Comparative proteomic analysis of Bacillus thuringiensis wild-type and two mutant strains disturbed in polyphosphate homeostasis

Filiz YEŞİLIRMAK1, Tuğrul DORUK2, Şerif YILMAZ2, Sedef TUNCA GEDİK2*

1 Department of Chemistry, Faculty of Science, İzmir Institute of Technology, Urla, İzmir, Turkey
2 Department of Molecular Biology and Genetics, Faculty of Sciences, Gebze Technical University, Gebze, Kocaeli, Turkey

Abstract: Polyphosphate polymer (polyP) plays a very important role in every living cell. Synthesis of this linear polymer of phosphate (P) residues is catalyzed by the polyphosphate kinase (PPK) enzyme. It was shown that high levels of intracellular polyphosphate stimulated endotoxin production by Bacillus thuringiensis subsp. israelensis (Bti). In this study, proteomic analysis of the wild-type and two mutant strains, overexpressing the ppk gene (Bti pHTppk) and without the ppk gene (Bti Δppk), were used to clarify the relation between polyP and endotoxin production. Intracellular proteins were separated by two-dimensional gel electrophoresis; 41 spots of interest (proteins differentially expressed) were obtained and 35 of them were identified by mass spectrometry. Analysis of the protein profiles showed that there is a general decrease in the expression levels of proteins related with energy metabolism, amino acid metabolism, and purine biosynthesis in both Bti pHTppk and Bti Δppk. Gluconeogenesis and fatty acid metabolism were also slowed down in both strains, whereas expression of stress response proteins increased compared to the wild-type. These results suggested that changes in polyP concentration cause a general stress condition inside the cell, which in turn stimulates secondary metabolite synthesis.

Key words: Polyphosphate polymer, polyphosphate kinase, two-dimensional gel electrophoresis, endotoxin, bioinsecticide, Bacillus thuringiensis israelensis, secondary metabolite, regulation, proteomics

1. Introduction
Polyphosphate (polyP), which is a linear polymer of phosphate (P) residues linked by high-energy phosphoanhydride bonds, is present in all organisms from bacteria to humans (Rao et al., 2009). PolyP has been shown to have important roles in a variety of cellular processes including regulation of enzyme activities, storage of Pi and energy, adaptive responses to physical and chemical stresses, bacterial survival during the stationary phase, chelation of cations, maintenance of optimal translation efficiency, gene transcription control, and formation and function of cell membrane, channels, and pumps (Kornberg et al., 1999). PolyP has also been shown to be necessary for motility, biofilm formation, and other virulence properties of different bacteria such as Salmonella spp. (Kim et al., 2002), Shigella flexneri (Kim et al., 2002), Pseudomonas aeruginosa (Rashid et al., 2000), and Mycobacterium tuberculosis (Singh et al., 2013). The effect of polyP on the biosynthesis of secondary metabolites by Streptomyces (Chouayekh and Virolle, 2002; Yalım Camcı et al., 2012) and Bacillus thuringiensis israelensis (Bti) (Doruk et al., 2013) has also been demonstrated. There are a few studies about the function of polyP in eukaryotes: it has a role in blood coagulation (Smith et al., 2006), inflammation (Muller et al., 2009), innate immunity, and cancer metastasis (Wang et al., 2003; Tammenkoski et al., 2008).

The main polyP synthetic enzyme in many bacteria is polyP kinase 1 (PPK1), which catalyzes the reversible transfer of Pi from ATP to polyP and from polyP to ADP (Brown and Kornberg, 2008).

Although polyP has been shown to have important functions in different organisms, to our knowledge, there have been only two studies on the role of this polymer in Bacillus. Shi et al. (2004) showed that polyP is important for motility, biofilm formation, and sporulation in Bacillus cereus. In another study, the Bti strain, which overexpress the ppk gene, was found to be about 7.7 times more toxic against late 2nd instar Culex quinquefasciatus than the wild-type (Doruk et al., 2013). To clarify the relation between polyP metabolism and endotoxin production, the proteomes of the wild-type and two mutant strains, one overexpressing the ppk gene (Bti pHTppk) (Doruk et al., 2013) and the other without the ppk gene (Bti Δppk) (Doruk and Gedik, 2013), were analyzed in this study.
2. Materials and methods

2.1. Media and growth conditions

*Bti* strains were grown in Difco sporulation medium (DSM) (4 g/L nutrient broth, 25 mM KH₂PO₄, 25 mM KH₂PO₄, 0.5 mM Ca(NO₃)₂, 0.5 mM MgSO₄, 0.5 mM FeSO₄, 10 µM MnCl₂, 5 g/L glucose) (Donovan et al., 1988) for endotoxin production and were grown in both DSM and Luria broth for proteomic analysis at 30 °C. Experiments were started from overnight cultures, which were diluted to equal inoculum size by using a spectrophotometer (OD₆₀₀). Where appropriate, 25 µg/mL erythromycin was added to growth media. Liquid cultures were aerated on a rotary shaker at 220 rpm.

2.2. Strains and plasmids

*Bti* ATCC 35646 and pHT315 were kindly provided by Gwo-Chyuan Shaw (National Yang-Ming University, Taiwan). The full list of strains and plasmids is given in Table 1.

2.3. Protein isolation

Protein extraction for toxin isolation was performed by the procedure of Donovan et al. (1988). Proteins of 10 mL of cells (spore and toxins) from cultures grown for 72 h were extracted and equal volumes of protein solution from each sample were serially diluted (from 50 µg/mL to 1.56 µg/mL) and used in the bioassay experiments.

For the total protein isolation for two-dimensional electrophoresis (2-DE), cultures grown for 10 h (in both DSM and LB media) were harvested and washed with a previously chilled TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA). The pellets were resuspended in lysis buffer (7 M urea, 2 M thiourea, 4% w/v CHAPS, cOmplete protease inhibitor cocktail (Roche, Switzerland)) and ruptured by sonication for 10 min at 0 °C. After adding 1 mg/mL DNase and 0.25 mg/mL RNase, the lysed cell suspension was centrifuged at 13,800 rcf for 10 min to precipitate the insoluble components. The supernatant was collected, its protein concentration was determined using the Bradford method (Bradford 1976), and it was then stored at –80 °C until used for 2-DE.

