MORPHOLOGICAL AND FUNCTIONAL VERSATILITY OF SOME BACTERIAL STRAINS UNDER CONTAMINATION STRESS

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Abstract. Various studies conducted over time in our laboratory on several bacterial strains isolated from areas contaminated with metal ions or petroleum hydrocarbons, the major environmental pollutants, as well as from unpolluted areas, but cultivated in the presence of a pollutant, allowed us to formulate a hypothesis regarding the *morphofunctional* versatility of microorganisms under stress of contamination. Some noticed morphofunctional aspects were related to cell size, but also to issues generated either by intracellular accumulation of products from the environment, either by releasing specific metabolites. This paper presents only a few aspects, supporting the hypothesis of bacterial avatars of biotechnological interest.

Keywords: bacterial variety, versatility, cell dimensions, contamination stress.

Rezumat. Versatilitatea morfologică şi funcţională a unor tulpini bacteriene în condiţiile stresului de contaminare. Diferite studii efectuate în decursul timpului, în laboratorul nostru, asupra unor tulpinii bacteriene, izolate din zone poluate cu ioni metalici sau cu hidrocarburi petroliere, *the major environmetal pollutants*, dar şi din zone nepoluate, crescute în prezenţa unui poluant, au stat la baza emiterii ipotezei versatilităţii morfo-funcţionale a unor microorganisme, în condiţiile stresului de contaminare. Unele aspecte morfo-funcţionale, constatate au fost legate de dimensiunile celulare, dar si aspecte generate fie prin acumularea intracelulară a unor produşi din mediu, fie prin eliberarea unor metaboliţi caracteristici. Lucrarea de faţă cuprinde doar câteva semnalări, constituind o ipoteză în demonstrarea avatarurilor bacteriene de interes biotehnologic.

Cuvinte cheie: varietate bacteriană, versatilitate, dimensiunile celulei, stress de contaminare.

INTRODUCTION

Environmental contamination with various allogenic substances most often occur as a result of human activity, accidental or accumulated in time. Thus, microorganisms from the environment have a rapid adaptation to the new pollutants (DIAZ, 2004), to the new conditions, and moreover, can use these xenobiotic chemicals as a growth and energy source (VAN DER MEER et al., 1992).

Most frequently, especially in recent years, the problem of genetic versatility has been brought into discussion. This versatility is due to mobile genetic elements (DIAZ, 2004).

Competition under these conditions becomes predominantly interspecific, but may also occur intraspecifically, within certain distress circumstances.

What differentiates, in this case, the resistant individuals from those that cannot stand the modified environmental parameters?

How can readjust and survive the individuals that have developed under polluted conditions, when contamination lowers or even disappears, and the environment remains "clean"?

What are the adaptation mechanisms to stress and reversion to the conditions previous to contamination?

Can all these stances be considered "competitive avatars"?

Where do the bacterial strains with bioremediative abilities come from, that reduce, by natural attenuation, the level of contamination by consuming or decomposing the allogenic compounds into easier or harder metabolizing constituents?

And to what extent bioremediation interventions based on augmentation of local microbiota with single stream bacterial augmented stream inoculum (DIAZ, 2004) and tested in laboratory conditions is beneficial to the environment, or is it insufficiently monitored in terms of duration in time and potential synergistic implications?

Of course, the answers to all these questions would be the understanding, explanation and mastering of the mechanisms which we are currently trying to study.

MATERIAL AND METHODS

The analysed bacterial strains were represented by several selected isolates from soil samples collected from the area of Copşa Mică town (C_1), Sibiu County, an adjacent area to the former industrial zone, with cadmium ions concentration exceeding the allowed level.

The samples under test consisted of Gram negative bacillary bacterial cultures, which were grown in liquid LB medium supplemented with $CdCl_2$ at concentrations varying between 250 and 3,000 mg‰, as compared to the controls, to simulate local pollution conditions.

Cultivation was carried out at 28°C for 120 hours, under continuous stirring.

