CHAPTER – II
Protein profiling of *Escherichia coli* and
*Pseudomonas aeruginosa* on exposure to
methomyl
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INTRODUCTION

The polyacrylamide gel electrophoresis has been used extensively for the separation of proteins in yeast, bacteria and higher organisms with the successful separation of whole cell extracts or specific proteins under selected conditions. This is an excellent method to attempt a global depiction of the cells protein profile. A single gel can provide large amount of diverse information about the physiological and genetic composition of a sample of cells. In fact many of the proteins on the gels are identified by PAGE (Shen et al., 2002). Thus, this technique is being extensively used to determine the in vivo amount of protein, its rate of synthesis, and its rate of degradation. The systematic analysis of the proteins of *Escherichia coli* has been already reported using this method to investigate the following in vivo processes and their regulation, protein regulation, protein stability, indirect effects of mutations, protein processing and message lifetimes (Nakayama et al., 1996). SDS–PAGE of extracted whole-cell and extracellular proteins is a relatively rapid and simple method that can be easily standardized (Berber, 2003).

Proteomics is a technique used to investigate whole proteins expressed by an organism, tissue or a cell at a specific time point under defined environmental conditions. This technique is based on gel electrophoresis. Proteomics has been used for many research purposes e.g. disease diagnosis, drug target and biomarkers (Lomthaisong et al., 2008). Proteomic investigations of microbial communities in their native environments not only provide the most realistic information about their function but also pose the greatest experimental and bioinformatics challenges (Valenzuela et al., 2006). It is one of the best strategies used to reveal the dynamic expressions of whole proteins in cells and their interactions (Shilev et al., 2007). Using proteomics, one can determine protein expression profiles related to research for both microbial isolates and communities. Proteomics provides a global view of the protein complement of biological systems and, in combination with other omics technologies, has an important role in helping uncover the mechanisms of these cellular processes and thereby advance the development of environmental biotechnologies (Lacerda and Reardon, 2009)
Protein electrophoresis has been of great value in bacterial classification and identification (Manchester et al., 1990). SDS-PAGE is an important molecular technique used for the identification at species level of whole cell proteins and it has the advantage of being fairly simple and rapid to perform (Leisner et al., 1994). Cellular proteins provide a second level of information about a cell, after the complete nucleotide sequence of the bacterial genome, and different types of electrophoresis have been used to explore relationships at this level. Analysis of whole-cell protein profiles by SDS–PAGE has been established as a useful method for the identification of a number of bacteria and unknown proteins (Berber et al., 2003; Shen et al., 2002). The identification of differentially expressed proteins could also clarify the gene response to some physiological actions and environmental conditions (Shilev et al., 2007).

Prokaryotic cells respond to environmental or chemical stress by inducing specific sites of proteins characteristic to each stress (Raheb et al., 2008). Stress proteins are expressed in response to a wide range of stress conditions in various bacteria (Gorg, 1993; Gross et al., 1990; Claudio et al., 1995). The proteins in each set of their coding genes constitute a stimulon, such as heat shock, SOS response and oxidation stress. In some other cases, proteins, which are associated with one stimulon, can be induced during other stresses, such as various heat shock proteins in Escherichia coli. These proteins are also synthesized when the cells are exposed to hydrogen peroxide, ethanol, UV light, puromycin or amino acid deprivation. In some stimulons, exposure to non-lethal levels of stress agents can confer protection against subsequent exposure to lethal levels of the same stress agents (Nystrom et al., 1992; Ronan et al., 2003). Although starvation for individual nutrients and other stress induce a unique and individual profile of protein expression, some proteins are common to different starvation and stress factors in Escherichia coli. However, the proteins of one stimulon do not respond coordinately to all the starvation and stress treatments and relatively few of the starvation- inducible proteins have been found to overlap with those induced by stress. This suggests that despite the regulation of a few specific proteins being interconnected, there are major difference in the regulatory pathways controlling the expression of starvation and different stress proteins (Lambert, 1997).
Studies on stress response and survival strategies of enteric bacteria have evolved a range of complex mechanisms, which use different regulatory structures and genetic components for their survival and virulence (Seputiene et al., 2004). *Escherichia coli* and *Pseudomonas aeruginosa* are the simplest bacteria that can be grown easily on their respective media and their whole cell protein profile can be easily studied on SDS-PAGE (Durrani et al., 2008). *Escherichia coli* has become one of the utmost popular models used for studying roles of various types of stress owing to its duplication time and rapid response to toxicants (Easton et al., 2006).