2.4. Mosquito larvicidal activity

The method for larvicidal activity was adapted from Promdonkoy et al. (2005). Basically, 10 late 2nd instar *Culex quinquefasciatus* larvae (supplied by Öner Koçak, Hacettepe University, Turkey) were exposed to serially diluted toxins in each well of 24-well plates (well diameter: 1.5 cm) containing 1 mL of sterile tap water. LC₅₀ values were determined by using probit analysis (Finney and Stevens, 1948) at the end of 24 h by taking the average results of three independent experiments.

2.5. Two-dimensional electrophoresis

Protein samples of 400 µg were mixed with a rehydration solution containing 7 M urea, 2 M thiourea, 4% CHAPS, immobilized pH gradient (IPG) buffer (2% v/v, pH 3–10), and 65 mM dithiothreitol (DTT) to a total volume of 400 µL. The mixture was loaded on IPG strips (17 cm, pH 3–10 nonlinear gradient, Bio-Rad, USA) and rehydrated without current for 24 h by passive rehydration and with current of 50 V for 16 h by active rehydration. Isoelectric focusing was carried out at 20 °C on the IPGphor unit under the following steps: 1) 200 V for 300 Vh, 2) 500 V for 500 Vh, 3) 1000 V for 1000 Vh, 4) 4000 V for 4000 Vh, 5) 8000 V for 24,000 Vh, and 6) 8000 V for 30,000 Vh. After focusing, the strips were subsequently equilibrated for 15 min in reduction solution (2% SDS, 6 M urea, 0.375 M Tris (pH 8.8), 20% glycerol, and 2% DTT) followed by 15 min in alkylation solution (2% SDS, 6 M urea, 0.375 M Tris (pH 8.8), 20% glycerol, and 125 mM iodoacetamide). After isoelectric focusing, the second dimension was performed in 12% polyacrylamide gels. After 2-DE (Bio-Rad), gels were stained with colloidal Coomassie brilliant blue (CBB) solution (Candiano et al., 2004) and the gel image was transferred to a computer using a digital imaging system (VersaDoc MP 4000, Bio-Rad).

2.6. Image analysis of 2-DE gels

PDQuest 8.0.1 2-DE gel analysis software (Bio-Rad) was used for spot quantification. Spot detection parameters were optimized in order to minimize false positive detection and maximize real spot detection. Analysis was performed to identify spots with qualitative (presence/absence) and quantitative ≥1.5-fold increase/decrease.

2.7. Protein identification by matrix-assisted laser desorption/ionization tandem time of flight (MALDI-TOF-TOF)

CBB-stained spots were excised from the gel, cut into pieces, and washed twice with 50% (v/v) methanol and Table 1. Strains and plasmids used in this study.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Strain/plasmid</th>
<th>Relevant genotype/comments</th>
<th>Source/reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHTppk</td>
<td></td>
<td>Contains ppk gene cloned into pHT315</td>
<td>(Doruk et al., 2013)</td>
</tr>
<tr>
<td>B. thuringiensis strains</td>
<td>Bti</td>
<td>Wild-type (ATCC 35646)</td>
<td>ATCC</td>
</tr>
<tr>
<td>Bti pHTppk</td>
<td></td>
<td>Bti carrying pHTppk plasmid</td>
<td>(Doruk et al., 2013)</td>
</tr>
<tr>
<td>Bti Δppk</td>
<td></td>
<td>Bti without ppk gene</td>
<td>(Doruk and Gedik, 2013)</td>
</tr>
</tbody>
</table>
5% acetic acid until they became colorless. Destained gel pieces were dehydrated with acetonitrile (ACN), treated with 10 mM DTT in 100 mM NH$_4$HCO$_3$ for 30 min at room temperature, and finally alkylated with 100 mM iodoacetamide in 100 mM NH$_4$HCO$_3$ for 30 min in the dark. After being dehydrated with ACN and rehydrated with NH$_4$HCO$_3$, gel pieces were digested with 30 µL of trypsin solution (20 ng/µL prepared in 100 mM NH$_4$HCO$_3$) and incubated at 37 °C overnight. The peptides were extracted twice from gel slices with 5% formic acid in 50% ACN. Desalting of peptide solution was performed by using a ZipTip.

Mass spectrometry (MS) analysis was performed on a MALDI-TOF-TOF instrument (Bruker Autoflex III Smartbeam, USA) and spectra were processed and analyzed using the BioTools software (Bruker Daltonics, USA). Database searching was carried out individually using an in-house MASCOT server (Matrix Science, London, UK).

3. Results

Previously, Doruk et al. (2013) showed that the toxicity of the ppk-overexpressing Bti pHTppk strain against late 2nd instar C. quinquefasciatus was about 7.7 times higher than that of Bti, as determined by a larvicidal activity test. Toxicity of Bti without the ppk gene (Bti Δppk) was found to be 2.4 times higher than that of Bti against late 2nd instar C. quinquefasciatus (Table 2) in the present study, suggesting that changes in polyP concentration (increases or decreases) stimulate secondary metabolite synthesis.

To clarify the relationship between polyP and endotoxin production, the proteomes of the wild-type, the ppk-overexpressing strain (Bti pHTppk) (Doruk et al., 2013), and the strain without the ppk gene (Bti Δppk) (Doruk and Gedik, 2013) were compared by 2-DE (Figure 1). Bti strains were grown in both LB and DSM media and samples were collected at the 6th and 10th hours of fermentation where the ppk activity of Bti is high (data not shown). All samples were analyzed using biological and experimental duplicates. The protein profiles of 6-h and 10-h samples grown in both LB and DSM were similar, although the spots were more clear in 10-h samples grown in LB. Figure 1 shows the gels of the proteins extracted from each strain grown in LB for 10 h. Forty-one proteins were found to be differentially expressed and 35 of them were identified by MS. Compared to the wild-type, expression of 9 proteins increased, that of 17 decreased, and 4 of them ceased in the Bti Δppk mutant strain (Figures 2 and 3; Table 3). The expression of 11 proteins increased, that of 12 decreased, and 1 of them ceased in the Bti pHTppk strain compared to the wild-type strain (Figure 2 and 4; Table 3). Proteins identified by MALDI-TOF-TOF were grouped according to their functions (Table 3; Figures 3 and 4).

