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SUSTAINABLE WINERIES THROUGH WASTE VALORISATION: NOVEL BIOSURFACTANTS (RHAMNOLIPIDS) FOR TREATING HYDROCARBON-CONTAMINATED SOILS

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CREIAMO-CIRCULARECONOMY The project CREIAMO is aimed at producing biosurfactants using agricultural wastes and, therefore, proving their potential applications for treating hydrocarbon-contaminated soils. In close collaboration with local agricultural stakeholders, our research activities attempt to apply the fundamental principles of the increasingly popular concept of "Circular economy" (CE) in the region of Lombardy, in Italy. The biosurfactant production strategy is based on identifying and physicochemical characterization of agricultural residues from wine and olive oil sectors suitable for microbial fermentation using selected non-pathogenic bacterial strains. Then, we are optimizing their capacities to grow on those hazardous agricultural wastes and to produce higher yields of these microbial surfactants before moving to a large-scale production phase. In addition, the biosurfactants' applications on hydrocarbon-contaminated soil will be carried out during further activities.

ABSTRACT

The first phase of the research establishes novel alternatives for winey wastes to produce safe biosurfactants with potential applications on contaminated soils. Given the nature of those agricultural wastes, non-fermented, fermented grape marc and grape lees were subjected to physic-chemical characterization for a better understanding of the designed microbial fermentation processes. In contrast to previous biosurfactant studies, several glycolipid-producing nonpathogenic strains were pre-evaluated for their abilities to produce microbial surfactants using the main components of winery wastes as carbon sources. After assessing numerous conditions, *Burkholderia thailandensis* E264 was found to be able to produce rhamnolipids, predominately Rha-Rha C14-C14, on grape marc wastes. It showed higher yields of rhamnolipids production, which are capable of reducing the surface tension to 33.2 mN/m with a low critical micelle concentration (CMC) and important emulsification capacities of various hydrophobic substrates (e.g., diesel oil).

RESULTS & DISCUSSION

A- Cell growth, surface tension reduction and the emulsifying capacity of strain E264 The cell growth (OD 600 nm), surface tension reduction (mN/m) and emulsification index (E24) (%) were monitored for the strain *Burkholderia thailandensis* E264 on MSM medium during 236 hours of incubation at 30 °C and 150 rpm (Fig. 3ABC). The strain E264 grew on several sugars including, glucose, fructose, galactose, arabinose, xylose as sole carbon, and energy source (Fig. 3A), while it was not able to grow on lactose. Interestingly, it displayed a high capacity to reduce the surface tension of cell-free supernatants to an average of 33-36 mN/m when growing on various sugars, including glucose, fructose, galactose, arabinose, xylose, and sucrose (Fig. 3B) ($p<0.01$). The emulsification index (E24) was found to be similar to that of chemical surfactants such as Tween 20 and Tween 80 (Fig. 3C). These results indicate that strain E264 is a good candidate for further fermentation attempts on the winery wastes with specific physicochemical properties.

