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INDUCTION OF ANTI-POLLUTANT REGULONS DURING CHEMICAL STRESS ON *BACILLUS SUBTILIS* AND ITS DESCENDENT PROGENIES

ABSTRACT:

Saprophytic soil *Bacillus subtilis* strain NC was isolated from an industrial region, at Shubra Al-Khima, north Cairo, Egypt. A progeny strain was isolated and adapted to metal bioremediation under stress of Cr^{6+} , Hg^{2+} and Fe^{3+} ions. Both parent (Pt) and progeny (Pg) were treated with separate nine heavy metals. According to their impact upon the bacterial viability, the heavy metals tested were assigned into three groups: Growth promoting elements (GPE) including Mn^{2+} , Co^{2+} , Zn^{2+} and Mg^{2+} ; growth retarding elements (GRE) including Li^+ , and B^{3+} and growth inhibiting elements (GIE) including Sr^{2+} , V^{5+} and Pb^{2+} . Fortifying these groups with traces of Cr^{6+} (C), Hg^{2+} (A), and Fe^{3+} ions (F), separately, resulted in a dissimilar influence on the groups. For the Pg lines, the toxic effect of GRE was antagonized and that of GPE was synergized while the inhibitory effect of GIE was prominent for Pt line. Consumption of heavy metals in the bacterial growth (%of residual control) was the highest with GPE and the least was GIE fortified with C, A and F, indicating the lyses of cells and impairment of permeability control. Pt and Pg proteinomes were subjected to SDS-PAGE analysis. Electrophoretic profile analysis indicated the presence of novel bands expression of regulons in Pg smear emerging the possibility of induction of proteins/enzymes capable of adapting the high concentrations of heavy metals.

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INTRODUCTION:

Rapid industrialization and urbanization have resulted in an elevated level of toxic heavy metals and metalloids entering the biosphere. The fate of toxic metallic cations in the soil environment (and its subsequent movement into ground water or uptake by plants) depends largely on the interactions of these metals with soil organic and inorganic components (Ochiai *et al.*, 2007).

Metal ions, including Mn^{2+} , Fe^{3+} , Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} , are both essential and potentially toxic. Therefore, homeostatic regulation of their intracellular concentrations is critical (Jakubovics and Jenkinson, 2001).

The occurrence of heavy metals in the soil results in the impairment of most plant processes and causes drastic drop in yield, in addition to the transposition of these heavy metals to the plant consumers. Mercury, chromium and iron deposits are the most hazardous heavy metals continuously added to the soil according to their customary exhaustion in many industries and human activities.

Detoxification of mercury by bacteria was investigated by Pilon-Smith and Pilon (2000) in their transformation of *Arabidopsis thaliana* with mercuric reductase (Mer A) and organomercurial lyase (Mer B) derived from Gram negative bacteria, where the double transgenic of the genes of both enzymes resulted in the highest tolerance to organic mercury up to 10 μM . The inconceivable accumulation of mercury in the soil, changes the flux of biologically available mercury in natural microbial communities where enzymatic activities underpin the biogeological cycling of mercury with consequence human exposure to toxic forms of this element (Ogunseitan, 2002).

The use of chromium in metal refinishing and electroplating is extensively increased leading to high contamination of soil with the high soluble toxic forms of Cr^{6+} anions; CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$. Some bacteria can reduce chromate (Cr^{6+}) to insoluble and less toxic Cr^{3+} , but many fundamental parameters to remediate chromate are not characterized (Keyhan *et al.*, 2004).

In many bacteria, the ferric uptake regulator (Fur) protein coordinates the expression of iron uptake and homeostasis pathways in response to available iron (Andrews *et al.*, 2003). Fur represses a small RNA, RyhB, which in turn, negatively regulates the expression of iron-rich enzymes such as succinate dehydrogenase, fumarase, and aconitase (Masse and Arguin, 2005). This allows the production of these enzymes to be activated in response to available iron.

Because of large surface area-to-volume ratio, microorganisms provide a large contact area which can interact with metals in the surrounding environment. They accumulate metals through various mechanisms including complexation, adsorption, precipitation, and active transport into the cell (Samuelson *et al.*, 2000). Many microorganisms are used for bioremediation of soils from heavy metals: *Trichoderma viride*, *T. Harzianum*, *Pseudomonas putida*, *P. syringae*, *P. florescence*, *Bacillus subtilis*, *Vibrio harveyi*, *E. coli*, *Schewanella putrefaciens*, *Deinococcus radiodurans*, *Staphylococcus aureus*, *Acinetobacter spp.*, and *Sporosarcina urea* (Keyhan *et al.*, 2004).