From these cultures, samples were prepared and subjected to electronic microscope examination. Observations and image captures were performed, comparing the controls to the bacterial strains grown at $CdCl_2$ concentrations of 250 and 3,000 mg‰, respectively.

The samples were obtained by centrifugation at 5,000 rpm for 10 minutes and analysed with a JEM-1400 Plus Transmission Electron Microscope. Pelleted cells were washed in distilled water, fixed to copper grids and examined.

RESULTS AND DISCUSSIONS

The dimensions of bacterial cells were significantly different both in terms of length and thickness, which is a first signal in an attempt to demonstrate the influence of environmental contaminants on the morphological appearance of some bacterial strains, as well as and their ability to return to the original size when contamination stress is mitigated.

The results of our observations in this first stage of research are limited just to the morpho-dimensional appearance of the bacterial cells subjected to stress of contamination conditions. To achieve a complete picture to characterize the cellular changes under various cultivation conditions and their reversibility and versatility upon cessation of stress conditions, requires further studies, addressing topics like physiological and cellular metabolism (Table 1).

Table 1. Dimensions of the bacterial cells under electron microscopy, at magnifications up to 5,000 nm.

	Bacterial cell dimensions (nm)	
Control (no $CdCl_2$)	**Length**	**Thickness**
	5,431.61	2,243.72
	5,365.21	1,986.04
C_1 with 250 mg/l $CdCl_2$	5,049.78	2,195.92
	5,542.87	1,959.10
	5,160.04	1,894.40
	6,709.45	**1,872.65**
C_1 with 3,000 mg/l $CdCl_2$	2,737.0	1,622.08
	11,368.02	**1,502.80**
	6,888	**2,133.81**
	8,645.43	**1,397.54**
	10,675.07	**1,706.61**
	5,982.18	1,459.17

Photo 1 shows a picture of control bacterial cells grown in LB medium without addition of "contaminant". It can be seen that the cells are short and thick, relatively uniform and an intracellular contents with a specific longitudinal layout. The measured bacterial cell size reaches values of 1,986.04/5,365.21 nm, and 2,243.72/5,431.61 nm, respectively.

