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Research Article

Influence of imidacloprid treatment on Protein Profiling of soil isolate *Bacillus weihenstephanensis*

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Abstract: Imidacloprid has gained great attention as a synthetic insecticide that acts in a similar manner as nicotine and is now widely used for the control of pests. Bacteria react to environmental or chemical stress by synthesizing specific sets of proteins and these can be analyzed by protein profiling using SDS PAGE. The present investigation was undertaken to reveal protein profile changes and differentially expressed proteins in soil isolate *Bacillus weihenstephanensis* on dose and durational exposure to imidacloprid. Protein profiling of *Bacillus weihenstephanensis* treated with different (10^{-7} to 10^{-3} M) concentrations of imidacloprid observed regularly at the duration of 24 hrs for a period of 96 hours, showed the over expressions of certain protein, expression of stress proteins and novel proteins. The proteins expressed were observed to be dose and durational dependent. The present investigation proves that imidacloprid exposure induces the expression of stress and novel proteins in soil isolate *Bacillus weihenstephanensis*. The expression of these proteins may be necessary for the protection of the *Bacillus weihenstephanensis* from toxic effects of imidacloprid. The specific protein profiles that are expressed in response to the stress induced by imidacloprid can be used to monitor the environmental samples for the presence of similar pollutants.

Keywords: Imidacloprid; *Bacillus weihenstephanensis*; Stress Proteins; protein profiles

Introduction

Prokaryotic cells respond to environmental or chemical stress by synthesizing specific proteins characteristic to each stress. Stress proteins are expressed in response to a wide range of stress conditions in various bacteria (Claudio *et al*., 1995). Electrophoretic separations of proteins are widely used in proteomic analyses, and rely heavily on SDS electrophoresis. Proteomics is the investigation of the protein content or the protein complement of the genome of a biological system, also termed the proteome (Wilkins *et al.,* 1996). The objective of proteome research is to identify and describe the complex responses of a biological system to different stimuli. Proteomic investigation of a given cell or other biological system should ideally detect all proteins and their functional responses to a stimulus (Bell and Hook, 1979). Proteomic investigations of microbial communities in the natural environments not only provide the most realistic information about their function but also pose the greatest experimental and bioinformatics challenges (Valenzuela *et al*., 2006). Proteomics reveal the dynamic expressions of whole proteins in cells and their interactions (Bell and Hook, 1979).

When organisms or cells are exposed certain harmful physical and chemical agents, the organisms acquire an induced tolerance against the adverse effects of these agents (Flahaut *et al*., 1996).

In response to a wide range of stress conditions certain specific stress proteins are expressed. The heat shock response in various bacteria is most widely studied and best characterized, but studies are carried out mainly in *Escherichia coli* (Givsko *et al*., 1994). The stress proteins expressed in *Escherichia coli* have been characterized by using heat, radiation, heavy metals, oxidizing agents, nutrient starvation, the SOS response and organic solvents (Kobayashi *et al*., 1998). Most work on stress proteins was carried out on the bacterium *Escherichia coli*. However, for extrapolation to environmental behavior, it is necessary to understand the responses in a diverse range of different bacteria. Heat shock and starvation stress responses in *Bacillus subtilis* and *Pseudomonas putida* is also well studied (Givskov, 1994). The chemically induced stress response in microorganisms is not well studied. To completely understand the stress response in microorganism more work and data is required (Claudio *et al*., 1995). The molecular mechanisms and responses of cells against various pesticides are not yet completely understood (Patcharee *et al*., 2009). The majorities of proteomic investigations of environmental microorganisms focus on model microorganisms cultured in the laboratory because of their ability to tolerate, degrades, or precipitate toxic compounds. These characters make these microorganisms attractive for environmental biotechnology applications, and

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proteomics can provide a better understanding of their functions in specific habitats (Lacerda and Reardon, 2009). Therefore, the present investigation was undertaken to elucidate protein profile changes and differentially expressed proteins in soil isolate *Bacillus weihenstephanensis* on dose and durational exposure to imidacloprid.