In the present study, some saprophytic *Bacillus* isolates, capable to accumulate part of the deposited heavy metals, were obtained and subsequently identified as *Bacillus subtilis* strains. Heavy metals analysis for soil samples was performed. The successful growing *Bacillus subtilis* isolate was subjected to gradient concentrations of Hg^{2+} , Cr^{6+} and Fe^{3+} to obtain progeny isolates. This present study aimed to adopt the saprophytic *Bacillus* isolates able to accumulate, internalize and reduce the toxic heavy metals Hg^{2+} , Cr^{6+} and Fe^{3+} by inducing novel regulons of enzymatic machinery systems.

MATERIALS AND METHODS:

Chemicals:

Most chemicals were purchased from Sigma chemicals co. U.S.A., Aldrich co. U.S.A. and ADWIC Egypt. Chemicals of gel electrophoresis were from Promega co. U.K. Buffers for the enzymatic activities of mercuric reductase were obtained from Bio-Rad Laboratories, N.Y.

Soil samples and isolation of the bacteria used:

Seven soil samples were collected from the industrial area at Shubra Al-khima (Athawrah suburb) 25 Km north Cairo. Soil samples were collected from distributed sites, beside the field area, where massive economical plants are established for the industry of glass, fiberglass, ceramics, batteries fabrication, iron smelter, textiles, semiconductors, and others. Soils were aseptically divided into portions and sieved (3-mm mesh). Samples from each portion

were heated (75°C, 10 min) and cultivated on a modified trypton LB medium (TLB) described by Simbahan *et al.* (2005). Chemical analysis of heavy metals in the soil samples were conducted at Micro Analytical center, Cairo Univ. Egypt using the flame atomic absorption spectrometry (FAAS) according to D'Haese *et al.* (1997).

Bacterial identification:

Bacterial isolates were routinely cultivated on TLB for identification tests and to select the Gram-positive spore forming rods. Strains producing colonies on TLB agar after heating of the soil inoculum were considered to have spores. Following morphological and biochemical schemes derived from Bergey's manual of systematic bacteriology; (Holt, 1986) bacterial isolates were defined morphologically and biochemically. The isolate was assigned to be *B. subtilis* NC strain.

Resistance of bacterial strains to heavy metals:

For induction of resistant progeny of the parent isolates, a grown culture was subjected separately and collectively to three heavy metal supplements (Cr^{6+} , Hg^{2+} and Fe^{3+}) in a gradient loading scheme (5, 10, 20, 30, 40, 50, 100 μ M) on liquid TLB with shaking for 48 hr. Then, the cultures were incorporated into the same fresh TLB - heavy metals containing media for another 3 cycles before plating on solid TLB -heavy metals containing media to isolate a clone of a successful growing progeny. Nine different heavy metals (Mn^{2+} , Co^{2+} , Zn^{2+} , Mg^{2+} , Li^+ , B^{3+} , Sr^{2+} , V^{5+} and Pb^{2+}) were added separately at a concentration of 50 μ M to TLB broth cultures of both Pt and Pg isolates to induce their growth under the stress of these heavy metals. Traces (5 μ M) of Cr^{6+} , Hg^{2+} and Fe^{3+} ions (chlorides) were added separately to each metal culture to persuade the enzymes machinery systems to achieve their highest productivity.

Growth, proteinome and nucleic acids analysis:

Bacterial growth (as dry weight) in all growing cultures were determined as described in Dawoud and Eweis (2006), while proteinome and nucleic acids were determined as described in Dawoud and Mawgoud (2008).

Proteinome characterization and enzyme assay:

Proteinome analysis on SDS-PAGE was conducted according to Laemmli (1970). Gel bands were analyzed using the computerized image analysis (Gel-Pro analyzer, version 4, Media Cybernetics, 2002). The $HgCl_2$ -dependent oxidation of NADPH (tetrasodium salt) was measured by mercuric reductase assay as described by Simbahan *et al.* (2005).

RESULTS AND DISCUSSION:

Living organisms depend strongly on soil and freshwater sources for their subsistence. Through the course of millions of years of Earth's history, these resources have acquired a well-defined chemical composition suitable for life (Martinez *et al.*, 2004). Accumulation of trace amounts of foreign organic and inorganic species to these resources alters their pristine composition. These alterations make them unsafe for consumption by living organisms (Dong *et al.*, 2003). Toxicity in aqueous and soil environments stems, in part, from an artificial influx of toxic metals generated by human industrial activity. Species of Pb, Hg, Cd, Cr, Cu, Fe, and Zn among others have been detected in such ecosystems and found to bioaccumulate in organisms in nanomolar to micromolar concentrations (Pandey *et al.*, 2000). Effective bioremediation of the polluted sites requires knowledge of genetic pathways for resistance and biotransformation by component organisms within a microbial community (Hu *et al.*, 2005). Some elements (e.g., Al, Cd, Hg, and Pb) seem to have no essential biological functions, but are taken up and accumulated by microorganisms, and occasionally they become toxic at their higher concentrations (Nriagu, 1990). The accumulations of these heavy metals, subsequently, intensify their hazardous impact, therefore, it is of great importance to drain heavy metals or, merely remove them, continuously before reaching their higher concentrations. Many of the heavy metals are important components of biological systems and serve several functions; they represent the prosthetic groups in many proteins and dictate the active site of the enzyme. They also act as cofactors for some enzymatic reactions and multidentate center for porphyrin molecules; in addition they act as redox centers for transferring electrons in microbial cells (Coppi *et al.*, 2007).

Bacillus subtilis provides a model system for the investigation of metal ion homeostasis in gram-positive bacteria (Moore and Helmann, 2005). The metalloregulatory proteins controlling iron, zinc, and manganese homeostasis of *B. subtilis* were identified and characterized the corresponding regulons by using transcriptional profiling and DNA-binding studies (Hantke, 2001). The largest class of metal-regulated genes in *B. subtilis* encodes three transport systems, which suggests the presence of multiple metal uptake pathways. However, the contributions of these metal-regulated operons to cell physiology have not been systematically assessed (Ollinger *et al.*, 2006).

In the present study seven soil samples were collected from a field area in the industrial region, north Cairo, and analyzed

for the quality and quantity of heavy metals dumped from the surrounding and nearby factories. Soils samples were subjected to a screening scheme for isolation of *Bacillus subtilis* strain naturally tolerant to such heavy metals pollution. Out of 247 colonies grown on TLB medium, 32 colonies survived after three replications on the same medium. Only 6 colonies were successfully grown and maintained from a single clone colony. Following the assigned procedures of Bergey's manual for systematic bacteriology (Holt, 1986) for identification of *Bacillus subtilis*, two strains were demonstrated to be similar clones, while the other four colonies were preserved for further identification profiles. The identified candidate strain was assigned to be *Bacillus subtilis* strain NC, the parent isolate (Pt). Pt was subjected separately and collectively to three heavy metal supplements (Cr^{6+} , Hg^{2+} and Fe^{3+}) in a gradient loading scheme (5, 10, 20, 30, 40, 50, 100 μM) to induce the bacterial growth under heavy metals stresses. Few colonies with faint growth were obtained in application of the three ions together and their advanced growth was maintained on TLB medium supplemented with lower concentrations of the employed heavy metals. Two survival isolates were finally obtained and assigned to be the stressed progenies, one of which was selected for the present study (Pg). The influences of different application strategies of nine different heavy metals (Mn^{2+} , Co^{2+} , Zn^{2+} , Mg^{2+} , Li^+ , B^{3+} , Sr^{2+} , V^{5+} and Pb^{2+}) on the growth of both Pt and Pg under the same conditions of maintenance and incubation were determined.

The results (Fig. 1) show that, the response of both Pt and Pg isolates coincide in that their growth under pure heavy metal elements (represented as % control of dry weight) was highly increased under the effect of 50 μM of Mn^{2+} , Co^{2+} , Zn^{2+} , and Mg^{2+} ; which are considered growth promoting elements (GPE). Growth of both Pt and Pg isolates were retarded under the effect of Li^+ and B^{3+} ; growth retarding elements (GRE). While their growth was drastically inhibited under the effect of Sr^{2+} , V^{5+} and Pb^{2+} , growth inhibiting elements (GIE). Generally, the observed increment in dry weight of Pg isolate was higher than Pt isolate; while GRE promoted the growth of Pg isolates. These results are in agreement with Chastukhina *et al.* (2004) where the growth and production of glutamyl endopeptidase of *Bacillus subtilis* was stimulated by Mg^{2+} and Co^{2+} . In addition, the results (Fig. 1) revealed that combination of trace amount (5 μM) of Cr^{6+} (chromization), Hg^{2+} (amalgamation) and Fe^{3+} (ironization) with pure heavy metal elements synergized the growth-stimulating effect of heavy metals and decreased the antagonistic effect of GIE on the bacterial growth (Pg isolates). In this connection, Saleh *et al.* (2005) found that

supplementation of growing cultures with metal ions ameliorated the toxic effect of salinity on *Bacillus subtilis*.

