# Novel biostimulant bacterial exopolysaccharides production via solid-state fermentation as a valorisation strategy for agri-food waste

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**Introduction**

During last years, a generalised concern about the need for supplying food to an exponential growing population has emerged as a primary global objective. Consequently, efficient land use, improved yields, higher-quality production, and reduced environmental and social impacts of agriculture have become the primary focus of scientists and producers (Chojnacka, 2015). For example, the production of conventional chemical fertilizers is not sustainable environmentally (Chen, 2006), whereas alternatively, organic fertilizers from diverse sources of nutrients are increasingly being used to reduce these inconveniences. However, some nutrients from organic fertilizers cannot be easily absorbed by plants due to their chemical bonding form (Halpern et al., 2015).

In this context, a group of different substances able to promote plant growth called biostimulants have emerged as key subjects to enhance nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality (du Jardin, 2012; European Commission, 2019). Exopolysaccharides (EPS), a novel classified substance as a plant biostimulant, have a great potential in biosorption, biodegradability and water retention (More et al., 2014).

The main objective of the present study is to produce EPS from specific bacterial strains through solid-state fermentation (SSF) using agri-food waste as a substrate. This goal aims to provide a new perspective on the value chain for the agri-food industry by turning usual by-products into an opportunity to produce a new product with added value, which can be self-profitable for particular applications.

**Materials and methods**

Six agri-food residues (Apple pomace (AP), Pomegranate seeds (PS), Pomegranate peels (PP), Vegetable milk waste (VMW), Beet juice waste (BJW) and Ginger juice waste (GJW)) were tested as potential substrates for EPS production via SSF. Also, five bacterial strains (*Leuconostoc mesenteroides*, *Azotobacter beijerinckii*, *Geobacillus thermodenitrificans*, *Alicyclobacillus acidocaldarius* and *Burkholderia cepacia*) were initially used in combination with the substrates to determine those with higher potential. With the best substrate-bacteria combinations, two time-course experiments (8 days) were performed to establish the behaviour of the system. Then, three operational parameters (Airflow rate, Inoculum size and Micronutrients concentration) were chosen to optimise EPS production at lab-scale. The SSF system consisted of a 0.5 L reactor (Erlenmeyer flask) connected to a dynamic respirometric system and immersed in a water bath to control each fermentation temperature. The outgoing air flow was linked to an oxygen sensor allowing a programmed data logger software to collect the oxygen concentration.

Along with the fermentations, different operational parameters were analysed and compared before, during and after the fermentation. Apart from the oxygen consumption, the final carbohydrates content in the extracted EPS, the bacterial biomass growth, consumed reducing sugars (RS) and the pH variation of the substrates were also determined following standard methodologies.

**Results and discussion**

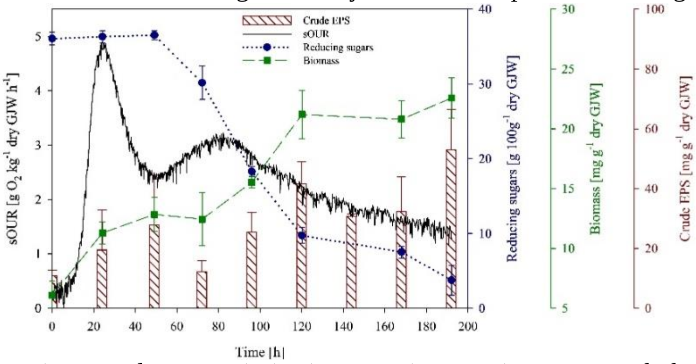
From the initial screening on strains/substrates, it was found that three combinations stood out among the other ones (Table 1). Since EPS production occurs during bacterial growth, and that usually comes from the RS consumption (Joulak et al., 2022), it could be expected that the higher the biomass growth, and the RS consumption, the higher the EPS production. Results show that, on one hand, the best combination for biomass production was *A. acidocaldarius* and AP, while on the other hand, the best combinations for RS consumption and the pH variation were *B. cepacia* with both juice wastes (GJW and BJW). After observing an increasing compaction on AP substrate, which was hindering the air flow through the reactor, both juice wastes combinations with *B. cepacia* were selected to perform a full SSF evaluation.

