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Proteomic Profiling of *Escherichia coli* in Response to Carbamate Pesticide - Methomyl

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1. Introduction

Since decades, there has been mounting concern regarding the adverse health effects of environmental contaminants in general and carbamate in particular. Methomyl is a carbamate and widely used throughout the world since it is effective as “contact insecticide” as well as “systemic insecticide” for fruits and vegetables and is well known established cholinesterase inhibitor ¹. Methomyl has been classified as a pesticide of category-I toxicity ². Methomyl is a metabolite of thiodicarb and acetimidate is suspected oncogen, which is a metabolite in animal tissues ³. It has been classified by the WHO, EPA (Environmental Protection Agency, USA), and EC (European Commission) as a very toxic and hazardous pesticide ⁴. Methomyl is highly soluble in water and can therefore, easily cause ground water contamination in agricultural areas ⁵. Bonatti *et al.*, ⁶ have shown genotoxic effects of methomyl in *in vitro* studies. Methomyl is potent genotoxic and is capable of inducing structural and numerical chromosomal aberration in mammalian cells ⁷.

Prokaryotic cells respond to environmental or chemical stress by inducing specific sites of proteins characteristic to each stress ⁸. 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 ⁹. The stress protein induced in response to four different pesticides viz. cypermethrin, zeta-cypermethrin, carbofuran and bifenthrin were analyzed by protein profiling of *Escherichia coli* by Asghar *et al.*, ¹⁰. Mechanisms of cellular adaptation and compensation against different kinds of toxic metals have been proposed. However, the molecular mechanisms and underlying responses of cells against various pesticides are not yet completely understood ¹¹.

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. Nowadays, proteomics has been used for many research purposes e.g. disease diagnosis, drug target and biomarkers of pollutants ^{12,13}. Proteomics, transcriptomics and metabolomics are powerful tools for acquiring information on gene/protein function and regulatory networks²⁸. 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 biotechnologies²⁹.

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. Thus, this technique is being extensively used to determine the *in vivo* amount of protein, its rate of synthesis, and rate its rate of degradation¹³. SDS-PAGE is an important molecular technique used for the identification of whole cell proteins and it has the advantage of being fairly simple and rapid to perform¹⁴. Therefore, the present investigation was undertaken to study the proteomic profiling of *Escherichia coli* on dose and durational exposure to methomyl by gel electrophoresis.

2. Materials and methods

2.1 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.

2.2 Maintenance and propagation of culture

The organism *Escherichia coli* was procured from NCL, Pune and the bacteria was maintained at 4°C on nutrient agar formulated by Lapage and Shelton¹⁵ and sub cultured very fortnight.

2.3 Medium used for the study

Synthetic sewage medium (S-medium) formulated by Babich and Stotzky¹⁶ was used as the medium for toxicity testing.

2.4 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.

2.5 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.

2.6 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. PAGE according to Laemmli¹⁷ analyzed these protein samples.

3. Results and discussion

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 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 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, 45, 48, 55, 63, 92 and 114 kDa (**Fig. 2**). The methomyl treated for 72 hrs showed expression at 29, 39, 45, 66 and 92 kDa (**Fig. 3**) and for 96 hrs the expressions was observed at 29, 35, 39, 45, 55, 63, and 92 kDa (**Fig. 4**) respectively. The expression of proteins were more conspicuous in our result which was obligatory, since the free *Escherichia coli* cells possess antioxidant enzymes, which are induced in response to the stress and are directly exposed to methomyl ¹⁸.