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., 1996). Studies in the microorganisms have provided evidence for increased longevity, cell division rate and survival when exposed to low doses of ionizing radiations and peroxide (Smith and Barbee, 1998). Stress proteins are expressed in response to a wide range of stress conditions. The most widely studied and best characterized of these responses is the heat shock response in various bacteria (Givsko et al., 1994; volker et al., 1994), but predominantly in *Escherichia coli* (Gross et al., 1990). In nature, there are numerous other conditions under which bacteria are under stress. The stress proteins in *Escherichia coli* have been characterized using heat, radiation, heavy metals, oxidizing agents, nutrient starvation, the SOS response and organic solvents (Kobayashi et al., 1998). Mechanisms of cellular adaptation and compensation against different kinds of toxic metals have been proposed (Patcharee et al., 2009). There are large numbers of specific proteins reported in various genera of bacteria that showed increase in the level of expression, upon adverse conditions, such as heat, toxic elements and nutrient limitations (Shilev et al., 2007). Bacteria of the genus *Pseudomonas* are well-studied and are of great interest not only because of high resistant to toxic substances, but also for their simple nutritional requirements and rapid growth on standard laboratory media (Pardo et al., 2003). *Pseudomonas aeruginosa* is isolated from different polluted
environments such as sewage, irrigation and agricultural drainage canals and soil (Shoriet and Sultan, 1992 and Soltan, 2001) therefore, it has been considered as a water quality indicator organism and studied for stress response against heavy metals (Soltan et al., 2008). Although not native to soil and water environments, *Escherichia coli* has been studied in the environmental context because of its role as a platform for metabolic engineering (Pferdeort et al., 2003). 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. Some limited work has been carried out on the heat shock and starvation stress responses in *Bacillus subtilis* (Givskov, 1994) and *Pseudomonas putida* (Volker, 1994), but thus far little is known regarding the chemically induced stress response in such microorganisms. In the case of the heat stress proteins and starvation-induced stress proteins, some similarities have been seen, but considerably more data are necessary to provide a detailed understanding of the stress response (Claudio et al., 1995). However, the molecular mechanisms and underlying responses of cells against various pesticides are not yet completely understood (Patcharee et al., 2009). Large majority of proteomic investigations of environmental microorganisms focus on model microorganisms cultured in the laboratory because of their interesting traits such as the ability to tolerate, degrade, or precipitate toxic compounds, or their versatility in the use of electron donors, electron acceptors, or carbon and energy sources. These qualities make these organisms attractive for environmental biotechnology applications, and proteomics can lead to a better understanding of their functions in specific habitats (Lacerda and Reardon, 2009). Therefore, the present investigation was undertaken to elucidate the proteomic profiling of *Escherichia coli* and *Pseudomonas aeruginosa* on dose and durational exposure to methomyl.
MATERIALS AND METHODS

Preparation of stock solution of methomyl

The sample of methomyl (Lannate ®) used in the experiment was supplied by E.I. Dupont India Pvt. Ltd., Haryana obtained. The stock solution of 1 M of methomyl was prepared and further diluted to give different required molar concentrations.

Maintenance and propagation of culture

The organism Escherichia coli, procured from NCL, Pune and the isolate Pseudomonas aeruginosa was maintained at 4°C on nutrient agar and sub cultured very fortnight.

Medium used for the study

Synthetic sewage medium (S-medium) formulated by Lackey and White (1970) was used as the medium for toxicity testing.

Preparation of inoculum for free cells

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

Experimental procedures

Free cells: Five ml of the pre-inoculum was inoculated to 250 ml Erlenmeyer’s flask containing 100 ml of sterilized S-medium amended with different molar concentrations of heavy metals. The flasks were incubated at 37°C for 96 hours under shaking conditions at 120 rpm on a rotary shaker (REMI – CIS-24). At regular intervals sample was taken out from each flask aseptically for analysis.