### Table 2. Mosquito larvicidal activity of Bti and Bti Δppk strains against late 2nd instar Culex quinquefasciatus larvae. LC$_{50}$ values were determined by using probit analysis.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mosquito larvicidal activity (24 h) (LC$_{50}$ ng/mL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bti (wild-type)</td>
<td>44.8 ± 2</td>
</tr>
<tr>
<td>Bti Δppk</td>
<td>18.4 ± 4.7</td>
</tr>
</tbody>
</table>

*: LC$_{50}$ is the concentration of inclusion that causes 50% mortality. n = 10.

3.1. Energy metabolism

Three proteins, dihydrolipoamide dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and inosine-5’-monophosphate dehydrogenase, were found to be less abundant in the Bti pHTppk strain (overexpressing the ppk gene) than in the wild-type and enolase was found overrepresented in the same strain.

Dihydrolipoamide dehydrogenase (DLD) (spot 5) is a vital enzyme of energy metabolism catalyzing NAD$^+$-dependent reoxidation of dihydrolipoamide in a number of multienzyme complexes, which are primarily involved in important steps of aerobic and anaerobic metabolism and also in the conversion of 2-oxo acids to their corresponding acyl-CoA derivative (Perham et al., 1987; de Kok et al., 1998). It is also known that DLD functions in the glycine cleavage multienzyme complex and in the acetoin dehydrogenase complex in some bacteria such as Bacillus subtilis and Clostridium magnum (Kruger et al., 1994; Huang et al., 1999). Moreover, mutations in the cell are known to stimulate the ability of DLD to produce superoxide radical and hydrogen peroxide in vitro (Ambrus et al., 2011) and this enzyme is an important source of reactive oxygen species (ROS) also in living cells, particularly under conditions that increase the NADH/NAD ratio (Starkov et al., 2004; Tretter and Adam-Vizi, 2004).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (spot 12) catalyzes the sixth step of glycolysis and also plays a role in gluconeogenesis. It is known that stress conditions cause the inactivation of GAPDH. This inactivation results in generation of more antioxidant cofactor NADPH, which is needed by some antioxidant systems (Ralser et al., 2007).

Inosine-5’-monophosphate dehydrogenase (IMPDH) (spot 16) is an important enzyme to regulate the intracellular guanine nucleotide pool, which is essential for maintaining normal cell function and growth. As a purine biosynthetic enzyme IMPDH is essential for DNA and RNA synthesis, signal transduction, energy transfer, glycoprotein synthesis, and other cellular proliferation processes (Shah and Kharkar, 2015).
Enolase (phosphopyruvate hydratase) (spot 19), which is overrepresented in Bti pHTppk, is a glycolytic enzyme involved in carbon metabolism. This metalloenzyme catalyzes the conversion of 2-phosphoglycerate (2-PG) to phosphoenolpyruvate (PEP). It is also involved in RNA processing and gene regulation. Recently it was shown that enolase influences tolerance to oxidative stress and virulence in Pseudomonas aeruginosa (Weng et al., 2016). Enolase is also known to be important for sporulation of Bacillus subtilis (Leyva-Vazquez and Setlow, 1994).

DLD, GAPDH, IMPDH, and citrate synthase proteins were found to be less abundant in the Bti Δppk strain. Nucleoside diphosphate kinase, on the other hand, was not expressed in this strain.

Citrate synthase (spot 24) catalyzes the first step of the Krebs cycle. Oxaloacetate and acetyl-CoA are the substrates of the reaction. High ratios of ATP:ADP, acetyl-CoA:CoA, and NADH:NAD are known to inhibit the enzyme (Wiegand and Remington, 1986).

Nucleoside diphosphate kinases (NDPKs) (spot 38) are enzymes required for the synthesis of nucleoside triphosphates (NTPs) other than ATP. They have important roles in bacterial growth, virulence, protein elongation, lipid synthesis, cell signaling, and polysaccharide synthesis

---

**Figure 1.** Representative 2-DE gels of proteins extracted from Bti (A), Bti pHTppk (B), and Bti Δppk (C) strains grown for 10 h in LB medium. The spots differentially represented are numbered and correspond to the proteins listed in Table 3. All samples were analyzed by using biological and experimental duplicates.
Attwood and Wieland (2015) discovered that NDPKs also act as a protein histidine kinase, which involves reversible histidine phosphorylation. This enzyme also serves an important role in the synthesis of (p)ppGpp, an alarmone of the stringent response (Kim et al., 1998).

3.2. Protein folding and stress response
Three proteins (chaperone protein dnaK, alkyl hydroperoxide reductase, elongation factor Ts) that function in stress response and two others (aconitate hydratase, trigger factor) that function in protein folding were found to be more abundant in the \( Bti \) pHTppk strain.

Chaperone protein dnaK (spot 18) is responsible for correct folding of proteins by inhibiting unsuitable molecular interactions (Deuerling and Bukau, 2004) and is a source of mutational robustness (Aguilar-Rodríguez et al., 2016).

Alkyl hydroperoxide reductase (peroxiredoxin) (spot 20) protects the cell against ROS, which are related to the TCA cycle and respiration chain, by reducing peroxides to water or alcohol. Moreover, this enzyme renews the NAD pool and protects the oxidation/reduction balance (Nishiyama et al., 2001; Seib et al., 2006).

Elongation factor proteins (spot 32) were shown to fold proteins like stress chaperones in \( E. coli \) (Caldas et al., 1998).

Aconitate hydratase (spot 30) functions in the TCA cycle and is also responsible for posttranslational modifications necessary for correct protein folding (Gupta et al., 2009).

