

**SYNTHESIS AND ESTIMATION OF TOTAL EXTRACELLULAR PROTEIN CONTENT IN *BACILLUS SUBTILIS* UNDER MILD STRESS CONDITION OF CERTAIN ANTIMICROBIALS**KHUSRO A<sup>1\*</sup>, SANKARI D<sup>2</sup><sup>1</sup>Department of Plant Biology and Biotechnology, (PG Biotechnology), Loyola College, Nungambakkam, Chennai, Tamil Nadu, India.<sup>2</sup>Department of Biotechnology, Faculty of Science and Humanities, SRM University, Kattankulathur, Chennai, Tamil Nadu, India.

Email: arman Khan0301@gmail.com

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**ABSTRACT**

**Objective:** The present study was investigated to determine the impact of certain antimicrobials on a novel bacterial isolate for the estimation of total extracellular protein.

**Methods:** In this regard, isolation and molecular characterization of the isolate from poultry farm feces soil sample was done by serial dilution, followed by morphological characteristics and biochemical tests of pure isolated culture. Further, the identification of bacterium as *Bacillus subtilis* strain KPA was confirmed by subjecting its amplicon (483 bp) to 16S rRNA gene sequence analysis and pairwise alignment through basic local alignment search tool. Different volumes of antimicrobial agents such as *Allium sativum*, ampicillin and mercuric chloride at their sub-minimal inhibitory concentration (MIC) values were added to the lag phase culture of strain KPA. Total extracellular protein estimation was done through Bradford test. Partially purified extracellular proteins were observed as spots through thin layer chromatography (TLC).

**Results:** The total extracellular protein content in strain KPA was found to be enhanced after 48 hrs of incubation in presence of antimicrobials tested here. Mercuric chloride was able to enhance total protein in the bacteria even after 24 hrs of incubation. Separation of partially purified extracellular proteins of treated samples by TLC was observed as different spots with different retention factor values, compared with non-treated or control sample.

**Conclusion:** The stress response is a metabolic program activated due to unfavorable conditions. Hence, *B. subtilis* strain KPA in the presence of sub-MIC of *A. sativum*, ampicillin and mercuric chloride could regulate bioactive proteins production.

**Keywords:** Antimicrobials, *Bacillus subtilis*, Sub-minimal inhibitory concentration, Total protein, Thin layer chromatography.

**INTRODUCTION**

Microorganisms are often exposed to stress in various challenging environmental conditions. They have adaptive defense mechanisms, which allow them to survive and function in unfavorable conditions. Adaptive mechanism works in response to various stress and harsh conditions. The biological purpose of the stress response is to protect cells against lethal environmental factors and to repair damage due to stress conditions. The stress response is manifested as a change in the metabolic activity of the cell, resulting from the repressions of synthesis of most of the proteins formed in the cell under normal physiological conditions and induction of the synthesis of a specific group of proteins enabling the cell to function in the new conditions [1]. Bacterial cellular responses to different stress conditions have attracted current interest in microbiology. A range of bacteria, including *Escherichia coli*, *Bacillus* sp. and *Pseudomonas* sp. [2-4] have been reported to elicit an array of survival strategies in response to such drastic changes. Bacteria have developed special physiologic mechanisms including the production and secretion of specific proteins in unfavorable and stress conditions. Hence, the production of proteins was higher than the normal condition [5]. The stress responses are very specific and the mechanisms include metabolic alterations, activation of chaperones and signal transduction cascade dedicated for sensing and responding to various stress [6]. Stress responses among microbial population against an array of environmental physico-chemical stimuli including temperature, salt, oxidative stress are well-known [7-9]. Antimicrobials are one of the stresses in the bacterial world. The antimicrobial agents may be medicinal plants, antibiotics, heavy metals, etc. Medicinal plants, antibiotics and toxic heavy metals provide stress to the bacteria below their inhibitory concentration values and modulate