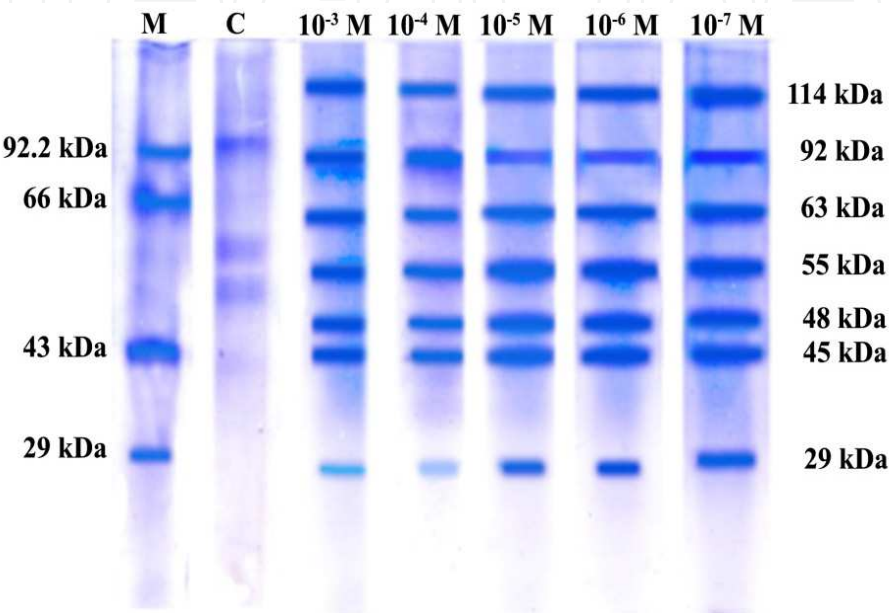


Fig. 1. Protein profile of *Escherichia coli* induced by methomyl for 24 hours.

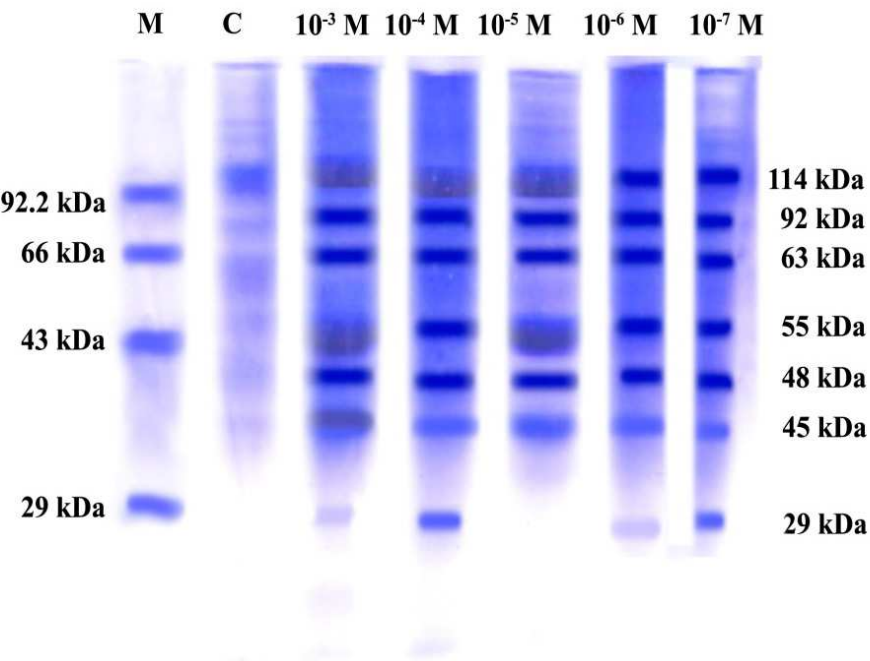


Fig. 2. Protein profile of *Escherichia coli* induced by methomyl for 48 hours.

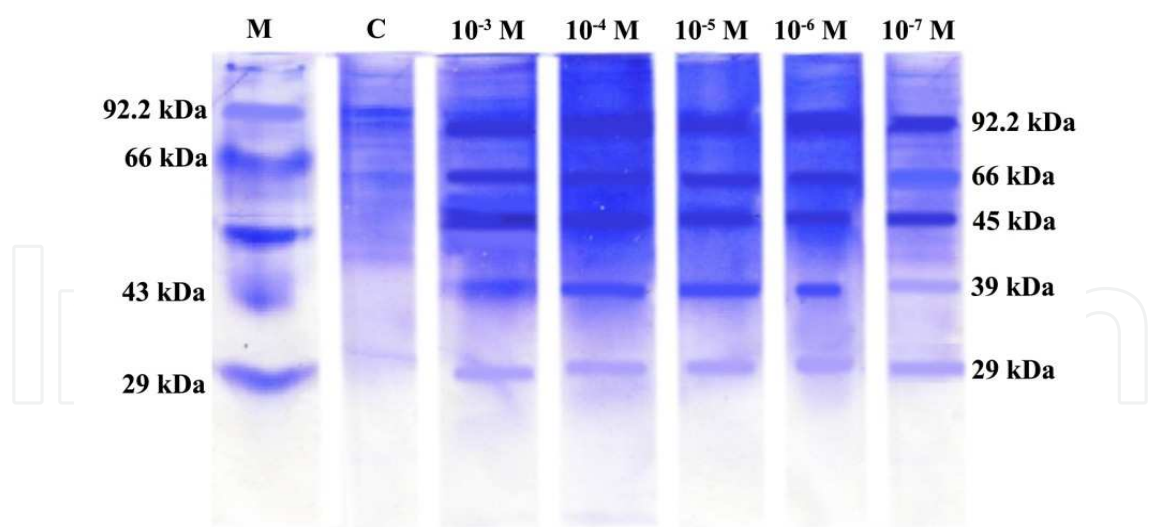


Fig. 3. Protein profile of *Escherichia coli* induced by methomyl for 72 hours.