Table 1. Summary of the main results for the best substrate-strain combinations from the screening experiment.

| Substrate | Microorganism | Temperature (ºC) | Biomass (mg X $g^{-1}$ DM) | Crude EPS (mg $g^{-1}$ DM) | Reducing sugars consumed (%) | pH variation |
|---|---|---|---|---|---|---|
| AP | *A. acidocaldarius* | 55 | 35.7 ± 7.7 | 88.1 ± 3.5 | 0.0 ± 0.0 | 1.1 ± 0.2 |
| BJW | *B. cepacia* | 30 | 15.1 ± 2.3 | 44.9 ± 8.6 | 71.4 ± 6.8 | 2.2 ± 0.1 |
| GJW | *B. cepacia* | 30 | 16.6 ± 5.4 | 55.4 ± 6.2 | 82.2 ± 3.7 | 1.5 ± 0.0 |

Once working with the selected combinations (*B. cepacia* with GJW and BJW) similar results were obtained in terms of biomass and EPS production, RS consumption and specific oxygen uptake rate (sOUR). Figure 1 shows that the highest EPS production in the scenario GJW with *B. cepacia* was observed on the fifth and the last day, coinciding with the plateau phases of RS consumption and biomass production. Additionally, two different sOUR peaks were observed, which were corelated to two increasing phases of biomass production. Based on this complete analysis of the fermentation dynamics, five days was selected as the time in which EPS are produced in significant amounts without affecting the productivity of the process.

Figure 1. Parameters evolution through an 8-days SSF of *B. cepacia* with Ginger Juice Waste.




Considering the previous results, an optimisation experiment using a Box-Behnken design was conducted using the combination *B. cepacia*-GJW to maximise EPS production while assessing the effects of the airflow rate, inoculum load and micronutrients addition (Mg, Mn, Zn, Fe and B). From those experiments, it was shown that, in order to increase RS consumption, biomass production and, consequently, EPS production, the most significant effect was found with the inoculum size, followed by the airflow rate. The observed trend indicates that to achieve the maximum EPS production it is needed to increase all the parameters to their highest values. Specifically, the most desired conditions according to the quadratic model given by the Box-Behnken design were an airflow rate of 0.058 (± 0.001) L $h^{-1}$ $g^{-1}$ DM, an inoculum of 4.41E+09 (± 0.34) CFU $g^{-1}$ DM, and a micronutrients concentration of 0.061 (± 0.005) ml $g^{-1}$ DM. Further analysis on the behaviour of other parameters is needed to fully understand which are best conditions at lab-scale.

**Conclusions**

This study presents a novel perspective on the value chain of the agri-food industry. Therefore, from a wide variety of bacterial strains and waste/by-products from the industry, the best combinations in terms of EPS production were selected. Straight experiments were executed to find the best conditions to produce EPS. The combination *B. cepacia* with GJW was chosen to perform a complete study of the process dynamics and to optimise three variables (airflow rate, inoculum size and micronutrients concentration) to maximise EPS production.

It has been evidenced that at lab-scale the EPS production through SSF is feasible if there is enough carbon source in the substrate and specific stress conditions that allow bacterial growth while also promote EPS formation. Consequently, in the short-term, a scaled-up operation will be tested to move closer towards an industrial perspective and complete the circular economy process. Finally, a pot-test or a field experiment will be done in order to see the impact and efficiency of the obtained EPS. It is highly needed in the agri-food industry to continue exploring innovative valorisation systems which enable, at the same time, to reduce the waste impact and to provide bio-based products that can be used in the same industry.

**References**

Chen, J.-H. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. *International Workshop on Sustained Management*, *October*, 1–11.

Chojnacka, K. (2015). Innovative bio-products for agriculture. *Open Chemistry*, *13*(1), 932–937.

du Jardin, P. (2012). The Science of Plant Biostimulants – A bibliographic analysis, Ad hoc study report. In *European commission* (p. 37).

European Commission. (2019). Regulation (EU) 2019/1009 of the European Parliament and of the Council. *Official Journal of the European Union*, *2019*(2003), 114.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., & Yermiyahu, U. (2015). The Use of Biostimulants for Enhancing Nutrient Uptake. In *Advances in Agronomy* (Vol. 130). Elsevier Inc.

Joulak, I., Concórdio-Reis, P., Torres, C. A. V., Sevrin, C., Grandfils, C., Attia, H., Freitas, F., Reis, M. A. M., & Azabou, S. (2022). Sustainable use of agro-industrial wastes as potential feedstocks for exopolysaccharide production by selected Halomonas strains. *Environmental Science and Pollution Research*, *29*(15), 22043–22055.

More, T. T., Yadav, J. S. S., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2014). Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Env. Management*, *144*, 1–25.

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