Trigger factor (spot 31) is a ribosome-related bacterial chaperone that folds proteins without ATP (Merz et al., 2008).

In the \( Bti \) \( \Delta ppk \) strain, elongation factor Ts, phage shock protein, aconitate hydratase, and trigger factor were found to be more abundant compared to the wild-type.

Phage shock protein (spot 39) plays important roles in the stress response in the cell, especially when shortages of nutrient and energy are present (Darwin, 2005).

3.3. Metabolic pathways
In both the \( Bti \) pHTppk and \( Bti \) \( \Delta ppk \) strains, phosphoenol pyruvate carboxykinase, acetate/propionate kinase, acyl-CoA dehydrogenase, and fructose 1,6-bisphosphatase proteins were found to be less abundant compared to the wild-type.

Other than those proteins, propionyl-CoA carboxylase beta chain, 3-ketoacyl-(acyl-carrier protein) reductase,
Table 3. MALDI-TOF-TOF results of differentially expressed proteins. “I” denotes proteins whose expression increased, “D” denotes proteins whose expression decreased, “N” denotes proteins that are not expressed, and “E” denotes proteins whose expressions are equal.

<table>
<thead>
<tr>
<th>Energy metabolism</th>
<th>Name</th>
<th>Bti pH</th>
<th>Bti ∆ppk</th>
<th>Parent ion</th>
<th>Accession</th>
<th>Mass</th>
<th>Score</th>
<th>Protein name</th>
<th>Title</th>
<th>Biological process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot 5</td>
<td>D</td>
<td>D</td>
<td>1848.8</td>
<td>gi/30022058</td>
<td>49409</td>
<td>109</td>
<td>Dihydrolipoamide dehydrogenase</td>
<td>A flavoprotein enzyme that oxidizes dihydrolipoamide to lipoamide.</td>
<td>Cell redox homeostasis</td>
<td></td>
</tr>
<tr>
<td>Spot 12</td>
<td>D</td>
<td>D</td>
<td>1703.7</td>
<td>gi/30023174</td>
<td>35176</td>
<td>112</td>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>Glycolysis and gluconeogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spot 16</td>
<td>D</td>
<td>D</td>
<td>1544.8</td>
<td>gi/6561887</td>
<td>55358</td>
<td>93</td>
<td>IMP dehydrogenase</td>
<td>Converts inosine monophosphate to xanthosine monophosphate, the first committed and rate-limiting step in de novo synthesis of guanine nucleotides, therefore playing an important role in regulation of cell growth</td>
<td>GMP biosynthesis, purine biosynthesis</td>
<td></td>
</tr>
<tr>
<td>Spot 19</td>
<td>I</td>
<td>E</td>
<td>1911.8</td>
<td>gi/30023172</td>
<td>46343</td>
<td>140</td>
<td>Enolase</td>
<td>Catalysis of the conversion of 2-PG to PEP, the ninth and penultimate step of glycolysis</td>
<td>Glycolysis, sporulation resulting in formation of a cellular spore</td>
<td></td>
</tr>
<tr>
<td>Spot 24</td>
<td>E</td>
<td>D</td>
<td>2081.9</td>
<td>gi/30020417</td>
<td>42358</td>
<td>60</td>
<td>Citrate synthase</td>
<td>Exists in nearly all living cells and stands as a pace-making enzyme in the first step of the citric acid cycle (or Krebs cycle)</td>
<td>Cellular carbohydrate metabolic process</td>
<td></td>
</tr>
<tr>
<td>Spot 38</td>
<td>E</td>
<td>N</td>
<td>975.6</td>
<td>gi/30019663</td>
<td>16631</td>
<td>54</td>
<td>Nucleoside diphosphate kinase</td>
<td></td>
<td>CTP biosynthetic process, GTP biosynthetic process, UTP biosynthetic process</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein folding and stress response</th>
<th>Name</th>
<th>Bti pH</th>
<th>Bti ∆ppk</th>
<th>Parent ion</th>
<th>Accession</th>
<th>Mass</th>
<th>Score</th>
<th>Protein name</th>
<th>Title</th>
<th>Biological process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot 18</td>
<td>I</td>
<td>E</td>
<td>1254.7</td>
<td>gi/118721</td>
<td>65211</td>
<td>53</td>
<td>Chaperone protein dnaK (heat shock protein 70)</td>
<td>Acts as a chaperone</td>
<td>Protein folding, response to stress</td>
<td></td>
</tr>
<tr>
<td>Spot 20</td>
<td>I</td>
<td>E</td>
<td>2008.9</td>
<td>gi/30018585</td>
<td>20693</td>
<td>52</td>
<td>Alkyl hydroperoxide reductase (peroxiredoxin) C22</td>
<td>Cell redox homeostasis, response to reactive oxygen species</td>
<td>Response to oxidative stress</td>
<td></td>
</tr>
<tr>
<td>Spot 32</td>
<td>I</td>
<td>I</td>
<td>1758.9</td>
<td>gi/16078713</td>
<td>32334</td>
<td>112</td>
<td>Elongation factor Ts</td>
<td>A set of proteins that are used in protein synthesis in the cell</td>
<td>Protein biosynthesis</td>
<td></td>
</tr>
<tr>
<td>Spot</td>
<td>pI</td>
<td>Mass</td>
<td>Accession #</td>
<td>Name</td>
<td>Description</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>----</td>
<td>------</td>
<td>-------------</td>
<td>------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>I</td>
<td>1286.7</td>
<td>gi/30265363</td>
<td>Acclinate hydratase</td>
<td>Cell cycle, cell division, protein folding, protein transport</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>31</td>
<td>I</td>
<td>903.5</td>
<td>gi/6809374</td>
<td>Trigger factor</td>
<td>Involved in protein export as a chaperone by maintaining the newly synthesized protein in an open conformation (cell division)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>E</td>
