CHARACTERIZATION OF SERPENTINE BACTERIA: BIOFILM PRODUCTION AND HEAVY METAL TOLERANCE

Mujo Hasanović*[1], Tatjana Vojinović[2], Jovana Jotanović[2], Esma Fočak[2], Anesa Ahatović Hajro[1], Adaleta Durmić-Pašić[1]

[1]University of Sarajevo - Institute for Genetic Engineering and Biotechnology, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina
[2]University of Sarajevo, Faculty of Science, Zmaja od Bosne 33-35, Sarajevo, Bosnia and Herzegovina
*Corresponding author: mujo.hasanovic@ingeb.unsa.ba

INTRODUCTION

Serpentine soils exhibit unique chemical properties, characterized by high concentrations of heavy metals (usually Ni, Fe, Cr, and Co) and limited availability of micronutrients and unfavorable water regime, rendering them unfavorable for numerous microbial species. In response, serpentine bacteria have evolved various adaptive strategies that enable their undisturbed proliferation. Furthermore, serpentine habitats have diverse bacterial communities with potential use in industrial biotechnology and agriculture.

AIM OF THE STUDY

The primary objective of this study was to examine the heavy metal tolerance and quantify biofilm production of bacterial serpentine isolates.

MATERIALS AND METHODS

24 sequenced bacterial isolates

14 rhizospheric bacteria (Rb)

10 endophyitic bacteria (Eb)

associated with *Robinia pseudoacacia* L.

Determination of heavy-metal tolerance

Tryptone yeast Agar

Tryptone yeast agar plates were supplemented with Ni, Cu and Co salts with final metal concentrations 100 mg/L and 200 mg/L.

Plates were incubated 15 days at room temperature.

Microtiter plate assay (MPA)

Isolates were incubated in Tryptic Soy Broth at 25 °C.

Incubation in microtiter plates for 24 h at 25°C

plate washing and fixing with absolute methanol

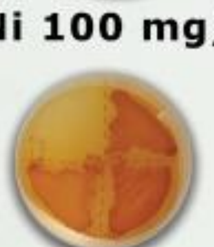
plate drying and dyeing with 4% crystal violet

Absorbance was measured at 570 nm using a Thermo Scientific MultiskanTM reader.

RESULTS

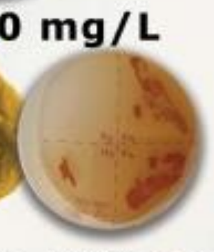
Heavy-metal tolerance


Ni 100 mg/L: Rb 14 (Rb1 - Rb14); Eb 3 (Eb2; Eb6; Eb8)


Ni 200 mg/L: Rb 13 (Rb1 -8; 10-14)


Cu 100 mg/L: Rb 13 (Rb1 -8; 10-14); Eb 4 (Eb1-4)


Cu 200 mg/L


Co 100 mg/L: Rb 13 (Rb1 -8; 10-14); Eb 4 (Eb1-4)

Rb - Rhizospheric bacteria   Eb - Endophytic bacteria

Biofilm production

moderate biofilm production: Rb5 - *Pseudomonas sp.*; Rb6 - *Pseudomonas sp.*

weak biofilm production: Rb1 - *Pseudomonas sp.*; Rb2 - *Pseudomonas sp.*; Rb3 - *Pseudomonas sp.*; Rb 4 - *Pseudomonas sp.*; Rb7 - *Pseudomonas sp.*; Rb8 - *Pseudomonas sp.*; Rb9- *Bacillus sp.*; Rb10 -*Pseudomonas sp.*; Rb11 - *Pseudomonas orientalis*; Rb12 - *Pseudomonas sp.*; Rb13 - *Pseudomonas sp.*; Rb14 - *Pseudomonas sp.*
Eb1 - *Bacillus* sp.; Eb2 - *Streptococcus* sp.

no biofilm production: Eb3 - *Bacillus sp.*; Eb4 - *Bacillus sp.*, Eb5 - *Bacillus proteolyticus*; Eb6 - *Bacillus proteolyticus*
Eb8 - *Brevibacterium/Peribacillus/Bacillus sp.*; Eb9 -*Lysinibacillus macroides*; Eb10 - *Bacillus mycoides*
Eb11 - *Bacillus sp.*

Rb - Rhizospheric bacteria   Eb - Endophytic bacteria

CONCLUSION

Rhizospheric *Pseudomonas* isolates exhibited strong heavy metal tolerance and prominent biofilm producing abilities as opposed to limited tolerance and weak biofilm production by endophytic *Bacillus, Brevibacillus* and *Lysinibacillus*. Bacteria produce biofilms as a survival strategy in response to environmental stressors. Biofilm production requires energy expenditure and endophytes, having procured nutrients and protection by plants, forego this ability. It is an important input when selecting isolates for biotechnological applications.

ACKNOWLEDGEMENT

This research was supported by Ministry of Science, Higher Education and Youth, Canton Sarajevo (grant No:27-02-35-37080-7/23).