ARCHITECTURE CIVIL ENGINEERING ENVIRONMENT
The Silesian University of Technology No. 4/2015

RESISTANCE TO MERCURY EPIPHYTIC BACTERIA *LEMNA MINOR*

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Received 30.08.2015, Revised 28.10.2015, Accepted 30.10.2015

Abstract
The developing of new alternative methods of purifying the environment of mercury is extremely important in terms of growing restrictions of legal norms issued by the European Union. One of such methods is phytoremediation.
The obtained results of the application *Salvinia natans* to remediate groundwater contaminated with mercury and the stimulation of this process through the active strains of epiphytic has interest for analogous effects phytoremediation by *Lemna minor*. Plants cavitation in physiological solution, corns on solid ground of morphological part of plants, seed stocks of homogenised tissue were used to guarantee the effectiveness of the isolation of epiphytic bacteria

Streszczenie
Poszukiwanie nowych alternatywnych metod oczyszczania środowiska z rtęci jest niezwykle ważne w aspekcie wzrostu restrykcji norm prawnych wydanych przez Unię Europejską. Jedną z takich metod jest fitoremediacja.
Uzyskane rokujące wyniki badań zastosowania *Salvinia natans* do remediacji wód skażonych rtęcią oraz stymulacja tego procesu przez aktywne szczepy epifityczne skierowały zainteresowania na poszukiwanie analogicznych efektów fitoremediacje przez *Lemna minor*. Dla zapewnienia skuteczności izolacji bakterii epifitycznych zastosowano kawitację roślin w roztworze fizjologicznym, odciski na podłoże stałe części morfologicznej roślin oraz posiewy zhomogenizowanych tkanek.

Keywords: **Contaminated water; Identification of bacteria; Isolation of bacteria; Phytoremediation.**

1. INTRODUCTION

Compounds of mercury entering the different elements of the environment: water, soil and air, are cumulative. The migration of mercury and its compounds between different elements of the environment is possible and can pose a potential threat to living organisms [1].

Mercury is a highly toxic element, however, its harmfulness is apparently and primarily depends on the form. It could be in natural minerals in volatile (Hg0), or in the form of ions in the aquatic environment and soil. Mercury could be transmitted in other forms and change its toxicological properties [2].

There are two basic sources of mercury in the environment. First is the natural emission of volcanic explosions and second source is associated with human activities. Anthropogenic origin of burning of municipal waste and fossil fuel production, metallurgy, industrial processes, which use mercury as well as lighting equipment and batteries [3,4].

Despite the limitations associated with significant technological changes in the industry, waste disposal,

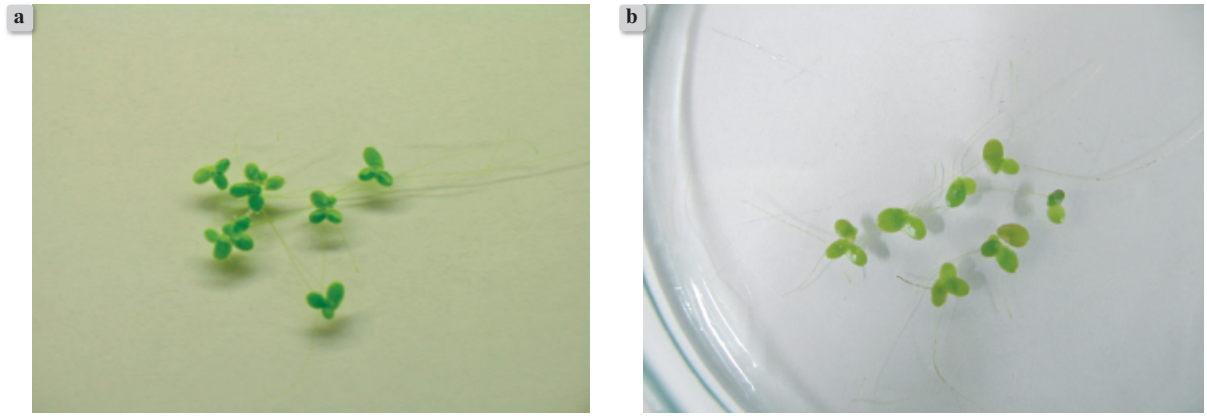

Figure 1.
Morphology of plants depending on the origin a) breeding *Lemna minor* b) *Lemna minor* taken from from the environment

as well as the purification of exhaust gases and an increase in restrictions on legal norms issued by the European Union- mercury is commonly present in the environment and its components and trophic chains [5,6,7].