transcription process in the bacterial cells. Medicinal plants are the important sources of potential antibacterial compounds [10]. The secondary metabolites of the medicinal plants at low concentration are one of the causative agents for providing mild stress conditions to the bacterium, resulting in physiological changes and other metabolic activities of the cell. Natural antibiotics are secondary metabolites of the microorganisms living in natural environment. The microorganism undergoes several changes such as filamentation, spheroplast formation and cell lysis in the presence of antibiotics [11]. The changes vary with the bacterial strain, the concentration of antibiotic, the length of exposure to the antibiotic and the inoculum size [12]. Many bacteria adapt to environmental stress through synthesis of proteins, especially by exposure to toxic heavy metals. Heavy metals influence the microbial population by affecting their growth, morphology, biochemical activities and ultimately resulting in decreased biomass and diversity [13]. Metabolic activities were induced when the bacterial cells were incubated with sub-inhibitory concentrations of certain toxic heavy metals. Bacteria utilize different strategies to adapt to varying environmental situations including exposures to high concentrations of heavy metals. As drastic changes of bacteria in response to stress including high temperature, nutrients unavailability, oxidative stress, low and high pH, salt concentrations have been studied but still there is less report on the estimation of total extracellular protein content of bacteria especially *Bacillus* sp. under mild stress condition of medicinal plants, antibiotics and heavy metals as antimicrobial agents. *Bacillus subtilis* is non-pathogenic and non-toxicogenic to humans, animals and plants. Thus, scientists pay more attention on the exploitation of *B. subtilis* for the secretion of various proteins of interest. In view of this the present study was investigated to isolate novel strain of *Bacillus* sp. and to enhance their extracellular

protein content under mild stress condition of *Allium sativum* (medicinal plant), ampicillin (antibiotic) and mercuric chloride (heavy metal). The preliminary study to determine the synthesis of extra proteins in the isolate was done by partial purification of treated and non-treated bacterial proteins and further these extracellular proteins was observed by thin layer chromatography (TLC).

## METHODS

### Sample collection, isolation and screening

Poultry feces sample was collected from poultry farm of Guduvanchery, Tamil Nadu (India). Feces sample was brought to the laboratory in aseptic condition. A serial dilution of the sample (1 g of feces soil) was made using sterile saline until a dilution of  $10^{-6}$  was obtained. 100  $\mu$ l of this dilution was spread over nutrient agar petri plates and incubated at 37°C for 24 hrs. Pure culture was isolated and subcultured in the same medium at 37°C. The culture was streaked and kept in incubator at 37°C for 24 hrs and was preserved in slants at  $4 \pm 2^\circ\text{C}$ .

### Morphological and biochemical tests

Purified isolate was characterized by biochemical analysis using indole test, methyl red test, Voges-Proskauer test, citrate utilization test, catalase test, urease test, oxidase test, and amylase test (according to the Bergey's manual of systemic bacteriology). Gram staining, endospore staining and motility test were performed under morphological tests.

### Isolation of genomic DNA

A volume of 2 ml of bacterial culture were centrifuged at 6000 rpm for 5 minutes. The supernatant was discarded. 1 ml of UniFlex™ buffer 1 and 10  $\mu$ l of RNase were added to the pellet obtained. Mixed well by pipetting and incubated for 30 minutes at 37°C in a water bath. To the lysed samples 1 ml of 1:1 phenol: chloroform was added and mixed well. The samples were centrifuged at 10,000 rpm for 15 minutes at room temperature. The aqueous layers were separated in a fresh 1.5 ml vial. To the aqueous layer 1 ml of UniFlex™ buffer two were added and mixed well by pipetting. The mixture was centrifuged at 12,000 rpm for 15 minutes at room temperature. The supernatant was discarded. To the pellet 500  $\mu$ l of 70% ethanol were mixed. Again it was centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatant was discarded. The pellet was air-dried for about 10-15 minutes till the ethanol evaporates. The pellet was resuspended in 50-100  $\mu$ l of UniFlex™ elution buffer. DNA was stored at  $-20^\circ\text{C}$ .

### Amplification of 16S rRNA genes by polymerase chain reaction (PCR), sequencing and alignment

The 16S ribosomal RNA was amplified by using the PCR (ependorfep gradient) with *Taq* DNA polymerase and primers 27F (5' AGTTTGATCCTGGCTCAG 3') and 1492R (5' ACGGCTACC TTGTTACGACTT 3'). The conditions for thermal cycling were as follows: Denaturation of the target DNA at 94°C for 4 minutes followed by 30 cycles at 94°C for 1 minute, primer annealing at 52°C for 1 minute and primer extension at 72°C for 1 minute. At the end of the cycling, the reaction mixture was held at 72°C for 10 minutes and then cooled to 4°C. PCR amplification was detected by agarose gel electrophoresis and visualized by alpha image gel doc after ethidium bromide staining. The PCR product obtained was sequenced by an automated sequencer (Genetic Analyzer 3130, Applied Biosystems, and USA). The same primers as above were used for sequencing. The sequence was compared for similarity with the reference species of bacteria contained in genomic database banks, using the NCBI basic local alignment search tool (BLAST) available at: <http://www.ncbi.nlm.nih.gov/>.