Immobilized cells: Immobilized beads were inoculated to 250 ml Erlenmeyer’s flask containing 100 ml of sterilized S-medium amended with different molar concentrations of pesticides to a final concentration equivalent to 5% of the pre-inoculum. The flasks were incubated at 37°C for 96 hours.
under shaking conditions at 120 rpm on a rotary shaker (REMI - CIS-24). At regular intervals, sample was taken out from each flask aseptically for analysis. For analysis the beads were dissolved in 0.1 M sodium citrate.

**Isolation of Protein**

The bacterial cell pellet was dissolved in 100µl of lysis buffer and incubated at 37°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

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

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-3.0 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-mercaptoethanol 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, DW 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 it in destaining solution (Methanol 4 ml, DW 5 ml, Glacial acetic acid 1 ml) and finally, the blue color protein bands were observed.

Marker proteins were used bearing molecular weight - 92.2, 66, 43, 29, 20.1 and 14.3 KD.
OBSERVATIONS

a) Protein profiling of *Escherichia coli* and *Pseudomonas aeruginosa* induced by methomyl

i) *Escherichia coli*

The present investigation was attempted to elucidate the protein profiling in *Escherichia coli* cells that were exposed to different concentrations of methomyl ranging from $10^{-7}$ M to $10^{-3}$ M for a period of 96 hrs, at regular intervals of 24 hrs, the proteins induced were analyzed. The protein expression was observed at 29, 45, 48, 55, 63, 92, and 114 kDa at 24 hrs (Fig. 1). On exposure to methomyl for 48 hrs the bands were observed at 29, 35, 39, 45, 63, 78, and 100 kDa (Fig. 2). The methomyl treated for 72 hrs showed expression at 29, 39, 45, 55 and 92.2 kDa (Fig. 3) and for 96 hrs the expressions was observed at 29, 35, 39, 45, 55, 63, and 92 kDa (Fig. 4) respectively.

ii) Immobilized *Escherichia coli*

The protein profiles of immobilized *Escherichia coli* showed the expressions at 35, 45, 55, 66, 92 and 114 kDa on treatment with methomyl for 24 hrs (Fig. 5), on exposure to methomyl for 48 hrs the bands were observed at 29, 35, 39, 45, 63, 78, 100 and 114 kDa (Fig. 6). The methomyl treated for 72 hrs showed expression at 29, 45, 66, 78, 100 and 114 kDa (Fig. 7) and for 96 hrs the expressions were observed at 35, 45, 63, 78, 100 and 114 kDa (Fig. 8) respectively.

iii) *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* was exposed to different concentrations of methomyl ranging from $10^{-7}$ M to $10^{-3}$ M of methomyl for a period of 96 hrs and at regular intervals of 24 hrs, the proteins induced were analyzed. The protein expression was observed at 19, 33, 38, 58, 85 and 115 kDa at 24 hrs (Fig. 9). On exposure to methomyl for 48 hrs the bands were observed at 16, 19, 33, 38, 58, 78 and 115 kDa (Fig. 10). The methomyl treated for 72 hrs showed expression at 19, 38, 58, 85, 97 and 115 kDa (Fig. 11) and for 96 hrs the expressions was observed at 19, 33, 38, 57, 97 and 115 kDa (Fig. 12) respectively.
DISCUSSION

a) Protein profiling of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to methomyl

Proteomics, transcriptomics and metabolomics are powerful tools for acquiring information on gene/protein function and regulatory networks (Phelps *et al.*, 2002; Singh, 2006). There are large numbers of specific proteins reported in various genera of bacteria that showed increase in their level of expressions, upon adverse conditions, such as heat, toxic elements and nutrient limitations (Shen *et al.*, 2002). The identification of differentially expressed proteins could clarify the gene response to some physiological actions and environmental conditions (Shilev *et al.*, 2005).