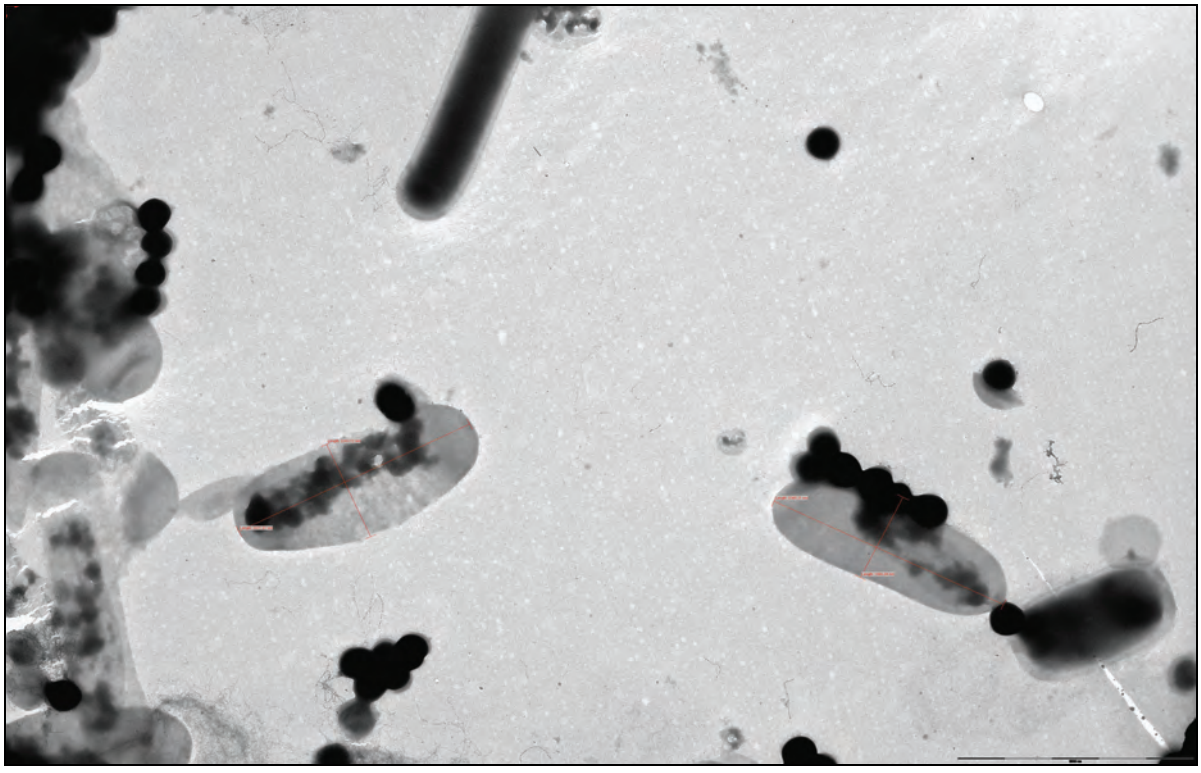

Photo 1. Electron microscopic image (JEM 140080 KV 12.6, 5,000 nm X) (original).

Photo 2a and 2b show the bacterial cells grown in LB medium containing 250 mg‰ $CdCl_2$, supplement which acted like a contaminant, leading to an increased cell size of about 1,000 nm in length (6,709.45/1,872.65 nm.).