Therefore, the search for new alternative purification methods of the environment with mercury is highly relevant. One of these methods is phytoremediation. In the report (Report: Evaluation of the phytoremediation efficiency to contaminated groundwater by mercury (II) by pleustofits from Lower Silesia) uses both water plants *Salvinia natans* – hyperaccumulator of mercury as well as *Lemna minor*. However, in order to intensify phytoremediation it was made the isolation of epiphytic bacteria resistant to high concentrations of mercury and supporting the life processes of plants only for Salvinia natans. The purpose of this work was to isolate epiphytes with *Lemna minor.*

2. MATERIALS AND METHODS

Samples for testing and isolation were collected from the environment of the Olawa river in Wroclaw at the end of September (end of the growing season – Fig. 1). It was made using sterile tweezers into bottles of 0.1 dm^3 each, in which there were 90 ml of 0.85% physiological solution. In turn, the plants were subjected to the action of ultrasound for 15 minutes and cultured on selective medium for Pseudomonas bacilli (Pseudomonas CFC agar Pseudomonas CHROMagar) to determine the membership of epiphitic of *Lemna minor* to a family of microorganisms.

To isolate all microorganisms resistant to mercury suspended in physiological solution they were seeded at the same time on plate with ground mineral minimum with 0.5% glucose concentrations of 0.15 $mgHg \cdot dm^{-3}$, 0.30 $mgHg \cdot dm^{-3}$ and 0.60 $mgHg \cdot dm^{-3}$ and identification were in search of the bacteria of the genus Pseudomonas. In addition, it also made prints of roots and leaves of the plant on culture media as well as the mineral medium with mercury.

Plate with selectively-differentiating ground was incubated at the optimum for the kind of Pseudomonas (approx. 26° C) for 24 hours. Growth on selective medium Pseudomonas CFC and blue colony on CHROMagar medium point to possible membership of the micro-organism to the family Pseudomonadaceae.

To determine the susceptibility of microorganisms to mercury (II) nitrate, colonies that formed in this way, were given on the plate with mercury concentrations of 0.15 $mgHg \cdot dm^{-3}$, 0.30 $mgHg \cdot dm^{-3}$ and 0.60 $mgHg \cdot dm^{-3}$, and then they were incubated in 37°C for 72 hours. The reading was made at a frequency of 24 hours. At the same time the colonies growing on medium with mercury were plated on the selective medium Pseudomonas CFC and CHROM agar Pseudomonas.

3. RESULTS

Salvinia natans and *Lemna minor* have formed ecological association, assuming that their epiphytic microflora might be similar. In addition, the *Lemna minor* usage of water in the wastewater treatment has led to research its epiphytic flora in the direction of resistance to the highest concentration of mercury 0.60 $mgHg \cdot dm^{-3}$.

Initially, the strains were isolated by a one-hour agitation in 0.85% NaCl, sonication, and homogeniza-

tion of tissue stamping) on mineral medium with 0.5% glucose as the carbon source at a concentration of 0.30 mgHg·dm^{-3}. Eight strains obtained in this way were stained with Gram method. Five of them belonged to gram-negative bacilli, while the other Gram positive bacilli (Table 1).