The present investigation was attempted to elucidate the protein profiling in *Escherichia coli* and *Pseudomonas aeruginosa* that were exposed to different concentrations of methomyl ranging from $10^{-7}$ M to $10^{-3}$ M for a period of 96 hrs. Protein profile of *Escherichia coli* and *Pseudomonas aeruginosa* exposed to methomyl showed induction of different stress proteins. It was observed that the proteins were expressed at different dose and duration of exposure and the intensity of the protein expressed increased with the dose of exposure of methomyl, indicating that the protein expressed is dose and durational dependant and methomyl induces stress (Kulkarni and Kaliwal, 2008; Kulkarni and Kaliwal, 2010). Similar observations were made by Asghar *et al.*, (2006), who analyzed the stress proteins of *Escherichia coli* induced in response to the pesticides cypermethrin, zeta-cypermethrin, carbofuran and bifenthrin. Their study has shown that there are many proteins synthesized in common for many stresses in *Escherichia coli*. Claudio *et al.*, (1995), have reported the stress response of *Pseudomonas putida* KT2442 on exposure to 2-chlorophenol and demonstrated different protein expression pattern where some of these proteins might play a major role in the stability of the cell under stress. It has been suggested that the over expressions of some of the proteins could be due to the fact that prokaryotic cells respond to environmental or chemical stress by inducing specific sets of proteins characteristic to each stress.
Exposure of *Escherichia coli* to pesticides is reported to induce the production of a large number of stress proteins to combat these harmful effects some of the proteins were induced by more than one pesticide (Asghar *et al.*, 2006). Further, it has been suggested that an increase in the intensity in protein expression may be due to the fact that the major protein modification is observed due to stress, loss of catalytic activity, amino acid modification, carbonyl group formation, increase in acidity, decrease in thermal stability, change in viscosity, fluorescence, fragmentation, formation of protein protein crosslink’s, s-s bridges and increased susceptibility to proteolysis (Cabisco *et al.*, 2000). A large number of proteins were induced exclusively in response to multiple nutrient starvations and could not be grouped into any of the individual nutrient starvation stimulons. This suggests that the multiple nutrient starvation stimulon is not only the sum of individual-nutrient starvation stimulons, and that, probably, additional sensors or signals are involved in the response to simultaneous starvation for carbon, nitrogen and phosphorus as has also been suggested by Nystrom (1990). In the present study, the types of stress patterns observed with the dose and duration of exposure of methomyl were identical which agreed with the earlier reports made by Asghar *et al.*, (2006) that the stress proteins produced in response to two different classes of pesticides showed same stress patterns for different substituent chemical groups within the same class and two different classes, indicating that the gene or set of genes responsible for stress expressions were the same irrespective of the class or nature of substituent’s on the pesticide. It has been reported that there was a clear temporal pattern to the synthesis of pesticides induced stress proteins indicating sequential gene expression as has been observed for starvation (Groat *et al.*, 1986), heat shock (VanBogelen *et al.*, 1987), SOS response (Nystrom and Neidhardt, 1992) and oxidation stress (Morgan *et al.*, 1986).

The induction of stress proteins and increase in the protein expressions observed in the present study may be due to the fact that prokaryotic cells respond to environmental or chemical stress by inducing specific sets of
proteins characteristic to each stress (Durrani et al., 2008), proteins that might play a major role in the stability of the cell under stress (Claudio et al., 1995), loss of catalytic activity, amino acid modification, carbonyl group formation, increase in acidity, decrease in thermal stability, change in viscosity, fluorescence, fragmentation, formation of protein protein crosslink's, s-s bridges and increased susceptibility to proteolysis (Cabisco et al., 2000).

The present study reveals that there are some commonly expressed proteins at 25 and 45 kDa in Escherichia coli, at 45 and 114 kDa in immobilized Escherichia coli and at 19, 38 and 115 kDa in Pseudomonas aeruginosa at all dose and durations of exposure to methomyl. It has been suggested that there are many protein synthesized in common with many stress in Escherichia coli and some of these proteins may play a major role in the stability of the cells under different stresses. The fact that specific patterns of proteins are expressed for a particular stress has led to the use of stress proteins to monitor environmental samples for the presence of particular pollutants (Sanders and Martin, 1993). In Escherichia coli there are already proteins which appear to be important under a wide range of stress conditions, including exposure to 2,4-dinitrophenol (Gage and Neidhart, 1993), and similar universal stress proteins are already known to exist in other bacteria (Volker et al., 1994).

