with a pH range of 6-10 (adjusted using 1N HCl and 1N NaOH).

Extraction of the metabolites

Different solvents were tested for the complete extraction of metabolites (intracellular in particular) and different dilutions were tried against DBM. The extraction of the secondary metabolites was done using various solvents. Different organic solvents namely; Ethyl acetate, Hexane, Butanol,Chloroform and Methanol were chosen to extract the metabolite of the isolated *Serratia*. The 1:1 solvent and broth culture ratio was taken and mixed for extraction of the metabolites, kept for 12hr under shaking condition. The solvent phase was collected for all the extractions (except in methanol) through a separating funnel, evaporated completely and resuspended in methanol:water (1:1) to make up the volume and was subjected to bioassay.

RESULTS

The *Serratia*, which was isolated from the gut of a dead DBM larvae, was subjected to various media components and culturing conditions for formulating the best cost effective media for obtaining the maximum growth and metabolite production having insecticidal activity.

Among the seven different media composition tested, the highest larvicidal activity was observed in composition 'b' (90%), followed by composition 'a' and 'e' (70%) and lowest observed in composition 'd' (20%), whereas, commercial nutrient broth media gave only 30% mortality(Table 1).

By maintaining the best media composition 'b', it was further tested for the best suited culturing conditions like incubation time, temperature, pH and extraction of the secondary metabolites.

A consistency of the highest mortality (90%) was observed at 5 days of incubation and the least was observed at 3 days (20%), indicating the culture duration for maximum production of metabolites having larvicidal activity against DBM (Table 2). Similarly, temperature plays a significant role in the growth as well as metabolite production in any microorganism. Among different temperatures tried,the highest larvicidal effect was observed at 30°C (70%) followed by 32°C (50%) and the least was recorded at 26°C (Table 2), indicating the best temperature for growth and metabolites production. The most important factor pH, showed varied insect mortality percentages, highest cell count (data not shown) and insect mortality was recorded at pH 6 (70%) followed by pH 7 (60%), where as least activity was seen at pH 10 (20%), indication the influence of pH on the growth and metabolites production (Table 2).

The effect of the various solvent extracts on the mortality of DBM was recorded and the highest mortality was recorded in 1:1 methanol:culture extract (80%) followed by ethyl acetate (35%) and least was seen in chloroform (10%), indication the effect of solvents on complete extraction of metabolites having insecticidal activity (Table 3). These results indicate improved larvicidal activity as compared to culture filtrate at respective dilutions, where as no mortality was seen in solvent controls (data not shown).

From the experiments carried out and the observations made, it has been concluded that the media composition 'b' is the best media and feasible in efficacy and cost, as cheaply procured raw materials were used. The incubation of *Serratia* in media 'b' for 5 days at the temperature of 30°C showed the highest mortality. The maintenance of media 'b' at a pH of 6 indicating requirement of slightly acidic condition to neutral condition and further extraction of the metabolite with methanol (1:1) brought about the maximum mortality of DBM larvae.

DISCUSSION

Diamond back moth was introduced into North America from Europe about 150 years ago. It now occurs wherever its host plants are grown. DBM larvae feed on all plants in the crucifer family,

including canola, mustard, and the vegetable cole crops such as broccoli, cabbage, also on several greenhouse plants. Extensive feeding on the flowers will delay plant maturity, cause the crop to develop unevenly and significantly reduce seed yield. In this regards, much attention is being given to the control of DBM. Pest management by chemical as well as natural controls have been showing potency against DBM. However, natural factors can have profound effect on DBM population due to its several advantages over chemical control agents.

In the present work, the isolated *Serratia* species from the gut of the DBM larvae has been shown to bring about mortality in the DBM larvae. Earlier works have also shown a significant efficiency of *Serratia marcescens* against DBM larvae. The liquid culture of *Serratia* is known to infect the 3rd and 4th instar larvae[8]. In order to ascend the efficacy of our isolated strain, several media formulation and optimization procedures have been carried out. The need to optimize the media composition is of foremost necessity. Several rich sources of carbon, nitrogen and trace elements have been brought together to come out with an effective novel media. The usage of glucose as a good source of carbon for the growth of *Serratia* has been demonstrated earlier[9]. Peptone is also commonly used for the growth of *Serratia* ,as it is a source of nitrogen and some organic and inorganic compounds[10]. Soy flour also manifests the same properties of peptone for its usage in the medium[11].

From the experiments conducted, it is shown that the medium 'b' composed of yeast extract,soy flour,glucose along with NaCl[12] demonstrated best result. However, no single medium composed of the components of our selected medium, has been reported so far anywhere in enhancing the growth of *Serratia*. The components can be easily and cheaply procured, hence proving to be cost effective. Incubation period plays a crucial role in the growth as well as in the production of metabolite by the microbe. It has been shown here that an incubation period of 5 days brought about maximum mortality in DBM and incubation time lesser or greater than that depreciated the mortality rate, indicating the production of secondary metabolites takes place at log phase as reported by earlier workers. Temperature is one of the most significant parameters that plays a major role in the growth of an organism. To conclude as to which temperature is most effective for growth as well as production of metabolite in *Serratia*, 30°C proved to be the best one by giving maximum insect mortality. Similarly, pH plays an important role in the growth and metabolism of an organism. In our experiment,the culture grown in the pH slightly acidic to neutral condition, conferred the highest colony count and insect mortality. Since the secondary metabolite produced by *Serratia* is responsible for the killing of the DBM, it is more prudent to extract the metabolites completely and put it for testing on DBM. Methanol has been proved as the solvent of choice for the extraction of the secondary metabolites for the mortality of DBM larvae, indicating complete recovery of the metabolites particularly intracellular ones.

The findings of the present experiment is a significant step towards the isolation of the potent microorganism, development of economically viable formulation against the devastating pest, DBM and characterization as well as optimization of the media as well as the growth factors for the microbe for maximum insecticidal metabolite production. This study will help in reducing chemical pesticide load on environment and can be made into a biocidal formulation for cost effect pest management. This can also be included in combination of other metabolites for more effective formulation development against DBM. The structural and chemical characterization of the metabolite having insecticidal property is in progress, which will throw more light on further improvement in the biocide formulation.

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