Materials and Methods

Preparation of stock solution of imidacloprid

The sample of imidacloprid 17% (confidor®) used in the experiment was supplied by Byers India Pvt. Ltd. The stock solution of 1 M of imidacloprid was prepared and further diluted to give 10^{-3} to 10^{-7} required molar concentrations.

Maintenance and propagation of culture

The soil isolates *Escherichia coli, Brevundimonas Sp.* MJ 15 and *Bacillus weihenstephanensis* were maintained at 4°C on nutrient agar and sub cultured very fortnight.

Medium used for the study

The medium used for toxicity testing was a optimized medium containing dextrose - 0.65 g l, Yeast extract - 1.05 g /l, KHPO - 0.30 g/l, and NaCl - 0.25 g /l and pH (7.0 -7.2).

Preparation of inoculum

Pre-inoculum was prepared by inoculating a loopful of bacteria from the overnight incubated nutrient agar slant cultures on a 100-ml sterilized optimized medium and incubated for 18-24 hours at 37°C under static conditions depending on the exponential phases of bacteria under test.

Experimental procedures

The pre-inoculum (5ml) was inoculated to 250 ml Erlenmeyer's flask containing 100 ml of sterilized optimized medium amended with different molar concentrations of imidacloprid. The flasks were incubated at 37°C for 96 hours under shaking conditions at 120 rpm on a rotary shaker (REMI – CIS-24) and at regular intervals sample was taken out from each flask aseptically for analysis.

Isolation of Protein

The bacterial cell pellet was dissolved in 100µl of lysis buffer and incubated at 37^{0}C for 15 min. the tubes were centrifuged and the supernatant was used as protein sample. These protein samples were analyzed by PAGE according to Laemmli (1970).

Procedure

Clean and dry, grease free PAGE glass plates were taken and assembled using spacer and clips. Bottom of the assembly was thoroughly sealed. Separating and stalking gel were prepared as follows

Contents	Volume Separating Gel (10% 10 ml)	Volume Stalking Gel (5%, 5 ml)
Distilled water		
30%	4	3.4
Acrylamide	3.3	0.830
1.5 M Tris-Cl	2.5 (pH 8.8)	0.630 (pH 6.8)
Buffer	0.100	0.05
SDS	0.100	0.05
APS	0.010	0.005
TEMED		

Stalking gel was casted on separating gel and immediately comb was inserted. Gel was left to polymerize and comb was carefully removed. The gel was assembled into the PAGE unit and electrophoresis buffer (Tris-3.g. Glycine 14.3 g. SDS 2.0 g for 1 liter) was added into upper and lower tank. protein sample was mixed with the sample buffer (1:1) (1.5 M Tris-Cl (pH 6.8) 0.625 ml. 20% SDS 1.0 ml. glycerol 1.0 ml, 2-mercaptoethenol 0.5 ml, 0.2% bromophenol blue) and boiled for 2-10 min. The sample was then loaded into the gel along with the markers and the gel was run at constant current of 50V till the sample dye reaches to the end of the gel. The gel was carefully removed and stained in staining solution (Methanol 4 ml, distilled water 5 ml, Glacial acetic acid 1 ml, 0.2% Coomassie Brilliant Blue R-250) with constant shaking for 2 hr the excess stain was removed by destaining with destaining solution (Methanol 4 ml, distilled water 5 ml, Glacial acetic acid 1 ml) and finally, the blue color protein bands were observed. Marker proteins were used bearing molecular weight of 97, 66, 44, 29 and 14 kDa.

