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Inoculum of authoctonous biosurfactant-producing bacteria in pesticide contaminated Agri Biobed: evaluation of pesticide abatement and microbial community structure

Andreolli Marco[1,2*], Righetti Davide[1], Ferrarese Alberto[3], Zerminiani Andrea[3], Lampis Silvia[1,2]

[1]*VUCC-DBT Verona University Culture Collection, Department of Biotechnology, University of Verona, Strada le Grazie, 15, Verona 37134, Italy*

[2]*Department of Biotechnology, University of Verona, Strada le Grazie, 15, Verona 37134, Italy*

[3]*Bio Soil Expert srl, Piazza Vicenza 8, Trento 38122, Italy*

*Email: marco.andreolli@univr.it

Introduction and Aims

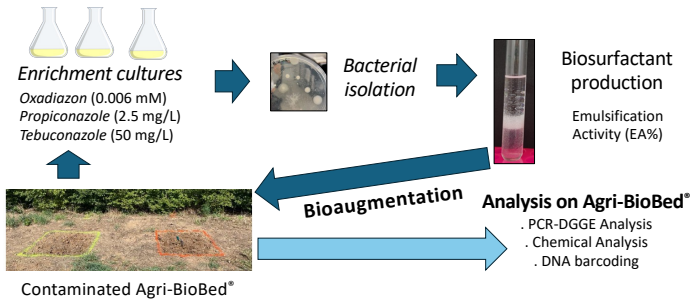
Pesticides, widely used in agriculture, harm the environment and health. Microbial ***biosurfactants*** can enhance their degradation. ***Agri-Biobeds***® treat accidental spills during equipment filling and washing.

Therefore, the ***aims*** were:

(i) isolate and characterize bacterial strains able to produce biosurfactans from a high polluted *Agri-Biobeds*®.

(ii) evaluate the effects in terms of either pesticide abatement or microbial community structure after a bioaugmentation treatment by using these biosurfactant- producing bacteria as inocula.

Research Workflow



Results

Isolation and identification of bacteria from enrichment cultures

Carbon source	Strain	Closest bacterial type strain	Accession Number	% of identity
Oxadiazon	**1Tox**	*Cupriavidus necator* N-1^T	CP002878	99.62
	2Tox	*Cupriavidus necator* N-1^T	CP002878	99.4
	3Tox (4Tox)	*Rhodococcus ruber* DSM 43338^T	LRRL01000064	99.78
	1Nox (2Nox)	*Cupriavidus necator* N-1^T	CP002878	99.63
Tebuconazole	**1Tteb**	*Micrococcus luteus* NCTC 2665^T	CP001628	99.62
	2Tteb	*Bosea robiniae* DSM 26672^T	Jgi.1085745	99.5
	3Tteb	*Rhodanobacter rhizosphaerae* CR164^T	FJ772032	99.34
	4Tteb	*Rhodanobacter ginsenosidimutans* Gsoil 3054^T	EU332826	98.59
	5Tteb	*Sphingopyxis taejonensis* JSS54^T	AF131297	98.76
	6Tteb	*Sphingopyxis taejonensis* JSS54^T	AF131297	98.8
	1Nteb	*Priestia aryabhattai* B8W22^T	EF114313	100
	5Nteb	*Bosea thiooxidans* DSM 9653^T	Jgi.1048902	99.33
	8Nteb	*Paenibacillus cineris* LMG 18439^T	AJ575658	99.62
Propiconazole	**1Tpro** (2Tpro)	*Diaphorobacter nitroreducens* NA10B^T	AB064317	99.9
	3Tpro (7Tpro)	*Brucella rhizosphaerae* PR17^T	NNRK01000031	99.05
	4Tpro	*Pseudomonas delhiensis* RLD-1^T	Jgi.1118306	99.39
	5Tpro	*Diaphorobacter nitroreducens* NA10B^T	AB064317	99.8
	6Tpro	*Diaphorobacter nitroreducens* NA10B^T	AB064317	99.8
	8Tpro	*Sphingomonas laterariae* LNB2^T	Jgi.1118286	98.71
	1Npro	*Diaphorobacter nitroreducens* NA10B^T	AB064317	99.9
	3Npro	*Brucella pituitosa* CCUG 50899^T	AM490609	99.38

Tab 1: Analysis of 16S rRNA genes obtained from each representative bacterial strain (in bolt) isolated from enrichment cultures using oxadiazon, tebuconazole or propiconazole as sole source of carbon and energy. The names of isolates belonging to each genotypic group are also shown.

