

Short Term Scientific Mission (STSM) 2014

Utilization of wastewater for the production of Extracellular Polysaccharides (EPS) by bacteria

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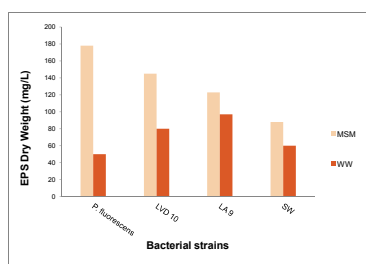
Objectives

1. Detection and selection of bacterial strains that produce EPS.
2. Tolerant strains that grow in wastewater and in addition to be able to produce EPS.
3. Extraction and quantitative measurements of EPS.
4. Quantitative analysis of protein and carbohydrate content of EPS

Methodology

1. A pre-screening qualitative method for the selection of potential EPS producers was carried out according to Subramanian *et al.* 2010, where strikes of the pure strains were carried out onto Plate Count Agar (PCA) and Sabouraud's dextrose agar (SDA) media. All seven strains were potential producers of EPS according to that method, although only four showed high production. The selected strains were *Enterobacter ludwigii* LA9, *Pseudomonas aeruginosa* LVD-10 and *Lelliottia amnigena* SW. The bacterial strains *Rhodococcus ruber* and *Pseudomonas fluorescens* were used for positive control.
2. Examination of EPS production in liquid cultures that were contained 25 g/L glucose and examination of EPS production in wastewater.
3. EPS extraction according to Bezawada *et al.*, 2013 and dry weight measurements.
4. EPS characterisation of the protein and carbohydrate content, using the Lowry (Lowry *et al.* 1951), and Anthrone (Trevelyan *et al.* 1952), methods, respectively.
5. Carbon Oxygen Demand measurement of the wastewater before treatment with the bacterial strains and after.

Results



Bacterial Strains	MSM		WW					
	Protein Concentration (mg/L)	Protein content (mg BSA/g EPS)	Carbohydrates content (mg glucose/g EPS)	Carbohydrates content (mg glucose/g EPS)	Protein Concentration (mg/L)	Protein content (mg BSA/g EPS)	Carbohydrates content (mg glucose/g EPS)	Carbohydrates content (mg glucose/g EPS)
<i>Pseudomonas fluorescens</i>								
LB	5	37.5	200	848	5.7	93.4	3	22.5
TB	2	44.4	9	200	5.7	126.7	0	0
<i>Pseudomonas aeruginosa</i> LVD-10								
LB	6	47.62	230	987	6	47.6	5	39.7
TB	2	106.3	9	203.2	8	19	2	109
<i>Lelliottia amnigena</i> SW								
LB	3	36.73	140	714	4	49	0	0
TB	2	300	10	800	4	600	0	0
<i>Enterobacter ludwigii</i> LA 9								
LB	6	58.1	200	989	8	77.4	5	48.4
TB	1	50.0	9	450	5	250	0	0

1. EPS production in mineral salt (MSM) medium compared to wastewater (WW) in 72 h (on the left). The strain LA9 showed only slight reduction of EPS production in WW compared to MSM.
2. The protein and carbohydrate content of the LB- and TB-EPS by each bacterial strain (table on the right).
3. The COD of the wastewater reduced from 266 mg/L to zero.

Highlights

The main objectives were achieved successfully, since the tested bacterial strains showed to be EPS producers. The three strains (*Enterobacter ludwigii* LA 9, *Lelliottia amnigena* SW and *Pseudomonas aeruginosa* LVD-10) that were tested in wastewater were able to tolerate wastewater and achieved complete mineralization, since the COD was reduced from 266 mg/L to zero within 72 hours.

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