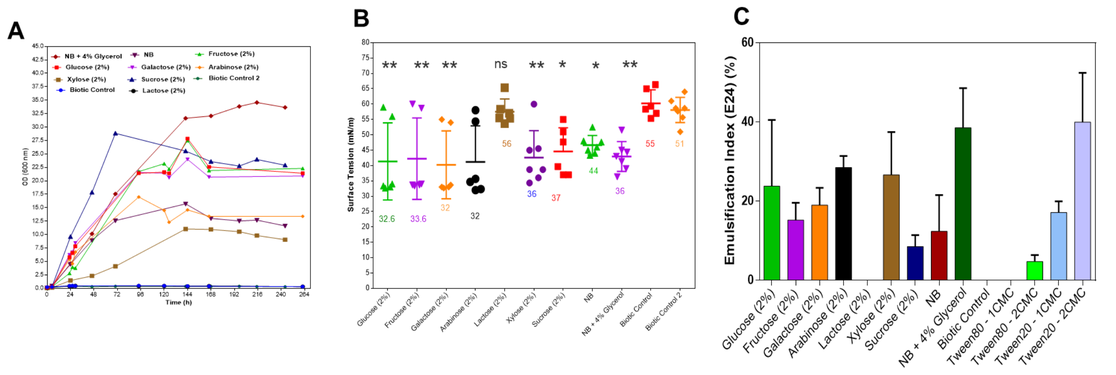
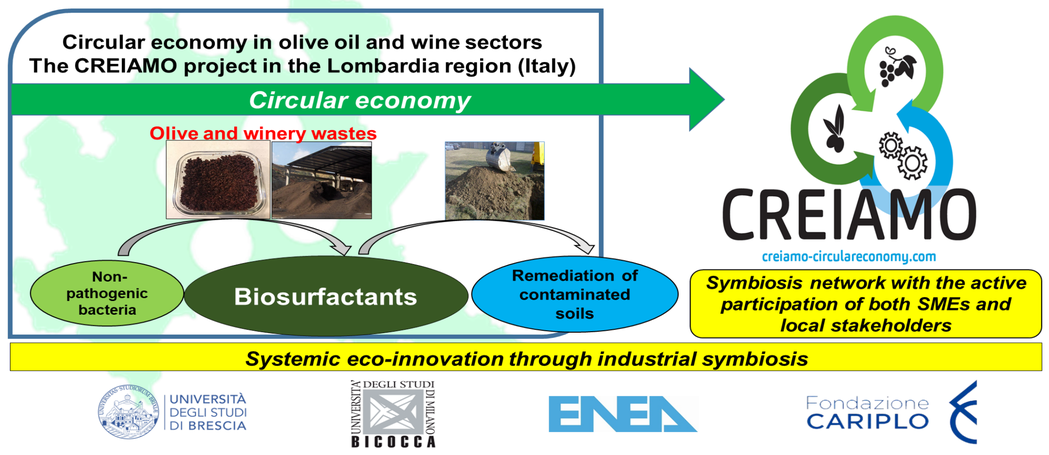


Fig. 3. Monitoring the cell growth (OD 600 nm) (A), surface tension reduction (mN/m) (B), and the emulsifying capacity (at 144 h of incubation) (%) (C) of the strain E264 after incubation during 263 h at 30 °C and 150 rpm. The values represent means ± SEM (Standard Error Mean). *p< 0.05, **(p< 0.01) are compared with biotic controls. ns: not significant. Each point in Figure 3B represents the means of a separate surface tension measurement during the incubation time.

TAKE-HOME MESSAGES

- Our results revealed that nonfermented grape marcs, derived from the white winemaking process, could be used as an economical substrates for the production of biosurfactants (e.g., long-chain rhamnolipids), which are in agreement with the concept of re-evaluation of agricultural wastes towards applying the CE principals.
- The nonpathogenic nature of the selected organism and the purity shown by the crude rhamnolipid suggested the possible use of these biomolecules directly in industrial practices or in soil remediation.
- The safe rhamnolipid from E264 has exhibited promising stabilities over a wide range of temperature, pH, salinities, similar to that produced by *Pseudomonas aeruginosa*.
- These biomolecules could be applied into bioremediation studies, which involve performance and stability characters e.g., treatment of contaminated soils. To replace the well-known *P. aeruginosa* as the rhamnolipid production using the strain E264 demonstrated interesting patterns and characteristics, which makes this strain as an excellent candidate for further studies on agricultural wastes as substrates.



B- Screening of a nonpathogenic rhamnolipids producer for further studies on winery wastes substrates The winery wastes, including fermented grape marc, nonfermented marc, and grape lees, are mainly characterized by high sugars contents and low pH. For these aspects, we performed a screening step using several bacterial nonpathogenic rhamnolipids' producers according to the industrial and commercial requirements (i.e., health and safety aspects) (Fig 2). The chemical compositions of these raw materials are currently being determined in our lab for better piloting the fermentation experiments.