<td>1627.8</td>
<td>gi/30019585</td>
<td>Phage shock protein A</td>
<td>Stress response</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>D</td>
<td>1939.8</td>
<td>gi/30022835</td>
<td>Phosphoenol pyruvate carboxykinase</td>
<td>Involved in the metabolic pathway of gluconeogenesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>D</td>
<td>1005.6</td>
<td>gi/30022712</td>
<td>Acetate/propionate kinase</td>
<td>Involved in the metabolic pathway of gluconeogenesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>D</td>
<td>1318.7</td>
<td>gi/30262538</td>
<td>Acyl-CoA dehydrogenase</td>
<td>Involved in the degradation of long chain fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>E</td>
<td>1761.8</td>
<td>gi/30023364</td>
<td>Fructose 1,6-bisphosphatase</td>
<td>Converts fructose-1,6-bisphosphate to fructose-6-phosphate in gluconeogenesis and the Calvin cycle, which are both anabolic pathways</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>E</td>
<td>1407.7</td>
<td>gi/30020616</td>
<td>Propionyl-CoA carboxylase beta chain</td>
<td>Catalyzes the carboxylation reaction of propionyl CoA (great importance as a glucose precursor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>I</td>
<td>973.6</td>
<td>gi/30009578</td>
<td>2'-5'-RNA ligase</td>
<td>Catalyzes a reversible reaction that splits the 3'-terminal ribose-phosphate (5'-phosphate) from the 2'-terminal ribose-phosphate (3'-phosphate) of RNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>E</td>
<td>1255.7</td>
<td>gi/9297081</td>
<td>Fructose-bisphosphate aldolase</td>
<td>Catalyzes a reversible reaction that splits the aldol, fructose-1,6-bisphosphate, into the triose phosphates, dihydroxyacetone phosphate and glyceraldehyde3-phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>E</td>
<td>1171.7</td>
<td>gi/30009486</td>
<td>3-Ketoacyl-(acyl-carrier protein) reductase</td>
<td>Catalyzes a reversible reaction that splits the aldol, fructose-1,6-bisphosphate, into the triose phosphates, dihydroxyacetone phosphate and glyceraldehyde3-phosphate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Table 3. (Continued).*
<table>
<thead>
<tr>
<th>Spot</th>
<th>pH</th>
<th>Tp</th>
<th>Parent ion</th>
<th>Accession #</th>
<th>Mass</th>
<th>Score</th>
<th>Protein name</th>
<th>Title</th>
<th>Biological process</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>D</td>
<td>D</td>
<td>1241.7</td>
<td>gi/30020418</td>
<td>53363</td>
<td>61</td>
<td>2-methyl citrate dehydratase</td>
<td>Participates in propanoate metabolism</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>E</td>
<td>1590.7</td>
<td>gi/6094396</td>
<td>56909</td>
<td>82</td>
<td>Glutamate-tRNA ligase</td>
<td>Participates in 3 metabolic pathways: glutamate, porphyrin and chlorophyll metabolism, and aminoacyl-tRNA biosynthesis</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>D</td>
<td>D</td>
<td>1507.7</td>
<td>gi/30022309</td>
<td>40200</td>
<td>77</td>
<td>Aminomethyltransferase</td>
<td>The glycine cleavage system catalyzes the degradation of glycine</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>D</td>
<td>N</td>
<td>1848.8</td>
<td>gi/30018617</td>
<td>35051</td>
<td>111</td>
<td>Carbamate kinase</td>
<td>Participates in 4 metabolic pathways: purine metabolism, glutamate metabolism, arginine and proline metabolism, and nitrogen metabolism</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>E</td>
<td>N</td>
<td>1796.9</td>
<td>gi/30018614</td>
<td>46850</td>
<td>131</td>
<td>Arginine deiminase</td>
<td>Participates in arginine and proline metabolism (expression could be regulated by various environmental factors)</td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>E</td>
<td>D</td>
<td>1175.6</td>
<td>gi/30023347</td>
<td>45230</td>
<td>52</td>
<td>Serine hydroxymethyltransferase</td>
<td>Catalyzes the reversible interconversion of serine and glycine with tetrahydrofolate (THF) serving as the one-carbon carrier; this reaction serves as the major source of one-carbon groups required for the biosynthesis of purines, thymidylate, methionine, and other important biomolecules</td>
<td></td>
</tr>
<tr>
<td>23</td>
<td>E</td>
<td>D</td>
<td>1812.9</td>
<td>gi/30018778</td>
<td>40209</td>
<td>96</td>
<td>Alanine dehydrogenase</td>
<td>Participates in taurine and hypotaurine metabolism and reductive carboxylate cycle (CO₂ fixation); catalyzes reversible oxidative deamination of L-alanine to pyruvate; this enzyme is a key factor in assimilation of L-alanine as an energy source through tricarboxylic acid cycle during sporulation</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>D</td>
<td>E</td>
<td>1577.8</td>
<td>gi/30022243</td>
<td>36760</td>
<td>74</td>
<td>2-Oxoisovalerate dehydrogenase alpha subunit</td>
<td>Oxidoreductase activity</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. (Continued).**

