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Evaluation of *Aneurinibacillus aneurinilyticus* isolated from corn steep liquor as source of Biosurfactants, Gramicidin S and Polyhydroxyalkanoates

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Abstract This study investigates the potential of *Aneurinibacillus aneurinilyticus* (*A. aneurinilyticus*), isolated from corn steep liquor, as a sustainable source of valuable bioproducts such us biosurfactants, Gramicidin S homologs, and polyhydroxyalkanoates (PHAs). Using phosphate-buffered saline (PBS), a biosurfactant extract was successfully extracted from *A. aneurinilyticus* cells, demonstrating its surface-active properties. In addition, ethanol was employed to extract Gramicidin S homologs from *A. aneurinilycus* cells, showcasing their potential to obtain new antibiotics against Gram-positive bacteria to address drug-resistant pathogens. Finally, vacuoles *in A. aneurinilyticus* were identified as potential reservoirs for PHAs, biopolyesters that can serve as sustainable alternatives to petroleum-based plastics.

Keywords: *Circular economy, antibiotic, biosurfactant, bioplastic, Bacillus, sustainability*

1. Introduction

Biosurfactants are surface-active compounds produced by microorganisms that reduce surface tension between liquids and solids. They are biodegradable, non-toxic, and have diverse applications such as environmental remediation, food, pharmaceuticals, and cosmetics (Vigil et al. 2024, Bhattacharya et al. 2017, Bjerk et al. 2021). These compounds are considered environmentally friendly alternatives to synthetic surfactants, which can be detrimental to ecosystems.

Gramicidin S is a cyclic peptide antibiotic with potent antimicrobial properties. It can be produced by sporulated bacillus like *Bacillus migulans*, *Bacillus brevis* or *Aneurinibacillus aneurinilyticus* or produced by chemical or enzymatic synthesis (Berdish et al. 2007, Kalyvas et al. 2024). Gramicidin S exhibits efficacy against Gram-positive bacteria and has been investigated as a potential treatment against virus and cancer cells (Choi et al. 2023, Enayathullah et al. 2022). Most of Gramicidin S is produced by enzymatic or chemical synthesis involving the use of trifluoroacetic (TF), that is contained in the final product what can increase the cytotoxicity of Gramicidin S (Wadhwani et al. 2006).

Polyhydroxyalkanoates (PHA) are biodegradable biopolyesters synthesized by bacteria as intracellular carbon storage compounds. They serve as a sustainable alternative to petroleum-based plastics, and they can be produced by sporulated bacillus (Naseem et al. 2024). After disruption, PHAs can be separated from other components through solvent extraction, where non-polar solvents such as chloroform or dichloromethane dissolve the PHAs. The PHA-rich solvent is then e evaporated, and the polymer purified (Pagliano et al. 2021).

2. Materials and Methods

A. aneurinilyticus was growth on Tryptic Soy Broth (TSB) medium for 7 days following the protocol established in a previous work and after that biomass was obtained, washed, and extracted with phosphate buffer saline (PBS) (López-Prieto et al. 2021) or ethanol (Lvova et al. 2024) at the optima conditions stablished in previous works.

Gramicidin S in the extracts was analyzed by Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS) using Electrospray Ionization Mass Spectrometry (ESI-MS) as ions source (Mass Bruker FTMS SolariX XR) coupled with Ultra Performance Liquid Chromatography (UPLC) (Lvova et al. 2024).

Biosurfactant in the extracts were determined based on the measurement of surface tension of PBS extracts following the protocol stablished in previous works (López-Prieto et al. 2021), using a tensiometer (K20 EasyDyne from KRÜSS Scientific).

After extraction of Gramicidin S biomass was examined for detecting vacuoles compatible with the storage of PHAs. Field Emission S Microscope (FESEM) with a Field Emission Scanning Electron Microscope (JEOLJSM6700F) equipped with a lower detector (LEI) was employed. Moreover, High-resolution Transmission Electron Microscopy (HRTEM) was applied to biomass

samples. The equipment consisted of a Transmission Electron Microscopy (JEOLJEM1010, 80Kv) equipped with a digital camera (CCD Orius Digital Montage Plug-in camera, Gatan Inc., Gatan, CA, USA) and software (Gatan Digital Micrograph, Gatan Inc.).

3. Results and discussion

After solid-liquid extraction with ethanol microbial biomass released Gramicidin S to the ethanolic, observing high concentration of Gramicidin S homologs (around 1000 mg/L), being an interesting alternative to the chemical or enzymatic synthesis of Gramicidin S. This extract possesses the advantage of being absent of TFA.

Regarding the extract obtained after PBS extraction the concentration of Gramicidin S was minimal (around 70 mg/L) but the extract contains high concentration of biosurfactants, that are able to reduce the surface tension of water up to 43.4 mN/m what could increase their potential bioactivity a low concentration of antibiotic reducing cytotoxicity.

Related the potential production of PHAs by *A. aneurinilyticus*. Figure 1 displays high-resolution microscopy images of a transverse section of *A. aneurinilyticus* cells, in which well-defined granules compartments, compatibles with the presence of PAHs, are visualized. The presence of granules in *A. aneurinilyticus* cells open the possibility to extract PHAs post-biosurfactant and Gramicidin S extraction by applying physical or chemical methods.

Figure 1. HRTEM photomicrographs of *A. aneurinilyticus* cells isolated from corn steep liquor

4. Conclusion

Based on the above information, this research highlights the industrial and environmental significance of *A. aneurinilyticus* in producing diverse sustainable metabolites (biosurfactants, Gramicidin S homologs and potential PHAs), promoting circular economy and industrial symbiosis.

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