A response regulator in Mycobacterium smegmatis which plays an important role in adaptation to oxygen-starved stationary phase was reported by Ronan et al., (2003) and a protein which is vital for growth and survival of Pseudomonas aeruginosa was reported by Boes, et al., (2006) and Schreiber et al., (2006). The over expressions of some of the proteins could be due to the fact that prokaryotic cells respond to environmental or chemical stress by inducing specific sets of proteins characteristic to each stress (Nystrom, 1992). It has been revealed that the secretion of extra cellular proteins, including toxins and cellular effectors, is one of the key contributing factors in a bacterium’s ability to thrive in diverse environments (Werner and Stephen, 2006). Flardh et al., (1992) have reported that during the transition on the stationary phase at temperatures around the optimum for growth, the concentration of proteins
which are involved in transcription and translation is greatly reduced. However, some proteins which have roles in energy metabolism are greatly increased in *Escherichia coli*. Applications of proteomics to study the physiology of *Bacillus subtilis* have been reviewed (Hecker *et al.*, 2004). Extensive proteomic work has been performed to understand the tolerance of *Bacillus subtilis* to extreme environments (Antelmann *et al.*, 2000; Bernhardt *et al.*, 2003). The proteins in each set of their coding genes constitute a stimulon, such as heat shock, SOS response and oxidation stress. In some other cases, proteins which are associated with one stimulon can be induced during other stresses, such as various heat shock proteins in *Escherichia coli*. These proteins are also synthesized when the cells are exposed to hydrogen peroxide, ethanol, UV light, puromycin or amino acid deprivation. In some stimulons, exposure to non-lethal levels of a stress agent can confer protection against subsequent exposure to lethal levels of the same stress agent (Nystrom *et al.*, 1992; Ronan *et al.*, 2003). Analysis of many proteins produced during the transition into stationary phase and under stress conditions (including starvation stress) 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 (Raheb *et al.*, 2008). It is suggested that the analysis of such stress proteins will aid in the development of more sensitive techniques for the pollutant analysis. The unique proteins could be purified and antibodies could be raised to enable quick detection, which could be used as biomarkers of xenobiotics in the environment (Patcharee *et al.*, 2009).

The over expression of some of the proteins observed in our study in *Escherichia coli* and *Pseudomonas aeruginosa* may be due to bacterium’s ability to thrive in diverse environments (Werner and Stephen, 2006), confer protection against subsequent exposure to lethal levels of the same stress agent (Nystrom *et al.*, 1992), novel proteins that are induced in common to each stress and could be the reason for cross protection in bacterial cells (Raheb *et al.*, 2008).
In the present study there are proteins uniquely expressed at 78 and 100 kDa in *Escherichia coli* and 16 kDa *Pseudomonas aeruginosa*. It has been suggested that the analysis of many proteins produced during the transition into stationary 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). The protein expressed is ascertained to the protein selective proteolytic degradation that appears to be rather significant in homeostasis maintaining and metabolism regulation in the cell (Beckwith and Strauch, 1988). 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). In some other cases, proteins, which are associated with one stimulon, can be induced during other stresses, such as various heat shock proteins in *Escherichia coli*. These proteins are also synthesized when the cells are exposed to different physical and chemical stress. In some stimulons, exposure to non-lethal levels of a stress agent can confer protection against subsequent exposure to lethal levels of the same stress agent (Nystrom et al., 1992). Although it has been reported that the starvation for individual nutrients and other stress induce a unique and individual profile of protein expression, some proteins are common to different starvation and stress factors in *Escherichia coli*. However, the proteins of one stimulon do not respond coordinately to all the starvation and stress treatments and this
suggests that despite the regulation of a few specific proteins being interconnected, there are major difference in the regulatory pathways controlling the expression of starvation and different stress proteins (Lambert et al., 1997). Studies in the micro-organisms have provided evidence for increased longevity, cell division rate and survival when exposed to stress (Smith et al., 1998).