### Antimicrobials of interest

*A. sativum* (medicinal plant), ampicillin (antibiotic) and mercuric chloride (heavy metal) were used to provide stress to the novel bacterial strain.

### Sample preparation

*A. sativum* (garlic) was purchased from local market of Nungambakkam, Tamil Nadu (India). The garlic bulbs without the outer skins were grinded

in a sterilized mortar and pestle. The fine garlic mesh was centrifuged at 6000 rpm for 10 minutes. The supernatant was filter sterilized by using a 0.2  $\mu$ m syringe filter to produce sterile supernatant. Heavy metal salt solution was prepared by mixing mercuric chloride ( $\text{HgCl}_2$ ) in sterilized distilled water at the concentration of 25 mg/L. Ampicillin (10  $\mu$ g) was prepared by mixing appropriate volume of dimethylsulfoxide.

### Minimal inhibitory concentration (MIC) and sub-MIC determination of antimicrobials

MIC and sub-MIC values of *A. sativum* juice, mercuric chloride and ampicillin were determined by microdilution method [14]. Serial dilutions of *A. sativum* juice, mercuric chloride and ampicillin were prepared from 100% to 1% concentration. Serial dilutions of antimicrobials were added to bacterial culture in microtiter plate. The plates were incubated at 37°C for overnight. The bacterial growth was detected by the addition of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solutions (10 mg/ml) in the wells. The highest dilution of antimicrobials inhibiting the bacterial growth was considered as MIC value. Half of the value of MIC was considered as sub-MIC value.

### Inoculum preparation

A volume of 50 ml of inoculum medium containing nutrient broth (pH- 7.0) was transferred to 250 ml of conical flask and cotton plugged. The flask was sterilized at 121°C, 15 lb pressure for 15 minutes. A loop full of bacteria was inoculated aseptically into the cooled medium and kept for incubation overnight at 37°C in a rotatory shaker.

### Shake flask fermentation

Estimation of total protein was carried out in 16 conical flasks (volume capacity 250 ml), each flask containing 50 ml of nutrient broth. The flasks were divided into three sets (each set containing five flasks). One flask was kept as control (parental or non-treated). One set was labeled as 50  $\mu$ l, 100  $\mu$ l, 250  $\mu$ l, 500  $\mu$ l and 1000  $\mu$ l of *A. sativum*. Second set was labeled as 50  $\mu$ l, 100  $\mu$ l, 250  $\mu$ l, 500  $\mu$ l and 1000  $\mu$ l of ampicillin. Third set was labeled as 50  $\mu$ l, 100  $\mu$ l, 250  $\mu$ l, 500  $\mu$ l and 1000  $\mu$ l of mercuric chloride. The flasks were sterilized at 121°C for 15 minutes. Each flask was inoculated with 500  $\mu$ l of overnight bacterial inoculum. The flasks were kept in the rotatory shaker at 37°C for 2 hrs (lag phase of novel strain; graph not shown here). The flasks were taken out and each flask except control was inoculated with appropriate concentration of antimicrobials (from sub-MIC value) as labelled on the flasks. All the flasks were again kept in rotatory shaker for 24-72 hrs of incubation.

### Cell-free supernatant preparation

The overnight bacterial cultures grown in the presence of different concentrations of antimicrobials were centrifuged at 7000 rpm for 10 minutes. The control flask containing only the bacterial culture was also centrifuged at the same rpm. The supernatants were collected and total extracellular protein content was estimated through Bradford test. The same procedure was repeated till 72 hrs.