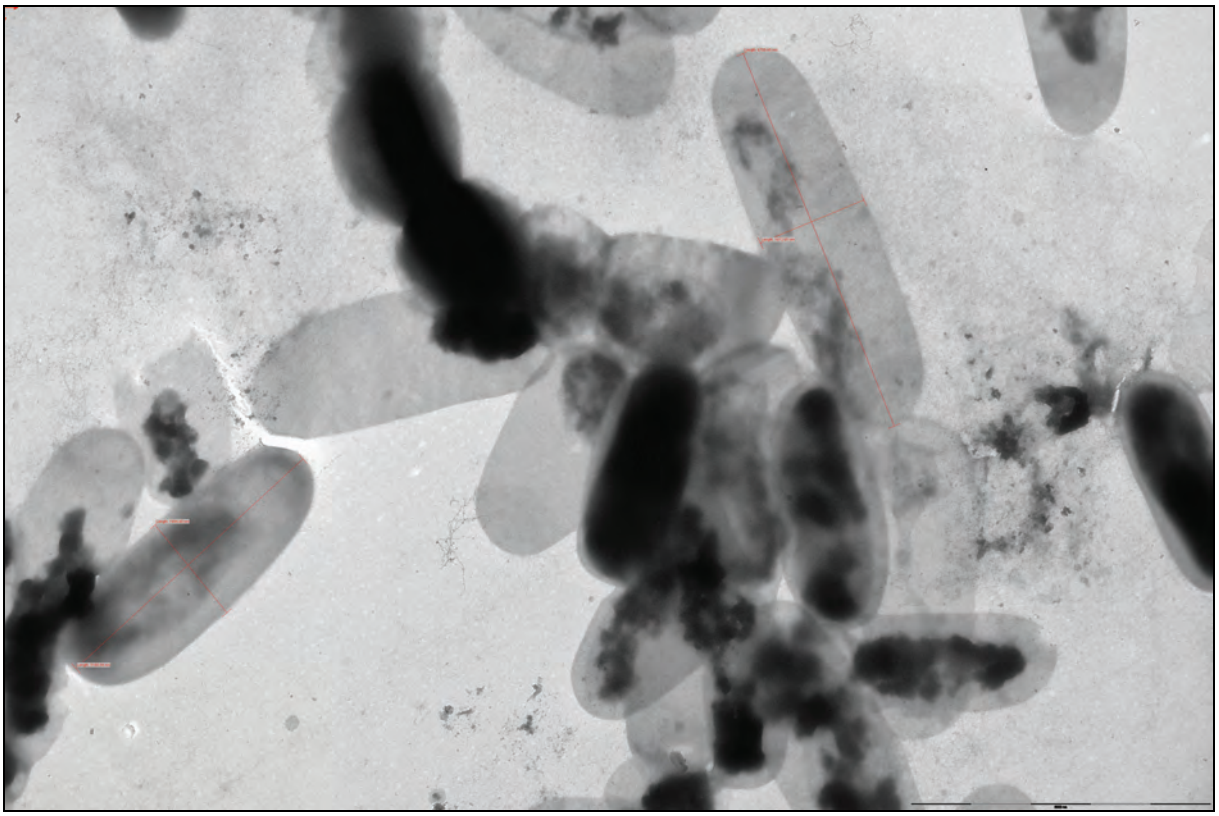

Photo 2a. EM imagine JEM 140080, 5,000 nm. X, cellular cultures in medium supplemented with 250 g‰ $CdCl_2$ (original).

