Improved rhamnolipid biosurfactant production by *Burkholderia thailandensis* E264 using agro-industrial wastes

Jéssica Correia, Eduardo J. Gudiña, Lígia R. Rodrigues, José A. Teixeira

CEB – Centre of Biological Engineering, University of Minho, Braga, Portugal

INTRODUCTION

Biosurfactants are amphiphilic surface-active compounds, produced by various microorganisms, that reduce surface and interfacial tension. These compounds are attracting increasing interest over their chemical counterparts due to their advantages (lower toxicity and higher biodegradability) and potential application in bioremediation, pharmaceuticals, cosmetics, agriculture, food and petroleum industries, among others. However, the high operational costs restrict their industrial-scale applications. Several attempts to reduce their production costs have been conducted and include the use of low-cost agro-industrial wastes and by-products as substrates[1]. Rhamnolipids (RL) are among the most widely studied biosurfactants; however, their main producer, *Pseudomonas aeruginosa*, is an opportunistic human pathogen, which can limit their application in several fields and makes necessary the study of alternative producers.

METHODS

B. thailandensis E264 was grown in standard (S) medium (glycerol 40 g/L, peptone 5 g/L, meat extract 3 g/L). Agro-industrial residues (corn steep liquor (CSL) and olive oil mill wastewater (OMW)) were obtained from local industries. RL production by *B. thailandensis* E264 was evaluated in flasks at 30 °C and 180 rpm, and their recovery was performed through adsorption chromatography (Amberlite® XAD®-2). Surface tension (ST) was measured using the Ring method (KRÜSS K20 Tensiometer). Emulsifying activity was determined against n-hexadecane. Critical micelle concentration (cmc) was calculated by measuring the ST of purified biosurfactant solutions at different concentrations. Substrates consumption was evaluated by high-performance liquid chromatography (HPLC) using an Aminex HPX-87H column. Bacterial growth was determined by the plate count technique and expressed as colony forming units (CFU) per mL.