### Estimation of total extracellular protein content

Estimation of total extracellular protein was performed through Bradford test. Bradford method is a simple and rapid method to estimate the protein content in a sample based on the ability of protein to bind with the dye Coomassie Brilliant Blue G250. The unbound dye has an absorption maxima of 495 nm, on binding with the protein, the absorption maxima becomes 595 nm. Thus from the absorbance at 595 nm, the protein in the sample solution can be estimated. Bovine serum albumin was used as standard. Different volumes (10  $\mu$ l, 20  $\mu$ l, 40  $\mu$ l, 60  $\mu$ l, 80  $\mu$ l and 100  $\mu$ l) of working standard were pipette out into a series of test tubes. 100  $\mu$ l of supernatant from each of the flask was pipette out into other tubes. Volume of the tubes was made up to 1 ml using sterilized distilled water. 5 ml of the Bradford reagent were added to all the tubes and mixed thoroughly. 1 ml of distilled water with 5 ml of Bradford reagent was used as blank. Absorbance at 595 nm was recorded against blank. Extracellular protein content in per milliliter

of test samples was determined against the standard curve. This procedure was used for determining the total extracellular protein content for all the flasks containing bacterial culture in stress condition till 72 hrs.

**Bacterial proteins production**

*B. subtilis* strain KPA colony was grown in 50 ml of Mueller-Hinton Broth and incubated at 37°C with gentle agitation. *A. sativum*, ampicillin and mercuric chloride at sub-MIC (0.5 × MIC) were added to the bacterial culture during lag phase (2 hrs of incubation) of cultivation period. Another culture not treated with *A. sativum*, ampicillin and mercuric chloride were served as a control. After 48 hrs of fermentation process, the production of bacterial proteins was further estimated.

**Bacterial proteins extraction**

Bacterial extracellular proteins extraction was done according to Lash *et al.*, [15] with some modifications in the procedure. The bacterial culture was centrifuged at 7000 ×g for 6 minutes at 4°C. The supernatant was collected and filter sterilization process was done by using 0.2 µm syringe filter in order to produce sterile cell-free supernatant. 60% (w/v) of ammonium sulfate was added to 50 ml of supernatant and mixed-well and left for 2-3 hrs at 4°C to precipitate the proteins. The precipitated proteins were pelleted by centrifugation at 10,000 ×g for 30 minutes at 4°C and suspended into 200 µl of phosphate-buffered saline (PBS, pH 6.8) before further centrifuged for another 20 minutes at 10,000 ×g at 4°C. The pellets were weighed and dissolved in known volume of PBS (according to the weight of pellets obtained) and preserved at -20°C for further use.

**Extracellular protein visualization by TLC**

Partially purified bacterial extracellular proteins were visualized by TLC using silica gel as adsorbent. A line was drawn on the TLC plate at a distance of 2 cm from the base. The samples were spotted with the help of capillary tube and it was allowed to dry. The plate was placed in jar with mobile phase (ethyl acetate:n-butanol:acetic acid:water = 8:10:5:5). After the solvent reaches more than half of the TLC plate it is taken out of the jar. The solvent run was drawn. The TLC plate was examined under the ultraviolet (UV) lamp at two different wavelengths (302 nm and 365 nm). After this the plate was viewed under the exposure of iodine vapors and the spots were observed. The spots were labeled and the distances from the origin were measured. The retention factor (Rf) values were calculated by the given formula.

$$\frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

**RESULTS**

**Isolation and identification of the new strain of bacteria**

The morphological and biochemical characteristic of the isolate were studied (Table 1). The isolated bacterial strain was identified as *Bacillus* sp. based on the taxonomical characteristics. Genomic DNA of the isolate was visualized under UV. The amplicon of 483 bp was observed using PCR amplification. In the present study, 16S rRNA gene sequencing of the isolate was investigated. The isolate was identified as *B. subtilis* strain KPA by comparing the similarity with the reference species of bacteria contained in genomic database banks, using the NCBI BLAST. The comparison showed that the similarity of 16S rRNA gene sequences was 99%. The identities of strain KPA were determined by comparing them with the available sequences of the strains and with high scored rRNA sequences in BLAST search. The novel isolated sequence was deposited in Genbank (Accession number-KC918878), maintained by NCBI, USA.

**MIC and sub-MIC determination of *A. sativum*, ampicillin and mercuric chloride**

The effects of antimicrobial on bacterial cell growth were studied to determine the MIC value, which could inhibit the bacterial growth. In this experiment, the bacterial viability was evaluated by using MTT

solution as an indicator. The results from Table 2 indicated that the MIC values of *A. sativum*, ampicillin and mercuric chloride on *B. subtilis* strain KPA was 10%, 10% and 20%, respectively. The sub-MIC values (which is 0.5 × MIC) for each treatments was further calculated as 5%, 5% and 10% for *A. sativum*, ampicillin and mercuric chloride respectively.