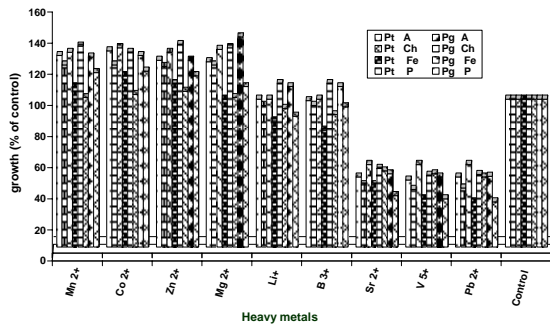


Fig. 1 Response of the growth of *Bacillus subtilis* NC isolates, Pt and Pg to the impact of different heavy metals stresses.

Induction of suitable regulon enzymatic systems in bacteria using different stresses was regarded by Keyhan *et al.* (2004). Budde *et al.* (2006) found that during the growth of *Bacillus subtilis* under stress of low temperature, its propagation in a minimal medium at 15 °C triggered the induction of 279 genes and repression of 301 genes in comparison to its growth at 37 °C. Antelmann *et al.* (2000) observed similar results in the stress of phosphate starvation response in *Bacillus subtilis*. On the other hand, it was postulated that the mode of action of heavy metals on the bacterial components may be traced in sugar transport, cell wall synthesis and construction, membrane function, carbohydrate metabolism, protein synthesis, and enzyme activation / inhibition. Moreover, combination of more than one heavy metal has a profound biological effect than a single element (Jjemba, 2004).

Proteinome and nucleic acids evaluation (Figs 2&3) revealed that pure heavy metal elements of GPE group (Mg^{2+} , Zn^{2+} , Mn^{2+} and Co^{2+}) increased the amount of proteinome and nucleic acids in the parent *Bacillus subtilis* strain NC and that would be resulted from the stimulatory effect of these elements, mainly, on the regulon enzymatic systems and that was reflected in the biosynthesis of these main components. However, it was recorded that glutathione synthase and phosphoenolpyruvate carboxylase were activated by Mg^{2+} (Wedding and Black, 1988), where carboxylases are bounded preferentially to nitrogen and phosphoryl groups via Mg^{2+} ions and Mg-ATP is the substrate for plasma membrane bound ATP-ase. In addition, Mg^{2+} is used also for charge compensation and osmoregulation processes, while, inadequate supply of Mg^{2+} leads to ceases of protein synthesis (Yazaki *et al.*, 1988). Zn^{2+} is essential elements in polymerization of amino acids into proteins where its shortage activates the RNA-degradation system by RNA-ase activity (Sharma *et al.*, 1982). Zinc metalloproteins

are involved in DNA replication, transcription and regulation of gene expression (Coleman, 1992). Zinc might bind to sulfhydryl group of cell membrane constituent or form tetrahedral complexes with cysteine residues of polypeptide chains and thereby protect membrane lipids and proteins against oxidative damage (Cakmak and Marschner, 1990). Mn^{2+} acts as cofactor for activation of more than 35 different enzymes (Sharma *et al.*, 1991) involved in redox reactions, decarboxylation, hydrolytic reactions and biosynthesis of aromatic amino acids.

The proteinome and nucleic acids increased in the presence of small traces of Cr^{6+} , Hg^{2+} and Fe^{3+} (Fig. 2) and that was reflected in the little amounts of heavy metals residuals (Fig. 3).

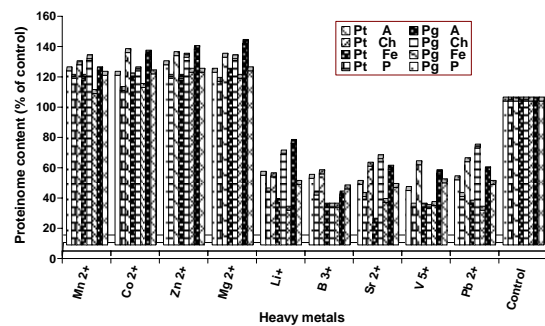


Fig. 2. Response of the proteinome of *Bacillus subtilis* NC isolates Pt and Pg to the impact of different heavy metal stresses.

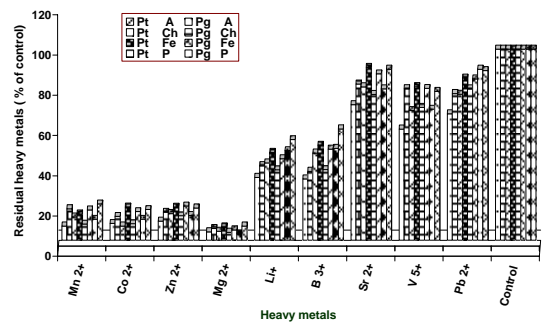


Fig. 3. Residuals of different heavy metals after bacterial growth of *Bacillus subtilis* NC isolates; Pt and Pg.