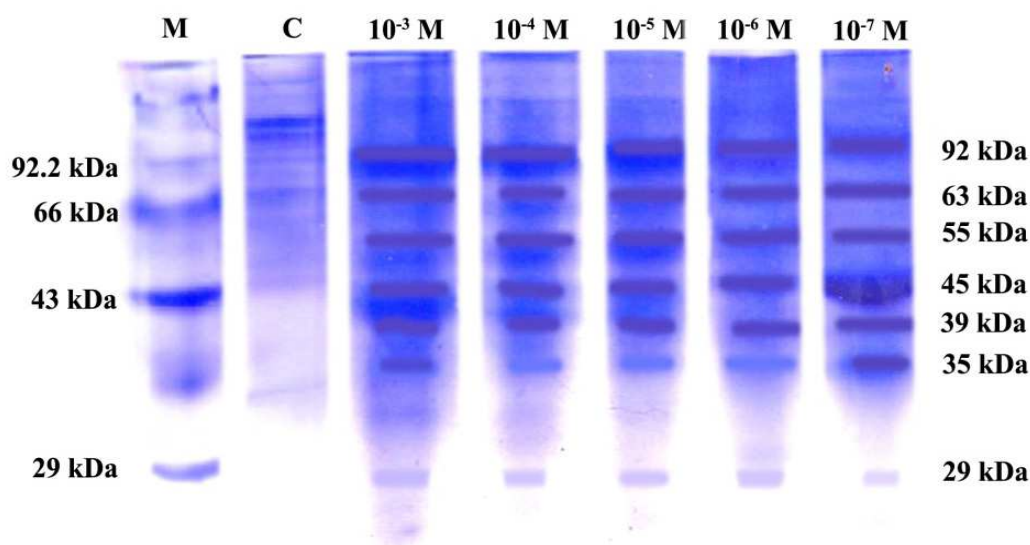


Fig. 4. Protein profile of *Escherichia coli* induced by methomyl for 96 hours.

The protein profiles were compared with the dose and duration of exposure of methomyl in *Escherichia coli* and the results revealed that the intensity of the proteins expressed increased with an increase in the dose and duration of exposure of methomyl when compared with those of the corresponding parameters of the control, indicating that the pesticide methomyl induces stress. Our results agreed with the observations made by Asghar *et al.*,¹⁰ who analyzed the stress proteins of *Escherichia coli* induced in response to the pesticides cypermethrin, zeta-cypermethrin, carbofuran and bifenthrin. The over expressions of some of the proteins observed in the present study at 29 and 45 kDa at all the dose and duration of exposure 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. It has been reported that 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 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 ¹⁹. Similarly, in the present study, the proteins expressed at 29 and 45 kDa could be unique or could be observed in the protein profiling of other micro-organisms exposed to various physical or chemical stress.

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 ²⁰. 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 ⁷. Hence, in the present study the high molecular weight proteins of 114 kDa at 24 and 48 hrs respectively observed in all the doses of exposure in comparison to their corresponding controls may be ascertained to the protein selective proteolytic degradation that appears to be rather significant in homeostasis maintaining and metabolism regulation in the cell ²¹. 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 ²² 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 ²³.

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 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 ²⁴. Studies in the micro-organisms have provided evidence for increased longevity, cell division rate and survival when exposed to stress ²⁵. Similarly 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 ¹⁰ that the stress proteins produced in response to two different classes of pesticides showed that the same stress patterns were obtained 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.

Further, an increase in the intensity in protein expression observed in the present study 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 ³. 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 ²⁶. Hence, the present study indicates that the protein expressions are dose and duration dependent. 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 ²⁷. It has been 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 raised to enable quick detection, which could be used as biomarkers of xenobiotics in the environment ¹¹.

4. Conclusions

The present study indicated the molecular weights of the various stress proteins induced in response to the dose and durational exposure of methomyl. Further, it indicates that the stress protein analysis is a promising alternative and more sensitive method for measuring toxic effects on the organisms at sub lethal levels. The study suggests that the proteomic profiling is a sensitive tool for environmental stress diagnosis, and that the stress proteins could be used as biomarkers for environmental pollution identification. 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.

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