



# **SIDEROPHORE PRODUCTION BY BACTERIA ISOLATED FROM *ROBINIA PSEUDOACACIA* L. ROOTS AND SERPENTINE SOIL**

**MUJO HASANOVIĆ<sup>1</sup>, ANESA AHATOVIĆ<sup>1</sup>, EMIR HRELJA<sup>2</sup>, ADALETA DURMIĆ-PAŠIĆ<sup>1</sup>**

<sup>1</sup>University of Sarajevo, Institute for Genetic engineering and biotechnology, Sarajevo, Bosnia and Herzegovina

<sup>2</sup>University of Sarajevo, Faculty of Science, Sarajevo, Bosnia and Herzegovina



# SIDEROPHORE PRODUCTION BY BACTERIA ISOLATED FROM *ROBINIA PSEUDOACACIA* L. ROOTS AND SERPENTINE SOIL

## AIM OF THE STUDY

Since siderophores can have a crucial role for plants in situations of low iron availability or nutrient deficiency, the main goal of this study was to isolate soil bacteria and assess their ability to produce siderophores.

Bacterial isolates were collected both from roots (*Robinia pseudoacacia* L.) and soil samples (Donja Paklenica, Bosnia and Herzegovina) (Fig 1, Fig 2).



Figure 1. *R. pseudoacacia* roots



Figure 2. Serpentine soil

## MATERIALS AND METHODS

Siderophore production was tested using qualitative (CAS Agar assay) and quantitative (spectrophotometric) methods (Fig 5, Fig 6).

Bacterial colonies from collected samples were isolated on yeast mannitol agar (YMA) and subsequently tested for the heavy metal tolerance on Trypton Yeast Agar (TYA) supplemented with Cu, Ni, Co in different concentrations (Fig 3, Fig 4).



Figure 5. CAS Agar



Figure 6. Multiskan GO Microplate Spectrophotometer



Figure 4. Trypton Yeast Agar (TYA) + Cu, Ni, Co

Figure 3. Yeast Mantol Agar (YMA)



# SIDEROPHORE PRODUCTION BY BACTERIA ISOLATED FROM *ROBINIA PSEUDOACACIA* L. ROOTS AND SERPENTINE SOIL

## RESULTS

The results of spectrophotometric method showed that all 26 selected metal tolerant isolates produced siderophores in a range from 10.96% to 96.66% siderophore units (SU) (Tab 1) (Fig 6.). After seven days of cultivation on CAS Agar, siderophore producing isolates exhibited an orange halo ranging from 3 to 20mm in diameter (Tab 2) (Fig 7.). One root isolate did not produce siderophores on CAS Agar but showed 32.86% SU of production performing the quantitative method.

## CONCLUSIONS

In comparison to root isolates, isolates collected from the serpentine soil showed more intensive siderophore production. The forthcoming study will include screening of other PGP strains and 16S rRNA sequencing for bacterial identification.

Table 1. CAS Agar qualitative method

Reference (CAS reagent)	Bacterial strain	Average absorbance	% siderophore units (SU)
2.547	IX <sub>1</sub>	0.084	96.702%
	IX <sub>3</sub>	0.097	96.192%
	IX <sub>4</sub>	1.223	51.983%
	IX <sub>5</sub>	0.872	65.764%
	IX <sub>6</sub>	0.213	91.637%
	IX <sub>7</sub>	1.084	57.440%
	IX <sub>8</sub>	0.097	96.192%
	IX <sub>9</sub>	0.097	96.192%
	IX <sub>10</sub>	0.744	70.789%
	IX <sub>14</sub>	1.026	59.717%
	IX <sub>15</sub>	0.869	65.881%
	IX <sub>16</sub>	1.168	54.142%
	IX <sub>17</sub>	1.335	47.585%
	IX <sub>18</sub>	0.904	64.507%
	X <sub>2</sub>	0.892	64.978%
	X <sub>4</sub>	0.670	73.695%
	X <sub>5</sub>	1.139	55.281%
	X <sub>6</sub>	1.128	55.713%
	X <sub>7</sub>	2.270	10.876%
	X <sub>8</sub>	2.223	12.721%
	X <sub>10</sub>	1.571	38.320%
	X <sub>12</sub>	1.444	43.306%
	X <sub>13</sub>	1.725	32.273%
	X <sub>14</sub>	1.872	26.502%
	X <sub>15</sub>	2.255	11.464%
	X <sub>16</sub>	2.275	10.679%

IX - serpentine soil isolates; X- *R.pseudoacacia* isolates

Table 2. Spectrophotometric quantitative method

Bacterial strain	Incubation period	24h	48h	72h	7d
IX <sub>1</sub>	3 mm	6 mm	7.5 mm	9 mm	
IX <sub>3</sub>	3 mm	7 mm	10 mm	12 mm	
IX <sub>4</sub>	2.5 mm	5 mm	6.5 mm	14 mm	
IX <sub>5</sub>	0 mm	3 mm	7 mm	18 mm	
IX <sub>6</sub>	3 mm	7.5 mm	9.5 mm	14 mm	
IX <sub>7</sub>	0 mm	3 mm	6 mm	19 mm	
IX <sub>8</sub>	3 mm	6.5 mm	8.5 mm	13 mm	
IX <sub>9</sub>	2 mm	5 mm	6.5 mm	20 mm	
IX <sub>10</sub>	0 mm	2.5 mm	7 mm	11 mm	
IX <sub>14</sub>	4 mm	7 mm	7.5 mm	8 mm	
IX <sub>15</sub>	5 mm	6 mm	7.5 mm	10 mm	
IX <sub>16</sub>	4 mm	5.5 mm	7 mm	12 mm	
IX <sub>17</sub>	4 mm	5.5 mm	6.5 mm	10 mm	
IX <sub>18</sub>	6 mm	8 mm	12 mm	16 mm	
X <sub>2</sub>	3 mm	4.5 mm	6.5 mm	11 mm	
X <sub>4</sub>	3 mm	5 mm	6 mm	10 mm	
X <sub>5</sub>	0 mm	4 mm	5 mm	8 mm	
X <sub>6</sub>	0 mm	4 mm	5 mm	7 mm	
X <sub>7</sub>	0 mm	1 mm	2 mm	5 mm	
X <sub>8</sub>	0 mm	1 mm	2 mm	4 mm	
X <sub>10</sub>	0 mm	1 mm	3.5 mm	6 mm	
X <sub>12</sub>	0 mm	1 mm	2 mm	4 mm	
X <sub>13</sub>	0 mm	0 mm	0 mm	0 mm	
X <sub>14</sub>	0 mm	1 mm	1 mm	4 mm	
X <sub>15</sub>	0 mm	1 mm	1 mm	4 mm	
X <sub>16</sub>	0 mm	1 mm	1 mm	3 mm	



Figure 8. Spectrophotometric method, cuvettes with CAS reagent and samples of isolates

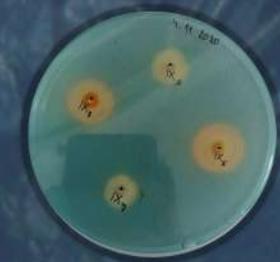


Figure 7. Isolates produced orange haze on CAS AGAR