These results suggest the occurrence of some activity in the regulating machinery of the enzymes. In this connection, Keyhan *et al.* (2004) observed that mixing various combinations of metals greatly affects the metal-enzymatic activities. The results (Figs 2&4) demonstrated different activation rate of combined heavy metals. Remarkably, the stressed Pg isolate exhibited more interaction with GPE than Pt, and that may be conferred as the existence of novel developed regulon enzymatic machinery and/or induction of mutant novel genes which facilitated the exploitation of those applied heavy metals. Data of GRE group (Li^+ and B^{3+}) revealed a decrease in proteinome content (Fig. 2) unparalleled with the nucleic acids production

and that would reflect the mode of action of GRE which may interfere with protein synthesis through the nucleic acids transcription and translation to produce amino acids polymerization and protein. Application of C, A and F resulted in sharp declines in the proteinome and nucleic acids of Pt isolate. On the contrary, application minimized the toxic effect of heavy metals in the Pg isolate which appoint a sort of antagonism for the effect of heavy metals or the restoring of the enzymatic activity machinery systems.

The third group of heavy metals (GIE) appeared to be very destructive for both cell constituents and metabolic activities of the studied isolates. The inhibiting consequence was reflected in the sharp decline of dry weights, proteinome quantity and nucleic acids synthesis (Figs 1-4). Toxicity of heavy metals are well understood in their influence on the binding bonds in the tertiary and quaternary structures of enzymes distorting their active sites and denature the enzymatic configuration leading to the complete irreversible inhibition of enzymatic machinery systems (Summer, 1992).

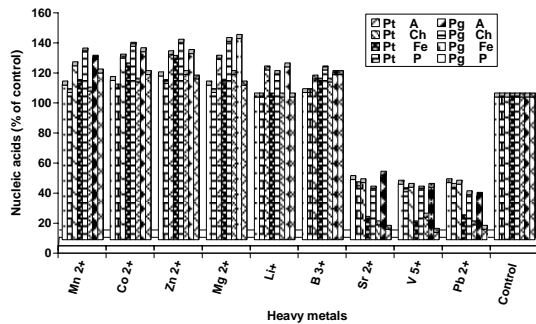


Fig. 4. Response of the nucleic acids of *Bacillus subtilis* NC isolates Pt and Pg to the impact of different heavy metals stresses.

The genes governing the metal resistance in bacteria are plasmid or chromosomal-born (Mahadevan *et al.*, 2006). The former expectation assists the transferring recombination process of these plasmids via conjugation between acceptable species in the same micro niche habitat (Xiong *et al.*, 2000). Although most resistance regulon systems function by energy-dependent efflux of toxic ions, some resistance engage enzymatic transformation such as mercurial resistance regulon system which involves mercuric reductase that converts soluble inorganic Hg^{2+} to Hg^0 where the latter eliminates as gas. In addition, there is mercurial lyase which cleaves the Hg-C bond of more toxic organo-mercurials to less toxic Hg^{2+} . Mercuric reductase was estimated with NADPH, tetrasodium salt (Fig. 5) which was more expressed in the traced amalgamated Mg^{2+} culture of Pg isolate. The Pg proteinome was investigated for the presence of novel expressed protein bands.

The electrophoretogram of the proteinome of both Pt and Pg isolates (Fig. 6) revealed the presence of new regulatory protein bands in Pg proteinome between 37.4 and 48.7 KD. Four intensive bands (36.1, 44.6, 57, 71.2 KD) compared to Pt smear were appeared, while many bands between 17 and 26.5 KD were disappeared. These changes are confirmed with the biochemical estimations and that support the hypothesis of the occurrence of novel enzymatic machinery system to cope with such elevated concentrations and varieties of applied heavy metals which induced the bacterial genome for a novel trend of metabolic processes. However, this point needs more exploration concerning the enzymatic regulatory proteins evolved in the present study.

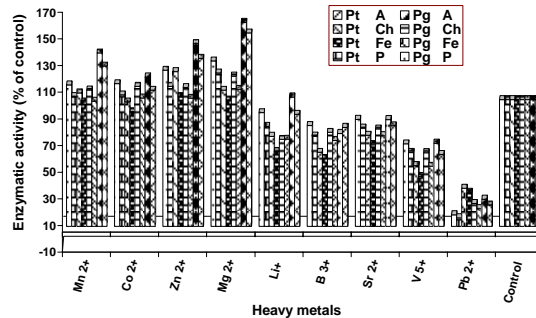


Fig. 5. Enzymatic activity of mercuric reductase in the growth cultures of *Bacillus subtilis* NC Pt and Pg isolates.