Biosurfactant production

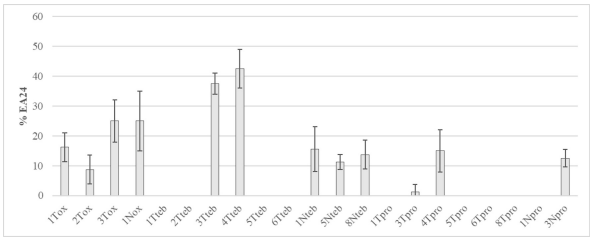


Fig. 1: Biosurfactant production evaluated by emulsification activity (% EA24)

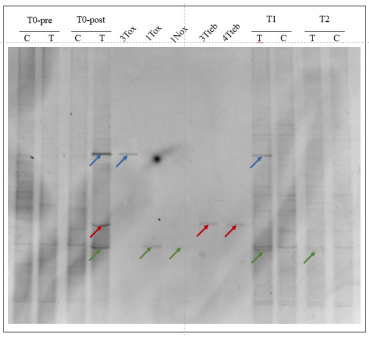
Chemical analysis

	T2 (83 days of treatment)		
	Treated	Control	Reduction
	mg/kg	mg/kg	%
Boscalid	< 0.01	0.060 ± 0.030	> 83.3
Chlorantraniliprole (DPX E-2Y45)	0.058 ± 0.029	2.5 ± 1.2	97.7
Difenoconazole	0.075 ± 0.038	0.51 ± 0.25	85.3
Flazasulfuron	< 0.01	0.01 ± 0.005	0
Fludioxonil	0.044 ± 0.022	0.39 ± 0.2	88.7
Flutolanil	0.029 ± 0.015	1.2 ± 0.6	97.6
Fluxapyroxad	0.23 ± 0.11	5.2 ± 2.6	95.6
Imidacloprid	< 0.01	0.22 ± 0.11	> 95.4
Oxadiazon	0.18 ± 0.09	1.3 ± 0.6	86.1
Pendimethalin	0.3 ± 0.15	3.8 ± 1.9	92.1
Propiconazole (sum of isomers)	0.036 ± 0.018	0.35 ± 0.18	89.7
Pyraclostrobin	0.14 ± 0.07	1.1 ± 0.6	87.3
Tebuconazole	0.022 ± 0.011	0.21 ± 0.1	89.5
	Total reduction		**93.1**

Tab. 2: Concentration of pesticides 83 days after the bioaugmentation treatment (T2). Results are expressed as the mean and standard deviation.

Bioaugmentation

Based on the results of biosurfactant production, four strains were chosen as inoculum in the bioaugmantation protocol: ***Rhodanobacter* sp. 3Tteb** and **4Tteb**, ***Cupriavidus* sp. 1Nox** and **1Tox** and ***Rhodococcus* sp. 3Tox**.



PCR - DGGE

Fig. 2: DGGE analysis from (T) treated (inoculated with biosurfactant-producing bacteria) and (C) particles at the beginning of the experiment either (T0-pre) before or after (T0-post) the inoculum and after 38 (T1) and 83 days (T2) of inoculum. The blue, red and green arrows indicate the bads related to *Rhodococcus* sp. 3Tox, *Rhodanobacter* sp. 3Tteb/4Tteb and *Cupriavidus* sp. 1Tox/1Nox respectively.

DNA metabarcoding

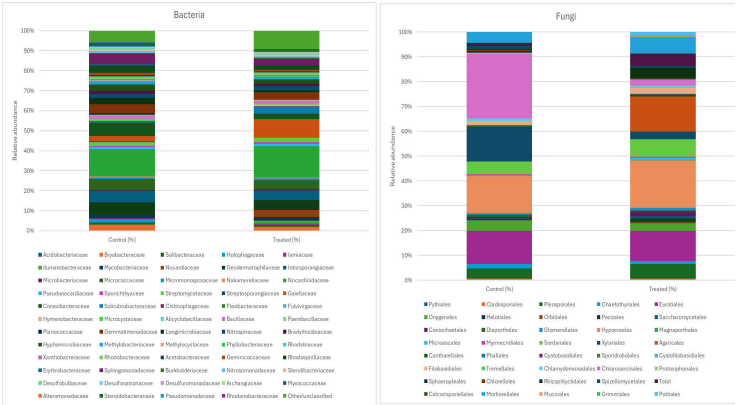


Fig 3: Bacterial (family level) and fungal (order level) composition at phylum in control and treated (inoculated with biosurfactant-producing bacteria) particles after 83 days of treatment

Conclusions

This work highlighted the importance of the biosurfactant-producing bacteria in pesticides degradation in soil. Moreover, the results suggested that the higher pesticides bioavailability shifted the microbial composition toward potential degrading population.