THE TOLERANCE OF SOME BACTERIAL STRAINS TO CADMIUM

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Abstract. Due to its properties of remanence in the environment, heavy metal pollution is recognized as a real problem, which affects the sites for long periods of time. Among heavy metals, cadmium is known as one of the most toxic, with negative effects on microorganisms, plants, animals and especially on human health. In this context, the present study was aimed to test the tolerance of different bacterial strains to cadmium, in order to select those that respond best for revealing the starting points of some new research directions in this area. Thus, three bacterial strains were tested at different concentrations of $CdCl_2$, of which only two showed the ability to grow in this medium. It was also observed that *Chm1* strain tolerates the highest concentration of cadmium chloride, up to 3,000 mg/l.

Keywords: bacterial tolerance, heavy metals, cadmium pollution.

Rezumat. Toleranţa unor tulpini bacteriene la cadmiu. Poluarea cu metale grele este recunoscută ca o problemă reală, datorită propietăţii de remanenţă în mediu a acestora, pentru perioade îndelungate. Dintre metalele grele, cadmiul este unul dintre cele mai toxice, cu efecte negative asupra microorganismelor, plantelor, animalelor, în special asupra sănătăţii oamenilor. În acest context, prezentul studiu a urmărit testarea toleranţei la cadmiu a diferitelor tulpini bacteriene, în vederea selectării acelora care ar putea constitui punctul de plecare al unor noi direcţii de cercetare. Astfel, au fost testate la diverse concentraţii de $CdCl_2$ trei tulpini bacteriene, dintre care doar două au prezentat capacitatea de a se dezvolta în astfel de condiţii. De asemenea, s-a observant că tulpina *Chm*$_1$ a tolerat o concentraţie de până la 3000 mg/l.

Cuvinte cheie: toleranţa bacteriană, metale grele, poluare cu cadmiu.

INTRODUCTION

Heavy metal pollution is one of the most widespread and serious environmental problems facing the biosphere in our times (SIRIPORNADULSIL & SIRIPORNADULSIL, 2013), potentially causing serious health issues to all categories of organisms (BHAGAT et al., 2016).

Among the heavy metals, cadmium presents a high stability in natural environments, being accumulated in soil and living organisms (JANKIEWICZ et al., 2000), has been responsible for a number of deaths (KEY et al., 1977).

Cadmium is used in various industrial processes (involved in producing plastic stabilizers, pigments for glass manufacturing, in electroplating processes, etc.), but the most important source of cadmium is reported to accumulate from cigarette smoke (JARUP, 2003, DONOVAN et al., 2016).

MATERIAL AND METHODS

Three bacterial strains were included for testing in this study-two isolated from soil contaminated with heavy metals, inclusively with cadmium loadings (*Chm*$_1$, *Chm*$_4$) from the area Copșa Mică and one from our laboratory collection belonging to *Shewanella* spp.

In order to determine their limits of tolerance to $CdCl_2$, the studied strains were grown on Luria-Bertani medium (LB), enriched with $CdCl_2$ in various concentrations (250; 500; 750; 1,000; 1,500; 2,000; 2,500 and 3,000 mg/l), at 28^0C, and shacked at 150 rpm, for seven days. The control was represented by bacterial culture in fluid medium without cadmium chloride. They were read at the spectrophotometer at 660 nm at intervals of 24 hours.

Their Gram affiliation (LAZĂR et al., 2004) and taxonomic identifications were further performed. We used the Biolog Sistem (with CN2 microplates for Gram-negative bacteria).

RESULTS AND DISCUTIONS

All the three bacterial streams taken into the study (*Chm1* and *Chm4* isolated from the soils polluted by heavy metals and *Shewanella* spp. from our laboratory collection), were described as Gram negative, with a bacillary shape (Fig. 1), but not identified at a species level within the performed tests.

We tried to demonstrate the tolerance of the three studied strains to cadmium, using cadmium chloride, because it is soluble and nontoxic (SIRIPORNADULSIL & SIRIPORNADULSIL, 2013).

In order to test their cadmium tolerance, the strains were grown in LB medium, gradually supplemented with cadmium chloride of different concentrations (Table 1). We found that the *Chm1* bacterial strain was able to tolerate the highest concentrations of the pollutant in its environment, up to 3,000 mg/l.