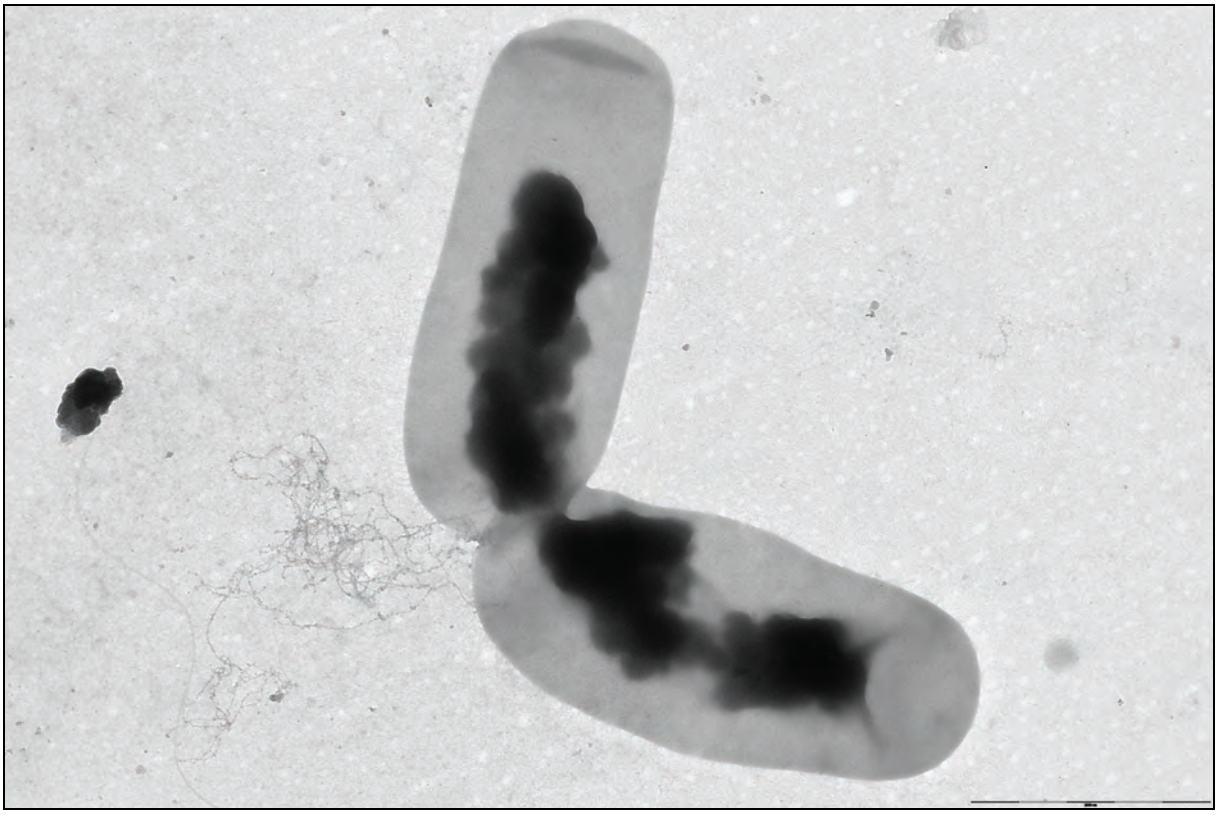

Photo 2b. EM image JEM 140080, 2,000 nm. X, cellular cultures in medium supplemented with 250 g‰ $CdCl_2$ (original).

In photo 3, the cultures that resisted to $CdCl_2$ concentrations of up to 3,000 mg‰ presented double size of the control cells (6,888/2,133.81 and 11,368.02/1,502.80, respectively). At this concentration, "ghost" cells were also noticed, devoid of content, which demonstrates a low viability at concentrations above certain limits of tolerance.

Viable cells grown under stress of contamination, which increased their dimensions, were re-passaged and grown in media with low $CdCl_2$ concentrations down to the total absence of the pollutant in order to determine phenotype reversibility of the cells in stressless conditions.

Alongside these experiments, the metabolic capacity of these strains was tested, including under stress of contamination conditions. Metabolic by products accumulated either intracellular or extracellular can be noticed.

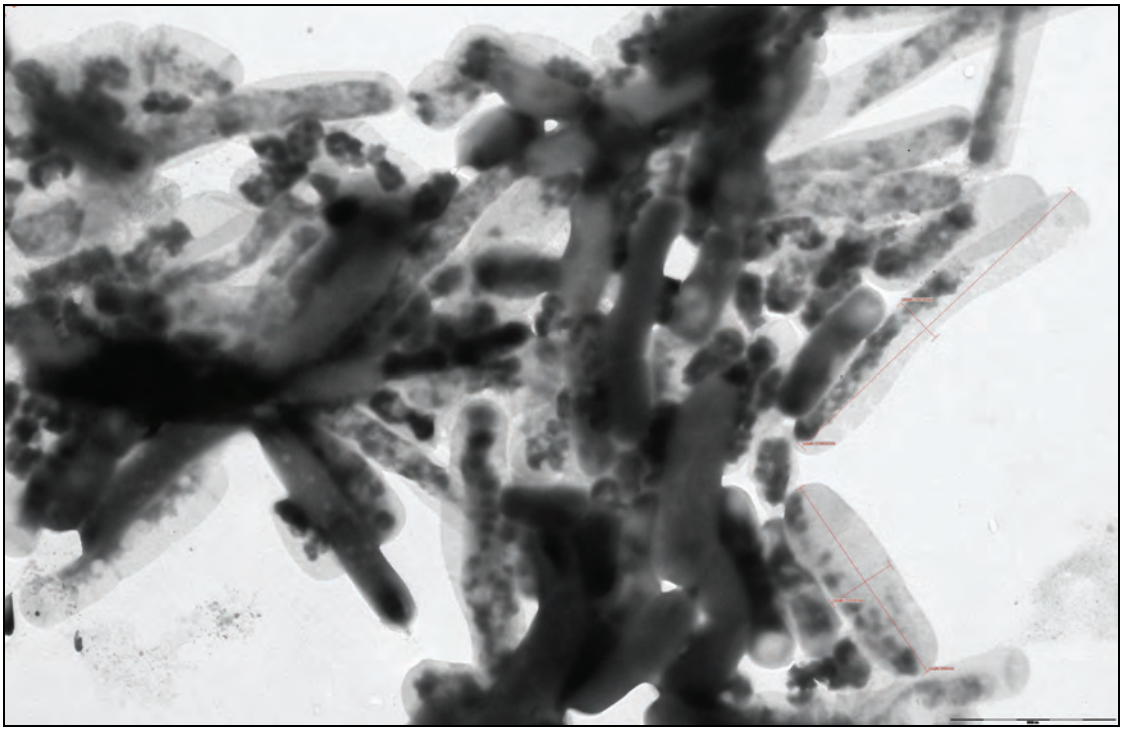

Photo 3. EM imagine JEM 140080, 5,000 nm. X, cellular cultures in medium supplemented with 3,000 g‰ $CdCl_2$ (original).