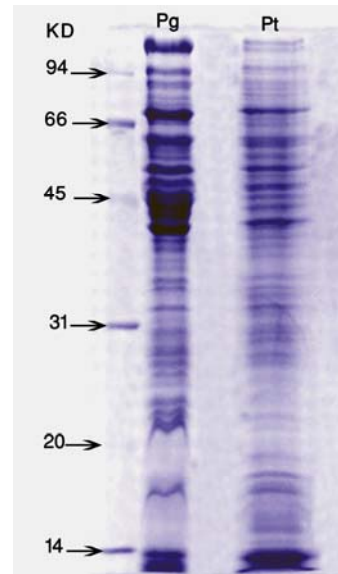


Fig. 6. Electrophoretogram of SDS-PAGE of *Bacillus subtilis* strain NC isolates; Pt and Pg.

KD: Marker of different Mol. Wt proteins.
Pt: Proteinome of parent *Bacillus* isolate.
Pg: Proteinome of progeny *Bacillus* isolate.

CONCLUSION:

The Gram positive saprophytic *Bacillus subtilis* has a definite effect in the plant growth enhancement; where it could dissolve P of weathered rocks and mobilize useful minerals (as P^{3+} , K^+ , Mg^{2+} , Mn^{2+} , Fe^{3+} , Cu^{2+}

and Zn²⁺) in minerals rock (Puente *et al.*, 2004). It was used by Kahru *et al.*, (2005) as a cadmium sensor in smelting area containing Cd²⁺, Pb²⁺ and Zn²⁺. Also, it could alleviate the adverse effect of salinity in the soil (Saleh *et al.*, 2005). Also, Korsten *et al.*, (1994) utilized *B. subtilis* as a field biocontrol agent spray to reduce the severity of Avocado black spot caused by *Pseudocercospora purpurea*. In the present study, the utilized heavy metals achieved different influences on the bacterial growth varied from growth promoting elements (Mn²⁺, Co²⁺, Zn²⁺ and Mg²⁺), growth retarding elements (Li⁺, and B³⁺) and growth inhibiting elements (Sr²⁺, V⁵⁺ and Pb²⁺). Combination of traces (5µM) of Cr⁶⁺, Hg²⁺ and Fe³⁺ with the

growing cultures of Pt and Pg isolates enhanced the growth yields, and the synthesis of proteinome and nucleic acids particularly with Mg²⁺ ions. The internalization of heavy metals was contentment with Pg isolate and this was confirmed by the decreased amounts of minerals residues acquired after the bacterial growth. The electrophoretogram of Pg proteinome display a great possibility for the presence of novel expression of enzymatic machinery systems responsible for the adaptation of the bacterium to such heavy metals stress and this was confirmed by the appearance of novel regulon bands of molecular weight range between 37.4 and 48.7 KD.

REFERENCES:

- Andrews SC, Robinson AK, Rodriguez-Quinones F. 2003. Bacterial iron homeostasis. *FEMS Microbiol. Rev.* 27: 215-237.
- Antelmann H, Scharf C, Hecker M. 2000. Phosphate starvation-inducible proteins of *Bacillus subtilis*: proteomics and transcriptional analysis. *J. Bacteriol.*, 182: 4478-4490.
- Budde I, Steil L, Scharf C, Volker U, Bremer E. 2006. Adaptation of *Bacillus subtilis* to growth at low temperature: combined transcriptomic & proteomic appraisal. *Microbiol. Read.*, 152: 831-853.
- Cakmak I, Marschner H. 1990. Decrease in nitrite uptake and increase in proton release in zinc deficient cotton, sunflower and buckwheat plants. *Plant Soil*, 129: 261-268.
- Chastukhina IB, Sharipova MR, Gabdrakhmanova LA, Safina NP, Kostrov SV, Ruden-Skaya GN, Leshchinskaya IB. 2004. The regulation of *Bacillus intermedius* glutamyl endopeptidase biosynthesis in the recombinant *Bacillus subtilis* strain AJ73 during sporulation. *Microbiol. Read.*, 145: 325-337.
- Coleman JE. 1992. Zinc proteins: enzymes, storage proteins, transcription factors, and replication proteins. *Annu. Rev. Biochem.* 61: 897-946.
- Coppi MV, O'Neil RA, Leang C, Kaufmann F, Methé BA, Nevin KP, Woodard TL, Liu A, Lovley DR. 2007. Involvement of *Geobacter sulfurreducens* SfrAB in acetate metabolism rather than intracellular, respiration-linked Fe (III) citrate reduction. *Microbiology*, 153: 3572-3585
- Dawoud MEA, Eweis M. 2006. Phytochemical control of edible mushroom's pathogenic bacteria. *J. Food Agric. Environ.*, 4: 321-324.
- Dawoud MEA, Mawgoud YA. 2008. Boron impact upon pathogenesis and some related proteinome components of *Pseudomonas syringae* pv. *syringae* during bacteriosis of some Triticum varieties. *N. Egypt. J. Microbiol.*, 19: 241-256.
- D'Haese PC, Landeghem GFV, Lamberts LV, Bekaert VA, Schrooten I, De Broe ME. 1997. Measurement of strontium in serum, urine, bone, and soft tissues by Zeeman atomic absorption spectrometry. *Clin. Chem.*, 43: 121-128.
- Dong D, Hua, X, Li Y, Zhang J, Yan D. 2003. Cd adsorption properties of components in different freshwater source coatings: the important role of ferromanganese oxides. *Environ. Sci. and Technol.*, 37: 4106-4113.
- Hantke K. 2001. Iron and metal regulation in bacteria. *Curr. Opin. Microbiol.*, 4: 172-177.
- Holt JG. 1986. *Bergey's Manual of Systematic Bacteriology*; 2 Gram-positive Bacteria other than Actinomycetes. 1st edition. Williams & Wilkins, Baltimore, MDV.
- Hu P, Brodie EL, Suzuki Y, McAdams HH, Anderson GL. 2005. Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. *J. Bacteriol.*, 187: 8437-8449.
- Jakubovics NS, Jenkinson HF. 2001. Out of the Iron Age: new insights into the critical role of manganese homeostasis in bacteria. *Microbiology*, 147: 1709-1718.
- Jjemba PK. 2004. *Environmental Microbiology: Principles and applications*. Science publishers, Inc. Plymouth, UK. Pp. 425-439.
- Kahru A, Hvasik A, Kasemets K, Pllumaa L, Kurvet I, Francois M, Dabourguier HC. 2005. Biotests and biosensors in ecotoxicological risk assessments of field soils polluted with zinc, lead and cadmium. *Environ. Toxicol. Chem.*, 24: 2973-2982.
- Keyhan M, Ackerley DF, Martin A. 2004. Targets of improvement in bacterial chromate bioremediation sediments 2003, proceedings of the second international conference on remediation of contaminated sediments. Venice- Italy.
- Korsten L, de Villiers EE, Duvenhage JA, M. Kotze J. 1994. Control of Avocado pre-harvest diseases with *Bacillus subtilis* and fungicide sprays. Year book South African Avocado Grower's Associations, 17: 32-34.
- Laemmli UK. 1970. Most commonly used discontinuous buffer system for SDS electrophoresis. *Nature (London)*, 227: 680-688.
- Mahadevan R, Bond DR, Butler JE, Esteve-Nunez A, Coppi MV, Palsson BO, Schilling CH, Lovley DR. 2006. Characterization of metabolism in the Fe (III)-reducing organism *Geobacter sulfurreducens* by Constraint-Based Modeling. *Appl. Environ. Microbiol.*, 72: 1558-1568.