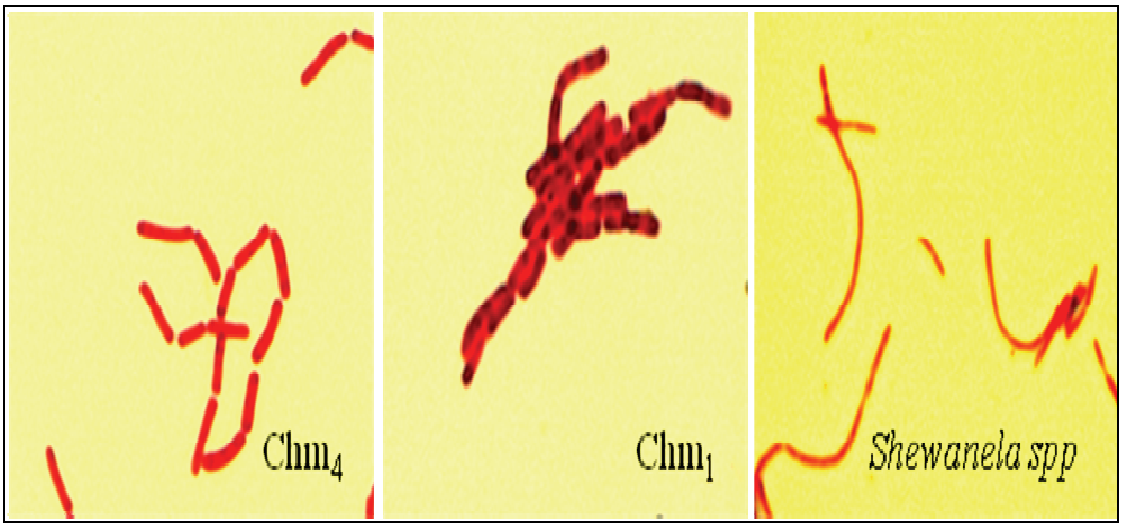

Figure 1. The aspect of the bacterial strains at the optical microscope (X 1000).

Table 1. The tolerance of *Chm1* bacterial strain to cadmium chloride.

$CdCl_2$ (mg/l)	**Optical Density (OD)**						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
250	0.763	0.931	1.028	1.073	1.05	1.014	1.119
500	0.732	0.898	0.982	1.018	1.153	1.194	1.316
750	0.739	0.978	1.1	1.166	1.175	1.273	1.427
1000	0.17	0.823	1.006	1.145	1.116	1.214	1.327
1500	0.015	0.708	0.947	1.095	1.009	1.249	1.368
2000	0.732	1.101	1.244	1.401	1.501	1.6	1.582
2500	0.673	0.988	1.194	1.341	1.451	1.512	1.556
3000	0.783	1.046	1.192	1.311	1.438	1.456	1.5
Control	0.752	1	1.165	1.254	1.329	1.392	1.537

It was also easy to observe the fact that *Chm1* developed quite similar in the medium with different concentrations of cadmium chloride and in the control one (Fig. 2). We could conclude that this bacterial strain owns the necessary mechanisms for adaptation to a toxic environment.

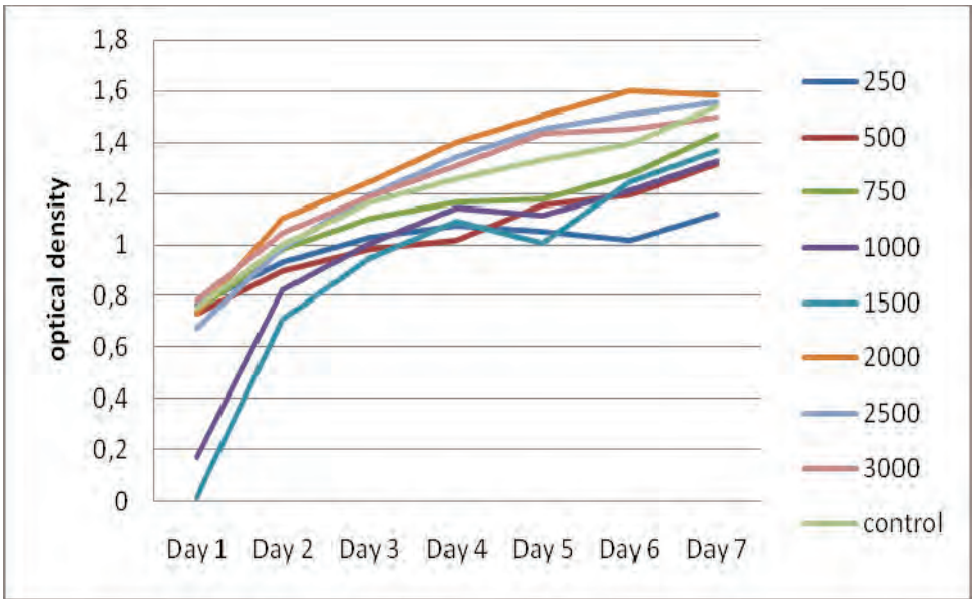

Figure 2. Development of *Chm_1* bacterial strain to different concentrations (mg/l) of $CdCl_2$.

Chm_4 bacterial strain, tested in the presence of the CdCl2 (Table 2), did not present any growth on the medium. These findings make us conclude that *Chm_4*, despite its similar provenance with *Chm_1* from a site polluted with cadmium, did not develop the necessary adaptive mechanisms for growing in the presence of this pollutant or lost these enzymatic adaptations during its maintenance in the laboratory conditions.

Table 2. The tolerance of the *Chm4* strain to cadmium chloride.