In photo 4 intracellular bacterial products accumulations can be seen. The *Pseudomonas* strain was grown in glucose congaing medium (20%); this strain has the ability to secrete polymers of polyhydroxyalkanoates type.

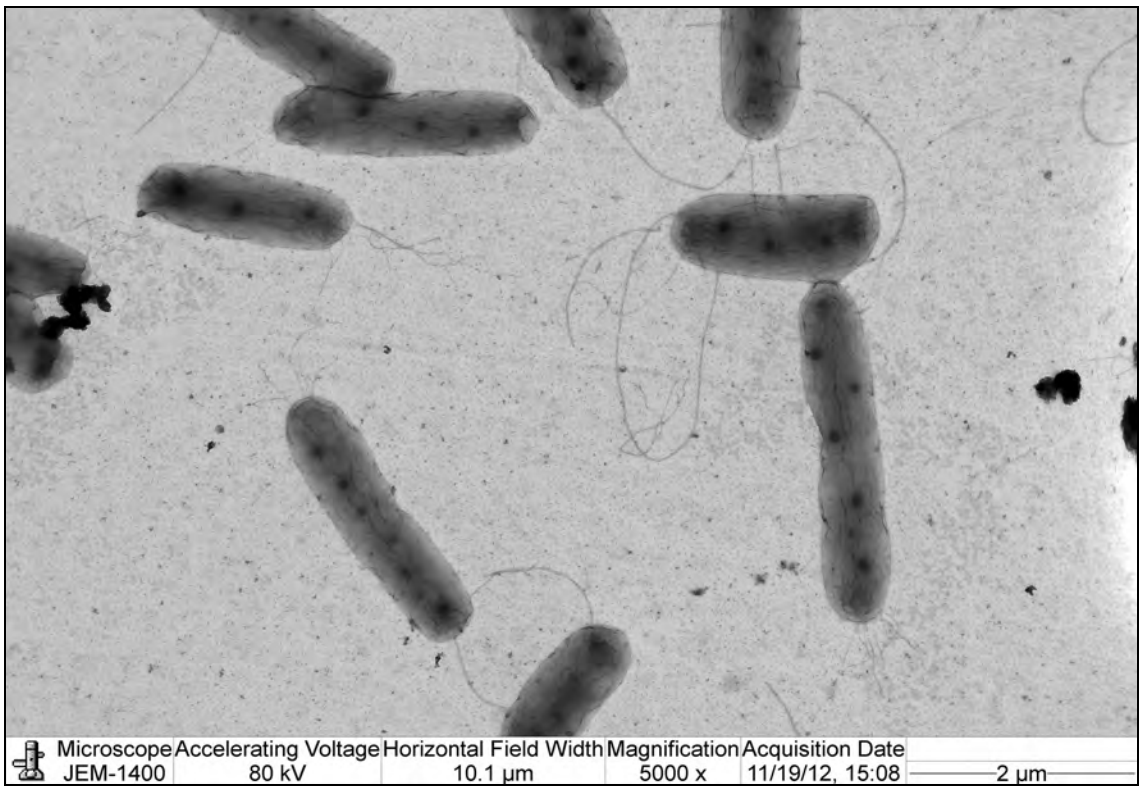

Photo 4. Intracellular accumulation of metabolic products (original)

As mentioned, extracellular metabolic products can be seen, such as surfactants (Photo 5). These products crystallize in different shapes, depending on the composition (ABDEL-MAWGOUD et al., 2009; STANCU, 2015), in this case.