Table 1.
Eight strains after isolation

Strain name	Methods of isolation	G(+)/ G(-)	Strain name	Methods of isolation	G(+)/ G(-)
1L	Shaking in NaCl for 1 hour	G(-)	**5L**	ultrasounds	G(-)
2L	Shaking in NaCl for 1 hour	G(-)	**6L**	ultrasounds	G(-)
3L	Shaking in NaCl for 1 hour	G(-)	**7L**	ultrasounds	G(+)
4L	Shaking in NaCl for 1 hour	G(+)	**8L**	ultrasounds	G(+)

For Gram-negative bacteria further analysis was carried out concerning belonging to the genus Pseudomonas and the test of resistance to the highest tested concentration -0.6 mgHg·dm^{-3} using gradient tiles and plate count. The tested microorganisms, except one -6L was characterized by growth in both media, the colonies on chromogenic media stained blue, which proves their belonging to *Pseudomonas spp*. (Fig. 2).

Evaluation of resistance and isolation could distinguish two strains -L1 and L3- which were insensitive to the concentration of mercury 0.60 mgHg·dm^{-3}. 1L strain had similar morphology colonies obtained from strain 2-26 *Salvinia natans* resistant to high concentrations of mercury and pertains to the species *Pseudomonas protegens* protecting plants against pathogens, DNA sequencing was performed in Macrogen laboratory in Netherlands.

As a result of the isolation as well as the evaluation of the resistance to high concentrations of mercury *Lemna minor* epiphytes was extracted strain invulnerable to 0.60 mgHg·dm^{-3}. Its taxonomic affiliation to the family Pseudomonaceae has been confirmed using the ground with chrome and dedicated base for Pseudomonas, such as King A and B and Pseudomonas CFC. Sequence analysis of the region of 16S ribosomal RNA using NCBI database qualified for the strain of *Pseudomonas rhizosphaerae* KF 147111.1 with 100% probability. The identification made on the basis of morphological and biochemical and DNA sequencing has shown that the affiliation to epiphytic strain of *Pseudomonas rhisosphera*, characterized by a positive impact on the plants.

The results presented for *Lemna minor* may indicate that, despite the high biodiversity of bacterial microflora of aquatic plants, the largest mercury androgen insensitivity are bacteria of the genus Pseudomonas. Taxonomic affiliation results are presented in Table 2.

Table 2.
Taxonomic belonging to the family Pseudomonadaceae based on a variety of substrates

Lemna-1	Strain name	
sticks	Cell shape	
G(-)	Gram-negative or Gram-positive	
+	King A	Substrates
+/-	King A	
+	Pseudomonas CFC	
+ (blue)	CHROMagar Pseudomonas	

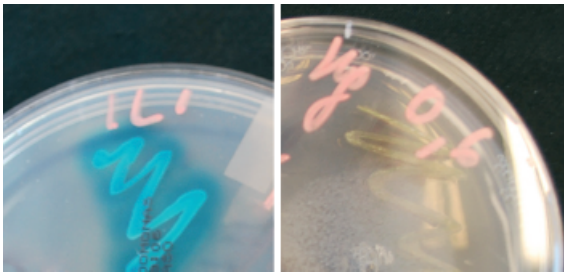

Figure 2.
The increase in the strain of *Lemna minor* chromogenic substrate and mineral substrate with a mercury concentration of 0.60 mgHg·dm^{-3}

Pseudomonas rhizosphaerae is a type of bacteria which was first isolated in Spain from roots area of grass, with the ability for phosphates solubilization [8]. For marine strains of this kind of proven ability to produce diketopiperasine and secondary metabolites with a high activity of antibacterial [9]. In addition, *Pseudomonas rhizosphaerae* belonging to this genus is epiphytic bacteria *Fragaria ananassa* (Strawberry) improving the transpiration of plants cuticular [10]. This demonstrates the positive effect and the action of protecting plants from pathogens.

4. CONCLUSIONS

- The growth of microorganisms with the blue colour of the individual colonies indicate taxonomic affiliation of microorganisms (recorded after incubation for 24 hours at 26° C).
- Simultaneous culturing microorganisms on selective medium and with the mercury increased the probability of the isolation of *Pseudomonas bacilli* resistant to high concentrations of mercury and eliminating any errors related to the physicochemical properties of the toxic metal.
- The largest mercury androgen insensitivity are bacteria of the genus Pseudomonas bacterial microflora among aquatic plants.

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