**Estimation of total extracellular protein content**

Total extracellular protein content of the strain under *A. sativum* stress was increased after 48 hrs of incubation. Maximum protein content of 0.820 mg/ml was determined after 48 hrs of incubation compared to the control (-0.051.2 mg/ml) at 50 µl of concentration. Total protein content was decreased after 72 hrs of incubation (Fig. 1). Ampicillin was able to enhance the protein content in strain KPA from -0.051.2 mg/ml to 0.862 mg/ml after 48 hrs of incubation at 1000 µl of concentration (Fig. 2). However, the stress provided by mercuric chloride was totally different from *A. sativum* and ampicillin. Total extracellular protein content in stressed strain KPA was increased (0.065 mg/ml) compared to the control bacteria (-0.051.2 mg/ml) at 250 µl of concentration even after 24 hrs of incubation. Total extracellular protein content in stressed strain KPA was also increased after 48 hrs of incubation. The protein content in stressed bacteria compared to control was also found to be increased after 72 hrs of incubation but the protein content was less than that of 48 hrs. Among all the antimicrobials tested here, mercuric

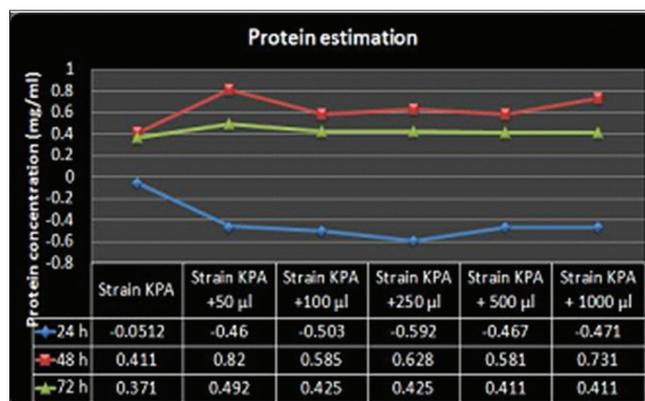
**Table 1: Morphological and biochemical test reports of strain KPA**

S. No.	Tests	Result
1	Morphology	Rod shaped
2	Gram staining	Positive
3	Motility	Positive
4	Indole	Negative
5	Methyl red	Negative
6	Voges-Proskauer	Positive
7	Citrate utilization	Positive
8	Urease	Negative
9	Catalase	Positive
10	Amylase	Positive
11	Oxidase	Negative
12	Endospore staining	Endospores observed

**Table 2: MIC and sub-MIC values for antimicrobials against strain KPA**

Antimicrobials	MIC value (%)	Sub-MIC value (%)
<i>Allium sativum</i>	10	5
Ampicillin	10	5
Mercuric chloride	20	10

*A. sativum*: *Allium sativum*, MIC: Minimal inhibitory concentration



**Fig. 1: Total extracellular protein content of strain KPA in presence of *Allium sativum***

chloride was found to be most effective as a signaling agent in order to induce total protein content (0.898 mg/ml) in strain KPA (Fig. 3).

**Extracellular protein visualization**

Partially purified extracellular proteins of strain KPA with and without stress conditions were weighed. *A. sativum* proteins were also partially purified. The weight of the normal bacterial extracellular protein pellet after purification was obtained as 20 mg. Although, the weight of *A. sativum*, ampicillin and Mercuric chloride stressed bacterial protein pellet was obtained as 30 mg, 22 mg and 22 mg, respectively. Partially purified garlic protein was weighed as 168 mg. The partially purified extracellular proteins were run on TLC plates to visualize extracellular protein pattern. Rf values for the partially purified extracellular proteins of the control bacteria (parental or non-treated), *A. sativum* stressed bacteria, mercuric chloride stressed bacteria, ampicillin stressed bacteria and *A. sativum* alone were determined as 0.16, 0.54; 0.27, 0.56, 0.64; 0.25, 0.56; 0.24, 0.56, 0.64 and 0.11, 0.33, 0.58 respectively using ethyl acetate:n-butanol:acetic acid:water as mobile phase in the ratio of 8:10:5:5 (Table 3 and Figs. 4a-c).