- Martinez RE, Pedersen K, Ferris FG. 2004. Cadmium complexation by bacteriogenic iron oxides from a subterranean environment: J. Colloid and Interf. Sci., 275: 82-89.
- Masse E, Arguin M. 2005. Ironing out the problem: new mechanisms of iron homeostasis. Trends Biochem. Sci., 30: 462-468.
- Moore CM, Helmann JD. 2005. Metal ion homeostasis in *Bacillus subtilis*. Curr. Opin. Microbiology, 8: 188-195.
- Nriagu JO. 1990. Global metal pollution: poisoning the biosphere. Environment, 32: 218-233.
- Ochiai A, Itoh T, Kawamata A, Hashimoto W, Murata K. 2007. Plant cell wall degradation by saprophytic *Bacillus subtilis* strains: Gene clusters responsible for rhamnogalacturonan depolymerization. Appl. Environ. Microbiol., 73: 3803-3813.
- Ogunseitan OA. 2002. Episodic bioavailability of environmental mercury: Implication for biotechnological control of mercury pollution. African journal of Biotech., 1: 1-9.
- Ollinger J, Song KB, Antelmann H, Hecker M, Helmann JD. 2006. Role of the Fur Regulon in Iron Transport in *Bacillus subtilis*. J. Bacteriol., 188: 3664-3673.
- Pandey AK, Pandey SD, Misra V. 2000. Stability constants of metal-humic acid complexes and its role in environmental detoxification: Ecotoxi. and Environ. Safety, 47: 195-200.
- Pilon-Smith E, Pilon M. 2000. Breeding mercury-breathing plants for environmental clean up. Trends Plant Sci., 5: 325-336.
- Puente ME, Li CY, Bashan Y. 2004. Microbial population and activities in the rhizosphere of rock-weathering desert plants. II. Growth promotion of Cactus seedlings. Plant Biol., 6: 643-650.
- Saleh SA, Heuberger H, Schnitzler WH. 2005. Alleviation of salinity effect on artichoke productivity by *Bacillus subtilis* F2B24, supplemented Ca & micronutrients. J. Appl. Bot. Food Qual., 74: 24-32.
- Samuelson P, W.-Rus H, Svedberg M, Stahli S. 2000. Staphylococcal surface display of metal-binding polyhistidyl peptides. Appl. Environ. Microbiol., 66: 1243-1248.
- Sharma CP, Sharma PN, Bisht SS, Nautiyal BD. 1982. Zinc deficiency induced changes in cabbage. In "Proceedings of the ninth plant nutrition colloquium, Warwick, England (A. Scaife, ed.), Common wealth Agricultural Bureau, Farnham Royal, Bucks. pp. 601-606.
- Sharma CP, Sharma PN, Chatterjee C, Agarwala SC. 1991. Manganese deficiency in maize effects pollen viability. Plant soil, 138: 139-142.
- Simbahan J, Kurth E, Schelert J, Dillman A, Moriyama E, Jovanovich S, Blum P. 2005. Community analysis of a mercury hot spring supports occurrence of domain-specific forms of mercuric reductase. Appl. Environ. Microbiol., 71: 8836-8845.
- Summer AO. 1992. Unwist and shout: a heavy metal responsive transcriptional regulator. J. Bacteriol., 174: 3097-3101.
- Wedding RT, Black MK. 1988. Role of magnesium in the binding of substrate and effectors to phosphoenolpyruvate carboxylase from a CAM plant. Plant Physiol., 87: 443-446.
- Xiong A, Singh VK, Cabrera G, Jayaswal RK. 2000. Molecular characterization of the ferric-uptake regulator, fur, from *Staphylococcus aureus*. Microbiology, 146: 659-668.
- Yazaki Y, Asukagawa N, Ishikawa Y, Ohta E, Sakata M. 1988. Estimation of cytoplasmic free Mg²⁺ levels and phosphorylation potentials in mung bean root tips in vivo ³¹P NMR spectroscopy. Plant Cell Physiol., 29: 919-924.

تخليق ناظمات- مضاده للتلوث من خلال الإجهاد الكيميائي لبكتيريا باسيلس سينتلس و سلالاتها البنوية

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تأثير المعادن المؤخرة للنمو في سلالة الآباء. و قد ازداد استهلاك البكتيريا للملوثات في وجود المعادن المحفزة للنمو ، المعصدة بأثار من الزئبق و الكروم و الحديد ، و اضمحل الإستهلاك في وجود المعادن المعيقة للنمو ، رغم تعضيدها بنفس العناصر ، مما يدل على تحلل الأغشية الخلوية البكتيرية ، و تعطل النفاذية بها. و عند تحليل البروتين الكمي لسلالات الآباء ، و السلالات البنوية على جل البولوي أكريلاميد ، باستخدام التفريد الكهربائي ، ظهرت حزم بروتينية جديدة نتيجة للإجهاد الكيميائي بالعناصر الثقيلة ، و التي تمثل ناظمات في الخلايا البنوية من المحتمل أن يؤدي التعبير النووي لها إلى إنتاج انزيمات قادرة على معالجة التلوث بالمعادن الثقيلة.

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تم عزل بكتيريا التربة المترمة باسيلس سينتلس "س" من المنطقة الصناعية بشبرا الخيمة شمال القاهرة ، و بعد استخدام الإجهاد الكيميائي لعناصر الزئبق، و الكروم، و الحديد على السلالة ، تم عزل بكتيريا بنوية متأقلمة للتخلص من تلوث المعادن الثقيلة. كذلك تم معاملة كلاً من الخلايا الآباء و الخلايا البنوية بسبعة عناصر ثقيلة ، و حيث تبين تأثير تلك المعادن على حيوية السلالات البكتيرية ، فقد تم تقسيم العناصر طبقاً لهذا التأثير إلى ثلاثة مجموعات: أ) مجموعة المعادن المحفزة للنمو مثل الكوبالت ، والقصدير ، و الماغنسيوم. ب) مجموعة المعادن المؤخرة للنمو مثل الليثيوم ، و البورون. ج) مجموعة المعادن المعيقة للنمو مثل التترانشيوم ، و الفانديوم ، والرصاص. و عند تعضيده هذه المجموعات بأثار قليلة من عناصر الكروم ، و الزئبق ، و الحديد ، تبين التأثيرات على السلالات البنوية ، فقد تلاشى تأثير المعادن المؤخرة للنمو، و تنامي تأثير المعادن المحفزة للنمو ، ولكن لم يتغير