$CdCl_2$ (mg/l)	**Optical Density (OD)**						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
250	0.006	0.008	0.008	0.008	0.008	0.008	0.008
500	0.008	0.008	0.006	0.006	0.006	0.006	0.006
750	0.008	0.008	0.006	0.006	0.006	0.006	0.006
1,000	0.007	0.006	0.007	0.008	0.008	0.008	0.008
1,500	0.008	0.005	0.004	0.007	0.007	0.007	0.007
Control	0.753	0.942	1.071	1.073	1.173	1.301	1.373

Regarding the *Shewnella* spp. patterns of development in a medium polluted with cadmium, it was found that a concentration of 1,000 mg/l of CdCl2 became no longer proper for this bacteria growth (Table 3, Fig. 3).

Table 3. The tolerance of the *Schwanella* spp. strain to cadmium chloride.

$CdCl_2$ (mg/l)	**Optical Density (OD)**						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
250	0.039	0.374	0.734	0.753	0.661	0.841	1.038
500	0.016	0.369	0.624	0.546	0.49	0.657	0.749
750	0.023	0.47	0.701	0.645	0.703	0.824	0.945
1,000	0.013	0.012	0.015	0.009	0.007	0.009	0.012
1,500	0.016	0.018	0.02	0.003	0.007	0.007	0.015
Control	0.432	0.736	0.694	0.801	0.849	0.938	1.147

Shewnella spp. developed as well comparable on the control medium and on the LB enriched with $CdCl_2$ of 250, 500 and 750 mg/l (Fig. 3), similar with the observations of *Chm1* behaviour of growing in these conditions.

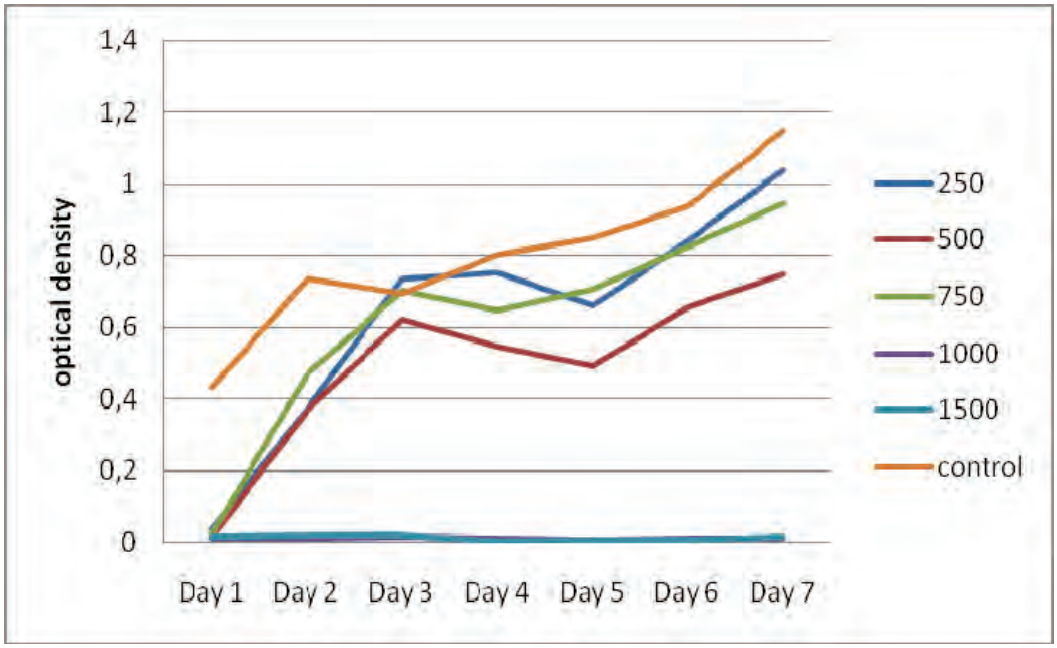

Figure 3. Development of *Shewnella* spp bacterial strain to different concentrations (mg/l) of $CdCl_2$.

Therefore, we can say that the bacterial strains *Shewnella* spp and *Chm1* have given positive results in testing cadmium chloride, so we could consider them cadmium tolerant.

The three studied bacteria strains have been identified by using Biologic Systems. Because the bacterial strains were Gram-negative, it was used Biolog Sistem CN2 microplates for Gram negative bacteria. The results based on this biochemical tests confirmed the gender strain *Shewnella*, but the strains Chm_1 and Chm_4 were not identified.

In future research, we intend to characterize and identify these strains.

CONCLUSIONS

Evaluating the results of the present study, we proved the ability of two bacterial strains (*Chm1* strain, isolated from a polluted site and *Shewnella* spp. from the laboratory collection) to grow in the presence of the cadmium chloride.

Moreover, we succeeded in selecting a bacterial strain able to tolerate well high concentrations of cadmium chloride (*Chm1*, which grown on medium with CdCl2 up to 3000 mg/l), in a similar way with its development on the control medium.

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