**DISCUSSION**

*B. subtilis* secretes high levels of proteins in unfavorable conditions. Bacteria under stress "switch on" a catalogue of genes, which affects the protein promoters. Antimicrobial agents at sub-inhibitory concentration could act as an inducer or signaling agent by causing up- or down expression of a large number of transcripts in different bacteria [16]. In this present study, *B. subtilis* strain KPA was grown in the presence of *A. sativum*, ampicillin and mercuric chloride at various concentrations (100-1%). The effects of these antimicrobials on bacterial growth were identified in order to determine the MIC and sub- MIC values against the strain. After addition of MTT to overnight grown culture in presence of these antimicrobials, the blue color showed bacteria growth due to the blue formazan formed, whereas the yellow colour indicated no bacteria growth. The MIC value was determined as the highest dilution or lowest concentration of *A. sativum*, ampicillin and mercuric chloride showing no bacterial growth. Microbial secondary metabolites, including microbial proteins are usually not produced during log phase, but are synthesized during a subsequent production stage (stationary phase), which is when primary nutrient source is depleted [17]. *B. subtilis* cells that has exhausted one or more essential nutrients and experienced fluctuations in the environment will enter the stationary phase of growth. At this stage, the production of extracellular proteins, enzymes and antibiotics are induced. These various phenomena reflect the complex response of the cells to stress and show that a rapid adjustment to environmental change is essential for survival [18]. In this study, bacterial cells were cultivated for 24-72 hrs in the presence of sub-MIC of *A. sativum*, ampicillin and mercuric chloride. After 24 hrs of incubation the stressed bacteria were not able to produce more amount of extracellular protein, but total extracellular protein contents of the treated bacteria compared to non-treated strain were increased after 48 hrs of incubation. This may be due to the reason that strain KPA entered into the stationary phase after 48 hrs when nutrients were depleted and the cells experienced more stress due to the presence of antimicrobials. On the other hand, Mercuric chloride at the particular concentration was able to enhance the total extracellular protein content in strain KPA even after 24 hrs of incubation. This may be because of different mode of action of mercuric chloride on strain KPA in order to provide stress, which results in secretion of more amounts of extracellular proteins after 24 hrs of incubation. Partially purified extracellular proteins of treated and non-treated bacteria were observed as different spots with different Rf values through TLC, indicating the synthesis of extra proteins in strain KPA under mild stress conditions of certain antimicrobials. Hence, it demonstrated that *B. subtilis* strain KPA cells were induced to secrete high level of extracellular proteins during stationary phase as an adaptation strategy in the sub-minimal stress condition caused by *A. sativum*, ampicillin and mercuric chloride. This study postulate that the bacteria will receive an alarm after sensing the antimicrobial agents and the mechanisms

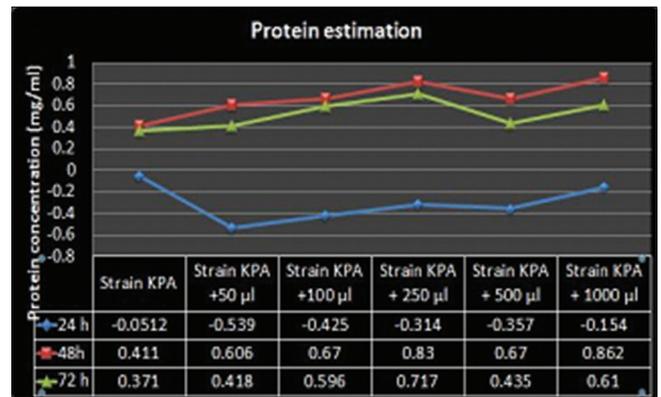


Fig. 2: Total extracellular protein content of strain KPA in presence of ampicillin

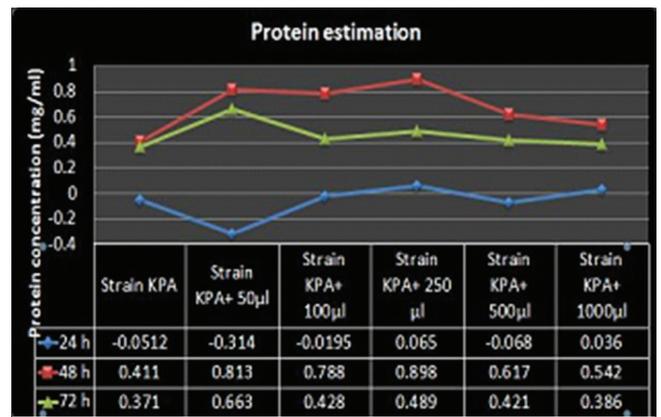


Fig. 3: Total extracellular protein content of strain KPA in presence of mercuric chloride