Nitrogen and amino acid metabolism
<table>
<thead>
<tr>
<th>Name</th>
<th>Bti pH Tppk</th>
<th>Bti Δppk</th>
<th>Parent ion</th>
<th>Accession #</th>
<th>Mass</th>
<th>Score</th>
<th>Protein name</th>
<th>Title</th>
<th>Biological process</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot 17</td>
<td>I</td>
<td>I</td>
<td>2486.2</td>
<td>gi/30018370</td>
<td>12510</td>
<td>136</td>
<td>50S ribosomal protein L7/L12</td>
<td>Forms part of the ribosomal stalk, which helps the ribosome interact with GTP-bound translation factors, and is thus essential for accurate translation</td>
<td>Translation</td>
</tr>
<tr>
<td>Spot 25</td>
<td>I</td>
<td>N</td>
<td>1449.8</td>
<td>gi/24474855</td>
<td>21588</td>
<td>129</td>
<td>Camelysin</td>
<td>Novel surface metalloprotease camelysin (casein-cleaving metalloprotease)</td>
<td>Proteolysis</td>
</tr>
<tr>
<td>Spot 27</td>
<td>D</td>
<td>D</td>
<td>973.5</td>
<td>gi/30019489</td>
<td>20252</td>
<td>61</td>
<td>Hypothetical protein BC 1339</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spot 29</td>
<td>I</td>
<td>I</td>
<td>1697.8</td>
<td>gi/75758638</td>
<td>21361</td>
<td>81</td>
<td>Hypothetical protein RBHT_07739</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spot 34</td>
<td>I</td>
<td>I</td>
<td>1573.8</td>
<td>gi/47565426</td>
<td>32684</td>
<td>125</td>
<td>Flagellin</td>
<td></td>
<td>Ciliary or bacterial-type flagellar motility</td>
</tr>
<tr>
<td>Spot 36</td>
<td>E</td>
<td>I</td>
<td>1943</td>
<td>gi/30021916</td>
<td>28775</td>
<td>118</td>
<td>Transcriptional repressor CodY</td>
<td>DNA-binding protein that represses the expression of many genes that are induced as cells making the transition from rapid exponential growth to stationary phase and sporulation</td>
<td>Regulation of transcription, DNA-dependent</td>
</tr>
</tbody>
</table>

Table 3. (Continued).
**Figure 3.** Summary of differentially expressed proteins in *Bti Δppk*. Red patterns show proteins whose expression increased, blue patterns show proteins whose expression decreased, and black patterns show proteins that are not expressed.

**Figure 4.** Summary of differentially expressed proteins in *Bti pHTppk*. Red patterns show proteins whose expression increased, blue patterns show proteins whose expression decreased, and black patterns show proteins that are not expressed.
and enoyl-CoA hydratase were also less abundant in the Bti Δppk strain. Fructose-bisphosphate aldolase enzyme, on the other hand, was expressed more in the same strain. The expression level of 2'-5' RNA ligase in the Bti pH7ppk strain was more than in the wild-type and vice versa for the Bti Δppk strain.

Phosphoenol pyruvate carboxykinase (spot 3) functions in gluconeogenesis and catalyzes the reversible decarboxylation and mononucleotide-dependent phosphorylation of oxaloacetate to PEP and CO₂ (Matte et al., 1996). It has been shown that this enzyme plays a role not only in gluconeogenesis but also in production of glutamine and lysine through the TCA cycle intermediates in Corynebacterium glutamicum (Aich et al., 2003).

Acetate/proionate kinase (spot 7), which is an important enzyme for glycolysis, is responsible for β-oxidation of fatty acids. It is known that the enzyme level increases in the case of high glucose concentrations (Grundy et al., 1993).

Acyl-CoA dehydrogenase (spot 11) functions in fatty acid and amino acid catabolism by catalyzing the α,β-dehydrogenation of acyl-CoA esters. Several members of the acyl-CoA dehydrogenase family are involved in fatty acid β-oxidation; these are short, medium, long, and very long chain acyl-CoA dehydrogenases. Short chain fatty acids are an important carbon and energy source in bacteria (Swigonova et al., 2009).

Fructose 1,6-bisphosphatase (spot 13) is a key enzyme of the gluconeogenic pathway, hydrolyzing fructose 1,6-bisphosphate to fructose 6-phosphate and inorganic phosphate (Hines et al., 2007).

Propionyl-CoA carboxylase beta chain (spot 15) catalyzes the carboxylation of propionyl-CoA. Propionyl-CoA, as an important metabolite in lipid metabolism and in valine, isoleucine, and methionine metabolisms, is crucial as a glucose precursor (Rodriguez and Gramajo, 1999; Rodriguez-Pombo et al., 2002).

3-Ketoacyl-(acyl-carrier protein) reductase (spot 40) functions in the biosynthesis of fatty acids by performing NADPH-dependent reduction of β-ketoacyl–ACP substrates to β-hydroxyacyl–ACP products (Nomura et al., 2005).

Enoyl-CoA hydratase (spot 41) is an important enzyme of fatty acid metabolism that catalyzes the second step of the β-oxidation pathway. It adds a water molecule to the double bond between the 2nd and 3rd carbon of acyl-CoA. This enzyme is responsible for production of acetyl-CoA and energy by metabolizing fatty acids (Agnihotri and Liu, 2003).

Fructose-bisphosphate aldolase (spot 33) is an important enzyme in carbohydrate metabolism. In glycolysis, this enzyme catalyzes the cleavage of fructose-1,6-bisphosphate (FBP) to a ketose, dihydroxyacetone phosphate (DHAP), and an aldose, glyceraldehyde-3-phosphate (G3P). In gluconeogenesis it catalyzes the reverse condensation reaction (Cooper et al., 1996).

2'-5'-RNA ligase (spot 28) is an important enzyme in the RNA metabolic process. It ligates half-tRNA molecules with 2',3'-cyclic phosphate and 5-hydroxyl termini, giving rise to a product containing a 2'-5' phosphodiester linkage. This enzyme has been shown to perform cleavage or ligation in vivo (Arn and Abelson, 1996).

3.4. Nitrogen and amino acid metabolism

In the Bti pH7ppk strain, 2-methyl citrate dehydratase, aminomethyltransferase, carbamate kinase, and 2-oxoisovalerate dehydrogenase alpha subunit proteins were found to be less abundant. Glutamate-tRNA ligase, on the other hand, was not expressed at all in the same strain.

In the Bti Δppk strain, 2-methyl citrate dehydratase, aminomethyltransferase, serine hydroxymethyltransferase, and alanine dehydrogenase proteins were found to be less abundant. Carbamate kinase and arginine deiminase, on the other hand, were not expressed at all in the same strain.

2-Methyl citrate dehydratase (spot 4) is required for propionate catabolism via the methyl citrate cycle. Propionate is oxidized via this cycle to pyruvate (Blank et al., 2002).

Aminomethyltransferase (spot 8) is one of the four enzymes of the glycine cleavage system, which catalyzes the oxidative decarboxylation of glycine in bacteria. This tetrahydrofolate-dependent enzyme is responsible for the synthesis of methylenetetrahydrofolate (Lee et al., 2004; Okamura-Ikeda et al., 2010).