In other situations (Photo 6), both intracellular and extracellular products were found in the same bacterial strain. The photos accompanying this material are original, belonging to the authors.

The obtained results represent a first signal within the set of studies expected to be able to prove the veracity of the hypothesis concerning the versatility of bacterial strains under stress of contamination.

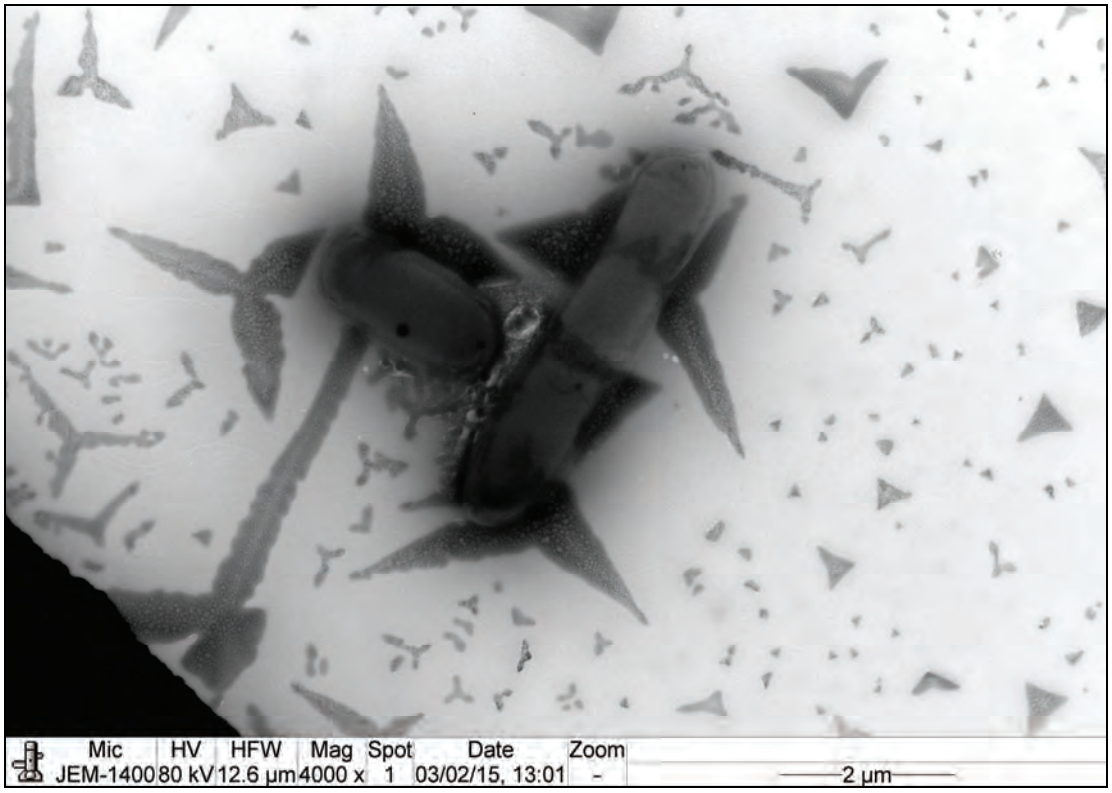

Photo 5. Extracellular secretions of metabolic products, in the form of needle crystals (original).