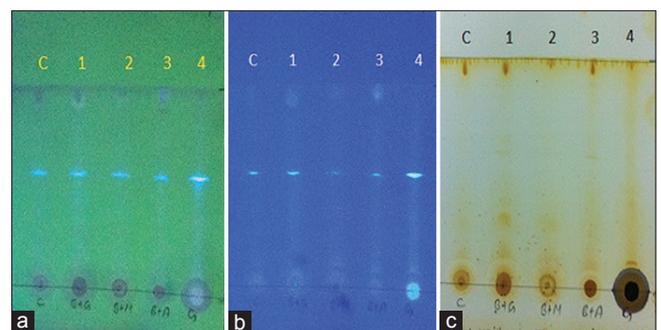


Fig. 4: (a) at 302 nm, (b) at 365 nm, (c) iodine vapour, (C: Extracellular proteins of strain KPA (control); 1: Extracellular proteins of strain KPA in presence of Allium sativum stress; 2: Extracellular proteins of strain KPA in presence of mercuric chloride stress; 3: Extracellular proteins of strain KPA in presence of ampicillin stress and 4: Allium sativum only)

Table 3: Rf values of the separated extracellular proteins (protein estimation) by TLC using ethyl acetate: n-butanol: Acetic acid: Water as eluting solvent

Partially purified proteins	Rf values
Control bacteria (strain KPA)	0.16, 0.54
Strain KPA+A. sativum	0.27, 0.56, 0.64
Strain KPA+mercuric chloride	0.25, 0.56
Strain KPA+ampicillin	0.24, 0.56, 0.64
<i>A. sativum</i>	0.11, 0.33, 0.58

*A. sativum*: Allium sativum, TLC: Thin layer chromatography

within them directly start working overtime to neutralize or pump out the undesired compound as well as to repair damaged molecules in the cell. This condition might trigger transcriptional changes in the bacteria and initiate the secretion of proteins. The proteins produced by the bacteria after exposure towards mild stress might be used by the bacteria to defend themselves against antimicrobial agents or stresses (Ismail *et al.*, 2013). It is supported by the previous study stated that the antimicrobials or antibiotics at sub MIC can act as signaling molecule or inducer in the bacterial metabolite process by modulating their transcriptional machinery process [19]. In this study, strain KPA was exposed to rapid changes in their environment after subjecting with *A. sativum*, ampicillin and mercuric chloride. The stress might induce the secretion of various extracellular proteins that related with metabolism. Notably, metabolism process is a vital process for microorganisms to obtain energy and nutrients it needs to live and reproduce. Metabolism alterations couple with gene activation leads to further cellular effects that require signal transduction. Hence, an initial stimulus can trigger the expression of a large number of genes which leading to physiological event like the production of new proteins related with metabolism networks in microbial cells [20]. Drastic changes in environmental conditions occur suddenly and therefore, a quick response is necessary to assure cell survival. Translational regulation of pre-existing mRNAs provides a fast and efficient way to control gene expression. Remarkably, the stress-induced attenuation of global translation is often accompanied by a switch to the selective translation of proteins that are required for cell survival under stress [21]. In this investigation, proteins involved in the translation process were differentially expressed by strain KPA cells under mild stress of certain antimicrobials. The findings presented that translation is regulated to synthesize more extracellular proteins as bacterial tolerance response due to drastic changes in environmental conditions.

## CONCLUSION

The present exploratory study described that *B. subtilis* strain KPA cells under mild stress condition of *A. sativum*, ampicillin and mercuric chloride at sub-MIC values were able to induce extracellular proteins production. The present findings led to the generalized assumption that the certain antimicrobial agents tested here at sub-MIC value were able to enhance extracellular proteins production by regulating the transcription process in bacteria. Identification of the purified extracellular proteins is essential to recognize the novel proteins or peptides synthesized in bacteria during mild stress condition. Hence, further study is necessary to know the novel proteins synthesized in strain KPA in presence of certain antimicrobials tested here by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and peptide sequencing by liquid chromatography-mass spectrometry (MS)/MS technique. Another study should also be continued to determine the antagonistic effect of synthesized proteins against certain pathogens including multidrug resistant microbes.

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