Such uniquely expressed proteins observed in the present study may be due to increased longevity, cell division rate and survival when exposed to stress (Smith et al., 1998), selective degradation of the modified proteins (Lee et al., 1988), the proteins induced for cross protection in bacterial cells and inaccuracy during protein biosynthesis, chemical or physical damage (Vasilyeva et al., 2000), major difference in the regulatory pathways controlling the expression of starvation and different stress proteins (Lambert et al., 1997).

The present study reveals some randomly expressed proteins at 39, 48, 63, 66, 92, 100 kDa in free and immobilized Escherichia coli and at 33, 78 and 85 kDa in Pseudomonas aeruginosa. It has been reported that the microbial cells under oxidative stress conditions present different dysfunctions due to the lesions caused by reactive oxygen species to DNA, proteins or lipids (Avery, 2001). It is suggested that the toxicant induced oxidative stress in cells is partially responsible for the toxic effects (Shanmuganathan et al., 2004) and these effects can lead to induction of outer membrane proteins in Pseudomonas aeruginosa (Said et al., 1987) or generally to alteration of membrane permeability (Modugno et al., 1994). In stationery phase, the formation of non-culturable but viable cells depends on production of special proteins (Trainor et al., 1999; Shleeva et al., 2004). It has been reported that the proteins expressed yield an insight into possible stress induced changes in gene regulation that coordinate the processes such as inducible resistant mechanisms, cell translation machine, metabolism and cell division (Seputine et al., 2004). The continued presence and expression of these proteins in cells subjected to long-term stress shows that the proteins have a continued role in the survival of the organisms under extreme nutrient limitation (Raheb et al., 2008).

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The randomly expressed proteins observed in our results may be due to different dysfunctions of microbial cells under oxidative stress (Avery, 2001), induction of proteins (Said et al., 1987), alterations of membrane permeability (Modugno et al., 1994), gene regulation that coordinate the processes such as inducible resistant mechanisms, cell translation machine, metabolism and cell division (Seputine et al., 2004).

The use of proteomic and metabolomic techniques may also provide the possibility to predict toxic potential of unknown chemicals by comparing specific patterns of protein/gene expression, reflecting the mode of action of unknown chemical, with the expression profiles of known toxicants. To conclude, by providing both, mechanism of action and predictive tools, ecotoxicogenomic approach seems especially promising for studying the effect of pollutants at low, environmentally relevant concentrations, improvement of toxic mixture analysis and long term exposure assessment of organisms (Logar and Vodovnik, 2007).
SUMMARY AND CONCLUSIONS

Proteomics is a technique used to investigate whole proteins expressed by an organism, tissue or a cell at a specific time point under defined environmental conditions. The prokaryotic cells respond to environmental or chemical stress by inducing specific sets of proteins that can be analyzed by protein profiling. Exposure of bacterial cells to pesticides is reported to induce the production of a large number of stress proteins to combat these harmful effects.

The *Escherichia coli* and *Pseudomonas aeruginosa* cells were exposed to different dose and duration of methomyl and protein samples were analyzed by standard protocol of poly acrylamide gel electrophoresis (PAGE). The present investigation was aimed to elucidate the protein profiles of *Escherichia coli* and *Pseudomonas aeruginosa* on exposure to methomyl.

1. Graded dose and durational exposure of methomyl to *Escherichia coli* and *Pseudomonas aeruginosa* showed induction of different stress proteins.
2. The types of stress patterns observed in the present study were identical revealing that these proteins are synthesized in common and may play a vital role in maintaining the stability of the cells under stress conditions.
3. Protein profiling of *Escherichia coli* and *Pseudomonas aeruginosa* treated with different (10^{-7} to 10^{-3} M) concentrations of methomyl observed regularly at the duration of 24 hrs for a period of 96 hours, showed the expressions of unique proteins indicating that such novel proteins induced may be necessary for the cross protection of the bacterial cells and methomyl induced intoxication in *Escherichia coli* and *Pseudomonas aeruginosa*.
4. Dose and durational exposure of methomyl to free and immobilized cells showed a close correlation between the toxic effects in both the cells and further revealed that immobilized cells are less sensitive to the toxicant and hence exhibited lesser stress.
5. Methomyl exposure to *Escherichia coli* and *Pseudomonas aeruginosa* showed the over expressions of some proteins which may be due to the bacterial ability to thrive and confer protection against the stress in diverse environment.
6. The present study on the protein induction and expression profiles suggests that the exposure of *Escherichia coli* and *Pseudomonas aeruginosa* to methomyl induced the production of stress proteins to combat the harmful effects of the toxicant. The proteins induced on exposure to methomyl were observed to be dose and durational dependent.