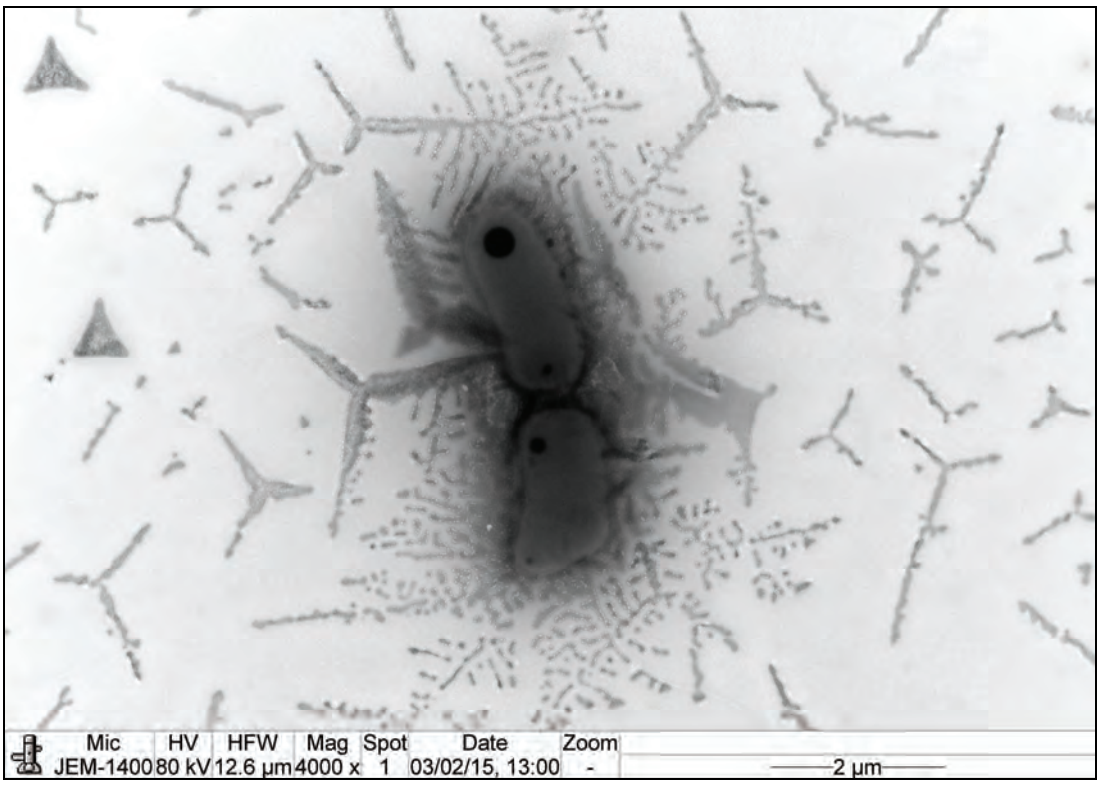

Photo 6. Intra and extracellular accumulation of products of metabolism (original).

CONCLUSIONS

The bacterial strains grown under stress conditions may manifest morphological and functional changes, sometimes reversible

Occasionally, in response to the stress of contamination, the size of bacterial cells exceeds the usual values. Proportional upon reduction of the contaminant concentration, cell sizes return to those specific to unpolluted environments.

It is worth noting that not all bacterial cells under stress of contamination exhibit dimension increase at the same growth rate.

A correlation might be set among bio-products, cell dimensions and presence of some contaminants at certain concentrations in the medium.

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REFERENCES

ABDEL-MAWGOUD A. M., ABOULWAFA M. M., HASSOUNA N. A. H. 2009. Characterization of rhamnolipid produced by *Pseudomonas aeruginosa* isolate Bs20. *Applied Biochemistry and Biotechnology*. Springer. Berlin. **157**: 329-345.

DIAZ E. 2004. Bacterial degradation of aromatic pollutans: a paradigm of metabolic versatility. *International Microbiology*. Spanish Society for Microbiology. Madrid. **7**(3): 173-180.

VAN DER MEER J. R., DE VOS W. M., HARAYAMA S., ZEHNDER A. J. 1992. Molecular mechanisms of genetic adaptation to xenobiotic compounds. *Microbiology*. *Molecular Biology*. Elsevier. London. **56**(4): 677-694.

STANCU M. M. 2015. Solvent Tolerance Mechanisms in *Shewanella putrefaciens* IBBPo6. *Water Air Soil Pollution*. Elsevier. Paris. **226**(39): 1-16.

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