RESULTS

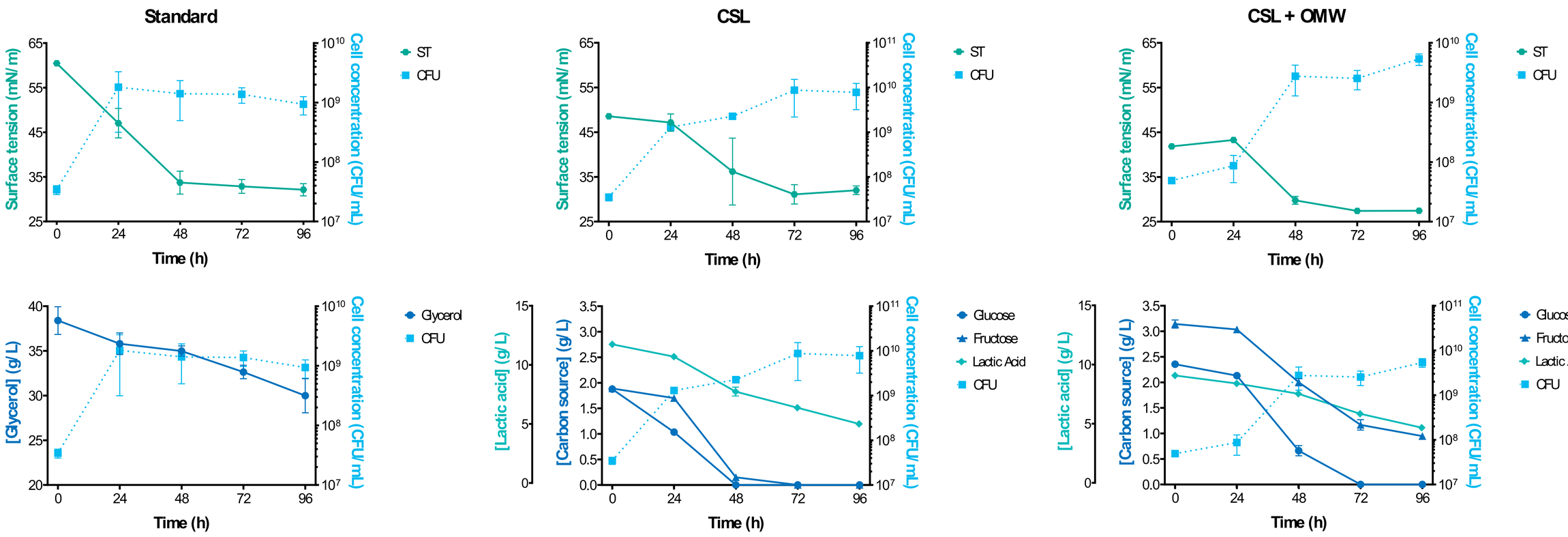

Figure 1. Top: surface tension values (mN/m) and bacterial growth (CFU/mL); Bottom: cabon source (g/L) and cell concentration (CFU/mL) over time of *Burkholderia thailandensis* E264 grown in standard, CSL and CSL + OMW media at 30 °C and 180 rpm.

RL production by *B. thailandensis* E264 was evaluated in several low-cost media containing as sole ingredients agro-industrial residues. CSL, the main residue of corn starch production, was evaluated as sole substrate for RL production at different concentrations. CSL is rich in proteins, amino acids and vitamins, and it also contains glucose and fructose. The best results were obtained at 7.5% (v/v), achieving ST values similar to those observed in the standard medium (31.1 mN/m) (Figure 1).

Subsequently, the effect of supplementing the CSL medium with different concentrations of OMW was studied. OMW is an environmentally hazardous residue generated during olive oil extraction and contains long-chain fatty acids that can act as inducers in RL production. The lowest ST value (27.4 mN/m) was obtained with the medium containing CSL (7.5% v/v) and OMW (10% v/v) as sole substrates (Figure 1).

HPLC analysis showed that in the standard medium, *B. thailandensis* E264 consumed 8.4 g/L of glycerol. In the CSL medium, glucose (1.9 g/L) and fructose (1.9 g/L) were fully consumed within the first 48 h of growth. In the CSL+OMW medium, which contains a higher concentration of both sugars (2.4 and 3.2 g/l, respectively), fructose was not completely exhausted. Furthermore, *B. thailandensis* E264 consumed between 4.9-6.8 g of lactic acid present in CSL and CSL+OMW media, that may be used as an additional carbon source when glucose is exhausted. The low-cost media also allowed a higher bacterial growth than the standard medium (Figure 1).

When grown in CSL medium, *B. thailandensis* E264 produced about 2.6 times the amount of RL produced in the standard medium. By supplementing the CSL medium with OMW, RL production was further increased, most likely due to the inductive effect of long-chain fatty acids present in OMW (mainly oleic, palmitic, linoleic and stearic acids) on RL production[2]. Furthermore, despite their lower emulsifying activity, RL produced in the low-cost medium (CSL+OMW) exhibited better surface-active properties when compared with those produced in the standard medium (Table 1).

Table 1. Rhamnolipid yield (g/L), lowest surface tension values (mN/m), critical micelle concentration (mg/L) and emulsifying indexes (%) obtained with *B. thailandensis* E264 in standard, CSL and CSL+OMW media at 30 °C and 180 rpm for 96 hours.

Medium	Rhamnolipids produced (g/L)	Surface tension (mN/m)	CMC (mg/L)	E_{24} (%)	E_{72} (%)
Standard	0.7	28.8 ± 0.2	460	63	56
CSL	1.8	28.2 ± 0.1	375	64	48
CSL + OMW	2.6	26.8 ± 0.1	280	56	40

CONCLUSIONS

A low-cost culture medium, containing as sole ingredients agro-industrial residues (CSL and OMW), was developed for RL production by *B. thailandensis* E264. This culture medium allowed the production of a higher amount of RL when compared with the synthetic standard medium, with better surface-active properties. Besides reducing the RL production costs, these results can allow the development of strategies for the valorisation of environmentally hazardous residues and promote the circular economy.

REFERENCES

1. Gudiña, E. J. et al. (2015). Bioresour. Technol. 177: 87–93
2. Gudiña, E. J. et al. (2016). Bioresour. Technol. 212: 144–15

ACKNOWLEDGEMENTS

This work was sponsored by PARTEX Oil and Gas and it was supported by the Portuguese Foundation for Science and Technology(FCT) under the scope of the strategic funding of UIDB/04469/2020 unit and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.