7. The specific patterns of the proteins that are expressed in response to the stress induced by methomyl could be used to monitor the environmental samples for the presence of such pollutants. Although the application of gene and protein expression analysis to ecotoxicology is still at an early stage, this holistic approach seems to have several potentials in different fields of ecological risk assessment. It can be concluded that such extensive work on proteomics can be performed in understanding the proteomic/genomic response and tolerance of the microorganisms to the extreme environment.
EXPLANATION TO PHOTOGRAPHS

Fig. 1 .. Protein profiles of *Escherichia coli* induced by methomyl for 24 hrs

Fig. 2 .. Protein profiles of *Escherichia coli* induced by methomyl for 48 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M, Lane 5 - $10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 1 - Protein profile of *Escherichia Coli* induced by methomyl for 24 hours

Fig. 2 - Protein profile of *Escherichia Coli* induced by methomyl for 48 hours
EXPLANATION TO PHOTOGRAPHS

Fig. 3  ..  Protein profiles of *Escherichia coli* induced by methomyl for 72 hrs

Fig. 4  ..  Protein profiles of *Escherichia coli* induced by methomyl for 96 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M,
Lane 5 - $10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 3 - Protein profile of *Escherichia Coli* induced by methomyl for 72 hours

Fig. 4 - Protein profile of *Escherichia Coli* induced by methomyl for 96 hours
EXPLANATION TO PHOTOGRAPHS

Fig. 5  Protein profiles of immobilized *Escherichia coli* induced by methomyl for 24 hrs

Fig. 6  Protein profiles of immobilized *Escherichia coli* induced by methomyl for 48 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M,
Lane 5 - $10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 5 - Protein profile of immobilized *Escherichia Coli* induced by methomyl for 24 hours

Fig. 6 - Protein profile of immobilized *Escherichia Coli* induced by methomyl for 48 hours
EXPLANATION TO PHOTOGRAPHS

Fig. 7  .. Protein profiles of immobilized *Escherichia coli* induced by methomyl for 72 hrs

Fig. 8  .. Protein profiles of immobilized *Escherichia coli* induced by methomyl for 96 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M,
Lane 5 - $10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 7 - Protein profile of immobilized Esherichia Coli induced by methomyl for 72 hours

Fig. 8 - Protein profile of immobilized Esherichia Coli induced by methomyl for 96 hours
EXPLANATION TO PHOTOGRAPHS

Fig. 9 .. Protein profiles of *Pseudomonas aeruginosa* induced by methomyl for 24 hrs

Fig. 10 .. Protein profiles of *Pseudomonas aeruginosa* induced by methomyl for 48 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M, Lane 5 - $10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 9 - Protein profile of *Pseudomonas aeruginosa* induced by methomyl for 24 hours

Fig. 10 - Protein profile of *Pseudomonas aeruginosa* induced by methomyl for 48 hours
EXPLANATION TO PHOTOGRAPHS

Fig. 11  .. Protein profiles of *Pseudomonas aeruginosa* induced by methomyl for 72 hrs

Fig. 12  .. Protein profiles of *Pseudomonas aeruginosa* induced by methomyl for 96 hrs

Lane 1 - M- Marker, Lane 2 - C- Control, Lane 3 - $10^{-3}$ M, Lane 4 - $10^{-4}$ M ,
Lane 5 -$10^{-5}$ M, Lane 6 - $10^{-6}$ M, Lane 7 - $10^{-7}$ M
Fig. 11 - Protein profile of *Pseudomonas aeruginosa* induced by methomyl for 72 hours

Fig. 12 - Protein profile of *Pseudomonas aeruginosa* induced by methomyl for 96 hours