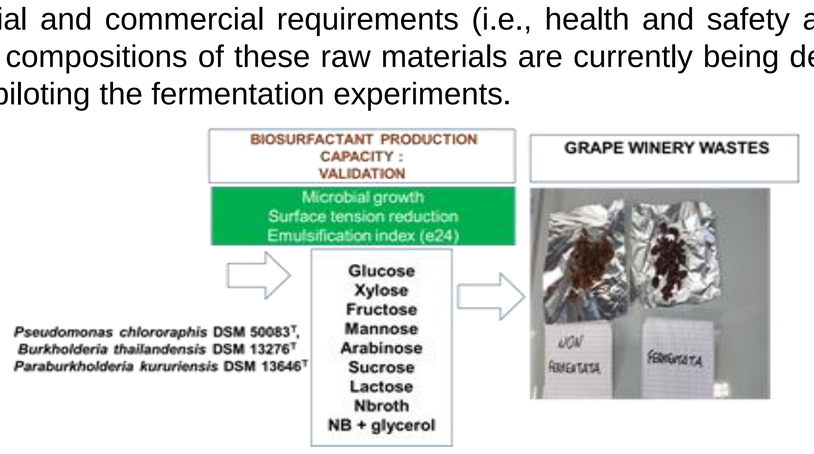


Fig. 2. Biosurfactant production strategy using sugar-containing wastes by non-pathogenic rhamnolipid producers.

C- Rhamnolipids production using winery wastes as substrates The strain E264 was found to grow well on the fraction higher concentration up to 80% NF, 100% NF and to reduce the surface tension to around 35 mN/m of the cell-free supernatant (Fig. 4AB) Interestingly, it showed a rhamnolipid production up to 1070 mg/l (13.37 mg/g of NF), with a higher purity, on raw grape marcs, predominately Rha-Rha C_{14}-C_{14}, in MSM medium (Fig. 4CD).

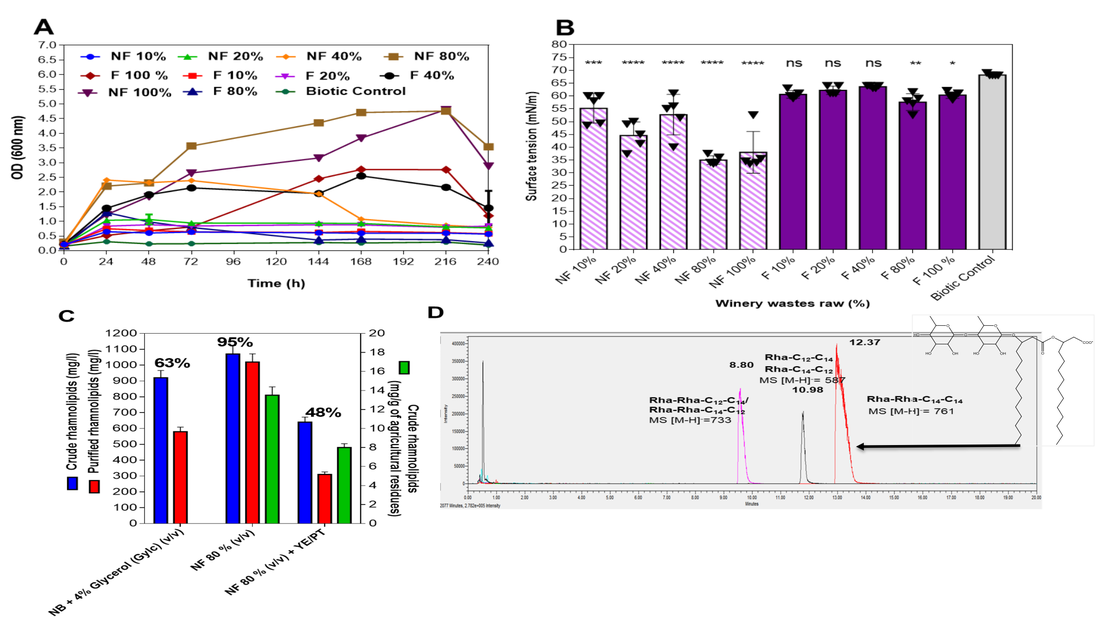


Fig.4. (A) Monitoring the cell growth (OD 600 nm) and) (B)the surface tension reduction (mN/m of *B. thailandensis* DSM E264, in the presence of nonfermented grape marc (NF) and fermented grape marc (F) fractions, in MSM medium during 240 hours at 30 °C and 150 rpm. (p< 0.05), ****(p< 0.01) are compared with biotic controls. (C) Crude and purified rhamnolipid yields (mg/) and percentage of purity and in the crude extracts from E264 fermentation on NF 80% (v/v), NF 80 % (v/v) +YE/PT and NB + 4% gly, after incubation during at 30 and 150 rpm. (D) Chromatograms UPLC-MS in negative ion mode ionization of rhamnolipid after SPE purification produced on nonfermented grape marcs at 80% (v/v) in MSM medium.**

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