Carbamate kinase (spot 14) functions in purine, glutamate, arginine, and proline metabolisms. It also has important functions in nitrogen metabolism (Marina et al., 1999).

2-Oxoisovalerate dehydrogenase alpha subunit (spot 35) functions in the catabolism of branched-chain amino acids isoleucine, leucine, and valine (Debarbouille et al., 1999).

Glutamate-tRNA ligase (spot 6) catalyzes the attachment of glutamate to tRNA(Glu) in a two-step reaction: glutamate is first activated by ATP to form Glu-AMP and then transferred to the acceptor end of tRNA(Glu). It functions in glutamate, porphyrin, and chlorophyll metabolisms. At the same time this enzyme also plays a role in aminoaeryl–tRNA biosynthesis (Breton et al., 1990; Gagnon et al., 1994).

Serine hydroxymethyltransferase (spot 22), which is an important enzyme that supplies single carbon units to the cell, catalyzes the reversible interconversion of L-serine into glycine and tetrahydrofolate into 5,10-methylenetetrahydrofolate.

5,10-Methylenetetrahydrofolate, being a vital link between amino acid and nucleotide metabolism, is a key
intermediate for the biosynthesis of purines, thymidylate, choline, and methionine (Pai et al., 2009).

Arginine deiminase (spot 21) functions in arginine and proline metabolisms. The expression of this enzyme is known to be regulated by environmental factors (Li et al., 2008).

Alanine dehydrogenase (spot 23) catalyzes the reversible oxidative deamination of L-alanine to pyruvate. This enzyme is important for energy generation during sporulation (Sironosian et al., 1993).

3.5. Other proteins

In the Bti pHTppk strain, 50S ribosomal protein L7/L12, flagellin, and camelysin proteins were found to be expressed more.

50S ribosomal protein L7/L12, flagellin, and transcriptional repressor CodY proteins were found to be expressed more in the Bti ∆ppk strain. Camelysin protein, on the other hand, was not expressed at all in the same strain.

50S ribosomal protein L7/L12 (spot 17), which is among the most important components of prokaryotic ribosomes, interacts with GTP-dependent translation factors during protein biosynthesis in bacteria (Pettersson and Kurland, 1980).

Flagellin (spot 34) is the most abundant (as many as 20,000) structural protein of flagella. Motility, chemotaxis, and biofilm formation require the expression of flagellin (Pratt and Kolter, 1998).

Camelysin (casein-cleaving metalloproteinase) (spot 25) is a zinc-containing metalloprotease that is responsible for proteolysis (Fricke et al., 2001). Camelysin preferentially cleaves peptide bonds in front of aliphatic hydrophobic and hydrophilic amino acids. It has been shown that this protein can activate cytotoxic toxin proteins (Cyt1Aa and Cyt2Ba) produced by Bti (Nisnevitch et al., 2010).

Transcriptional repressor CodY (spot 36) is a highly conserved DNA binding protein in gram-positive bacteria with low G+C content. It regulates the expression of many genes involved in the transition of cells from rapid exponential growth to the stationary phase and sporulation (Ratnayake-Lecamwasam et al., 2001).

4. Discussion

In a previous study, polyphosphate metabolism of Bti was manipulated by overexpressing the ppk gene (encoding polyP synthesizing enzyme) under the control of its native promoter (Doruk et al., 2013). This strain (Bti pHTppk) was found to produce more toxin than the wild-type and exhibited significantly higher toxicity against late 2nd instar Culex quinquefasciatus larvae (7.7 times) (Doruk et al., 2013) and 3rd instar wild Culex pipiens larvae (10 times) (Doruk et al., 2016) than control strains.

The Bti strain without the ppk gene (Bti ∆ppk) was also found to be more toxic (2.4 times) than the wild-type against late 2nd instar C. quinquefasciatus larvae in the present study. These results clearly demonstrate that overexpression or deletion of the ppk gene affects toxicity of Bti in a positive manner, although Bti pHTppk is much more toxic than Bti ∆ppk.

The elicitation mechanism of secondary metabolism is a complex process and the precise mechanism is poorly understood. Changes in the levels of intracellular molecules such as ions (Murphy et al., 2011), alarmones (Kawai et al., 2007), or ROS (Radman et al., 2006) serve as internal signals to direct the regulation of secondary metabolites either directly influencing the transcription of the secondary metabolite gene cluster or inducing a transcriptional activator of the target gene cluster (Kawai et al., 2007; Rigali et al., 2008; Nair et al., 2009; Tanaka et al., 2010).

To explain the phenotypes of Bti pHTppk and Bti ∆ppk, proteomes of wild and mutant strains were analyzed. For this, intracellular proteins were separated by 2-DE and 41 spots differentially expressed were determined; 35 of those were identified and analyzed by MS. Compared to the wild-type, expression of 9 proteins increased, that of 17 decreased, and 4 of them ceased in the Bti ∆ppk mutant strain. The expression of 11 proteins increased, that of 12 decreased, and 1 of them ceased in Bti pHTppk.

When we look into the whole proteome, the protein profile of the Bti pHTppk strain was found to be closer to the wild-type compared to the Bti ∆ppk strain, which has more dramatic changes in its proteome. There is a general decrease in the expression levels of proteins related to energy metabolism, amino acid metabolism, and purine biosynthesis in both Bti pHTppk and Bti ∆ppk. Gluconeogenesis and fatty acid metabolism were also slowed down in both strains. Decrease in expression levels of a larger number of proteins functioning in those metabolisms were seen in Bti ∆ppk.