Results

Protein profiling of *Bacillus weihenstephanensis* induced by imidacloprid

Bacillus weihenstephanensis was exposed to different concentrations of imidacloprid ranging from 10^{-7} M to 10^{-3} M of imidacloprid for a period of 96 hrs and at regular intervals of 24 hrs, the proteins induced were analyzed. The protein expression was observed at 40, 46, 64, 75, 78, 83 and 101 kDa at 24 hrs (Fig.1). On exposure to imidacloprid for 48 hrs the bands were observed at 25, 46, 67, 75, 85, 93 and 101 kDa (Fig. 2). The imidacloprid treated for 72 hrs showed expression at 25, 42, 55, 64, 75 and 93 kDa (Fig. 3) and for 96 hrs the expressions were observed at 17, 41, 46, 54, 60, 63, 75, 83, 89 and 91 kDa (Fig. 4) respectively.

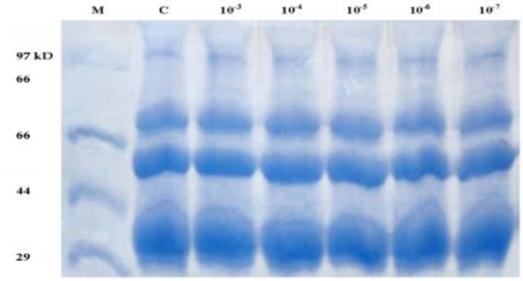

Fig. 1. Protein profiling of *Bacillus weihenstephanensis* after 24 Hours of exposure to imidacloprid

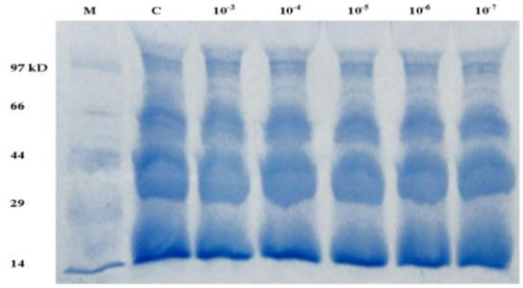

Fig. 2. Protein profiling of *Bacillus weihenstephanensis* after 48 Hours of exposure to imidacloprid

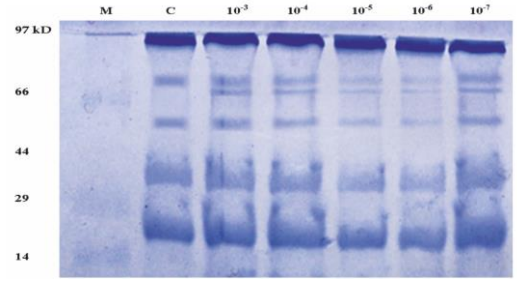

Fig. 3. Protein profiling of *Bacillus weihenstephanensis* after 72 Hours of exposure to imidacloprid

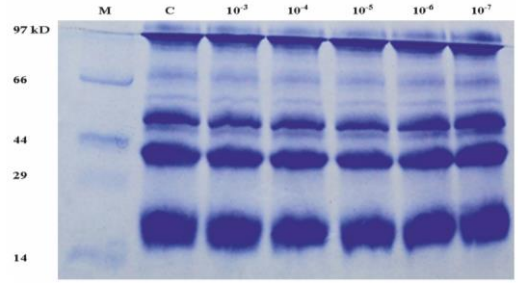

Fig. 4. Protein profiling of *Bacillus weihenstephanensis* after 96 Hours of exposure to imidacloprid

Discussion

Protein profiling of *Bacillus weihenstephanensis* on exposure to imidacloprid

The utility of protein gel electrophoresis in microbial characterization has been established for 20 years. A second level of information for a microorganism is given by the cellular proteins, and different types of electrophoresis are used to explore the relationship at this level (Vouterin *et al.,* 1993). Proteomic and metabolomic techniques can also be used to predict toxic potential of unknown chemicals by comparing specific patterns of protein and gene expression, reflecting the mode of action of unknown chemical, with the expression profiles of known toxicants. To conclude, ecotoxicogenomic approach seems promising for studying the effect of pollutants at low, environmentally relevant concentrations and long-term exposure assessment of organisms (Logar and Vodovnik, 2007).