Contrary to our results, Varela et al. (2010) found that polyP deficiency in Pseudomonas sp. B4 caused an increase in the energy-generating pathways (TCA, β-oxidation, oxidative phosphorylation). As it is a sporulating bacterium, we know that a great majority of genes involved in the glycolysis and TCA cycle are significantly downregulated during sporulation, implying an overall decrease in the activities of these pathways (Wang et al., 2013). Decrease in energy metabolisms of both Bti recombinant strains can be associated with sporulation, although we found that Bti ∆ppk and Bti pHTppk are sporulating as well as the wild-type (data not shown). Shi et al. (2004) suggested that polyP depolymerization may induce or at least promote efficient sporulation in B. cereus. Although sporulation efficiency was impaired in
the ppx mutant strain, sporulation did not appear to be defective in the ppk mutant in their study. Similarly, we also found that pppk is not necessary for sporulation in Bti. Transcriptional repressor CodY (spot 36), which regulates expression of many genes involved in the transition of cells from rapid exponential growth to the stationary phase and sporulation (Ratnayake-Lecamwasam et al., 2001), was expressed more in Bti Δppk. The reason for efficient sporulation of the ppk mutant strain could be the high expression of this protein.

Although a general decrease in the expression of proteins functioning in all metabolic pathways was seen in both recombinant strains, an increase in the expression of proteins that function in protein folding and stress response was observed. For example, elongation factor Ts, which can fold proteins like stress chaperons, and aconitate hydratase and trigger factor, which are protein folding proteins, were found to be expressed more in both Bti Δppk and Bti pHTppk. Moreover, stress response proteins chaperone protein dnaK and alkyl hydroperoxide reductase were also found to be expressed more in the Bti pHTppk strain. In the Bti Δppk strain phage shock protein, which is known to have very important tasks in the case of shortages of nutrient and energy, was also expressed more than in the wild-type.

There is other evidence for the occurrence of stress conditions inside both mutant cells. Dihydrolipoamide dehydrogenase (DLD or E3) is an enzyme of energy metabolism, but it is also an important source of ROS in living cells (Starkov et al., 2004; Tretter and Adam-Vizi, 2004). It is possible to hypothesize that decreasing the expression of DLD by Bti pHTppk and Bti Δppk strains can be a protection mechanism to avoid ROS accumulation inside the cell, which is already under stress because of the disturbance of polyP homeostasis. It is known that chemical molecules repressing ROS production in microbes could serve as elicitors of microbial secondary metabolites (Abdelmohsen et al., 2015), although we do not know if there is a specific chemical that negatively affects DLD expression and elicits toxin production in this specific case.

Another energy metabolism enzyme, glyceraldehyde-3-phosphate dehydrogenase, is inactivated under stress conditions. This inactivation results in the generation of more antioxidant cofactor NADPH, which is needed by some antioxidant systems (Ralser et al., 2007). This enzyme is also less abundant in both mutant strains.

Moreover, it was shown that enolase, which is a glycolytic enzyme involved in carbon metabolism, influences tolerance to oxidative stress and virulence in Pseudomonas aeruginosa (Weng et al., 2016). High expression of this enzyme in Bti pHTppk could give us a clue to claim that this strain may be under oxidative stress.

High expression of camelysin protein in Bti pHTppk could be another reason for the hypertoxicity of this strain. Nisnevitch et al. (2010) demonstrated that camelysin enzyme can activate the protoxins Cyt1Aa and Cyt2Ba by truncating their polypeptide chains. Therefore, protoxin activation is carried out not only by insect gut proteases (Al-Yahyae and Ellar, 1995), but also by the camelysin protein in Bti. It is interesting that this protein was not expressed at all in the Bti Δppk strain, which can explain the difference of the toxicity levels of both recombinant strains.

Deletion of the pppk gene caused more severe effects in Bti compared to the strain overexpressing the pppk gene. NDPK could be one of the responsible enzymes for these effects since it is not expressed at all in the Bti Δppk strain. This enzyme has important roles in bacterial growth, virulence, protein elongation, lipid and polysaccharide synthesis, and cell signaling by synthesizing NTPs other than ATP (Chakrabarty, 1998). NDPK also plays a critical role in the synthesis of (p)ppGpp during stringent response (Kim et al., 1998).

One of the proteins whose expression increased in both Bti pHTppk and Bti Δppk was flagellin. This is the unit that constitutes the filament of the bacterial flagella. It is known that prior to sporulation Bti cells increase their flagellin levels inside the cell in order to search for nutrients. Increased expression of flagellin protein was particularly seen in Bti Δppk (spot 40).

Fructose-bisphosphate aldolase catalyzes the cleavage of fructose-1,6-bisphosphate to DHAP and G3P (Cooper et al., 1996). In E. coli, to replenish a low concentration of needed inorganic phosphate, DHAP is converted to methylglyoxal and an inorganic phosphate is given off by this reaction (Kayser et al., 2005). It can be speculated that one of the reasons for high expression of fructose-bisphosphate aldolase in the Bti Δppk strain is to replenish the cell with inorganic phosphate.

In summary, the results of this study suggest that changes in polyP concentration (increases or decreases) cause a general stress condition inside the cell, which in turn stimulates secondary metabolite synthesis. General stress in cells caused by lack of polyP has been shown in previous studies (Varela et al., 2010; Le Marechal et al., 2013). However, creation of a general stress condition by increased concentration of polyP was shown for the first time in this study.

Acknowledgments
This work was supported by three different grant supplied by the Scientific and Technological Research Council of Turkey [TBAG-107T812, TBAG-111T047, KBAG-113Z898]. We are grateful to Dr Öner Koçak.
for his support in bioassay experiments carried out at Hacettepe University (Ankara, Turkey), to Dr Talat Yağcı for MALDI-TOF-TOF analysis carried out at the İzmir Institute of Technology (İzmir, Turkey), and to Dr Duygu Özal Demiralp for 2-DE analysis (Ankara University Biotechnology Institute, Ankara, Turkey).

References


Blank L, Green J, Guest JR (2002). AcnC of Escherichia coli is a 2-methylcitrate dehydratase (PrpD) that can use citrate and isocitrate as substrates. Microbiology 148: 133-146.


Doruk T, Girgin Ersoy Z, Öncel MS, Gedik ST (2016). High levels of polyphosphate kinase affect not only endotoxin production but also acid tolerance of Bacillus thuringiensis. Turk J Biol 40: 1168-1177.


101