The present investigation was attempted to elucidate the protein profiling in *Bacillus weihenstephanensis* that were exposed to different concentrations of imidacloprid ranging from 10^{-7} M to 10^{-3} M for a period of 96 hrs. Protein profile of *Bacillus weihenstephanensis* exposed to imidacloprid showed synthesis of commonly expressed protein, uniquely expressed, different stress proteins and over expression of some of the proteins. Different stress conditions induce expression of specific set of bacterial genes and several functions are repressed. Bacterial responses to different stress conditions studied mostly under *in vitro* provided useful information on survival under stress conditions and expression of different genes (Chowdhury *et al.,* 1996). The mechanisms for sensing environmental conditions and the resulting cellular signaling routes in microorganisms are still undefined. The regulation could be organized either globally or be stress specific. *E. coli* and *B. subtilis* are known for expression of specific genes for different environmental stress conditions.

The present investigation reveals that there are some commonly expressed proteins at 46 and 75 kDa in *Bacillus weihenstephanensis* at all dose and durations of exposure to imidacloprid. *E. coli* and *B. subtilis* are known for expression of specific genes for different environmental stress conditions. The stress proteins are used to monitor environmental samples for the presence of particular pollutants because of the fact that specific patterns of proteins are expressed for a particular stress (Sanders and Martin, 1993).

In the present study, there are proteins uniquely expressed at 14, 38, 54, and 56 kDa in *Escherichia coli* 57, 74, 82 and 95 kDa in 64, 83, 85 and 87 kDa in *Bacillus weihenstephanensis*. To overcome the stress, organisms generally might have two types of defense strategies: (i) mechanisms that prevent interaction with the stress factors and (ii) those that counteract the stress-induced damages (Saha *et al.,* 2003). It has been suggested that the analysis of many proteins produced during the transition into stationery phase and under stress conditions demonstrated that a number of novel proteins were induced in common to each stress and could be the reason for cross protection in bacterial cells. It is necessary to investigate the synthesis of these proteins during different stress conditions (Vasilyeva *et al.*, 2000). Similarly, it has been mentioned that when organisms or cells are exposed to low levels of certain harmful physical and chemical agents, the organisms acquire an induced tolerance against the adverse effects (Flahaut *et al.*, 1992). It has been reported, that along with short-lived regulatory proteins, the polypeptide chains with disrupted or changed structures are selectively hydrolyzed. Such defects might arise from inaccuracy during protein biosynthesis, chemical or physical damage

(Vasilyeva *et al.*, 2000) and moreover, the extracts of *Escherichia coli* have been shown to degrade rapidly the damaged enzyme, but not the native protein, and several preliminary reports have appeared concerning the *Escherichia coli* protease that may be responsible for selective degradation of the modified proteins (Lee *et al.*, 1988). Studies in the micro-organisms have provided evidence for increased longevity, cell division rate and survival when exposed to stress (Smith *et al.*, 1998). *Bacillus cereus* showed expression of nine new proteins of 97.1, 67, 64, 57, 54, 40, 39, 36, and 30 KDa in presence of Trichloroethylene (Mitra and Roy, 2011).

Conclusion

The present investigation undertaken to elucidate protein profile changes and differentially expressed proteins in soil isolates *Bacillus weihenstephanensis* on dose and durational exposure to imidacloprid revealed expression of commonly expressed protein, uniquely expressed and over expression of some of the proteins in all the three soil isolates. The different proteins expressed soil isolates may be due to the bacterium's ability to thrive in diverse environments by production of novel proteins or the increased production of already existing proteins, which are only produced under stress conditions due to stress response in bacteria and other organisms. The study shows that imidacloprid induced intoxication results in differential protein expression in *Bacillus weihenstephanensis*.

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