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NOVEL STRATEGY FOR THE RECOMBINANT PRODUCTION OF SWEET-TASTING PROTEIN BRAZZEIN

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In response to global initiatives directed at reducing the consumption of sucrose and synthetic sweeteners, there has been a greater focus on natural sweeteners with enhanced nutritional quality, such as Brazzein. This plant-derived protein sweetener is notable for its small molecular size (6.5 kDa), high resistance to pH and temperature fluctuations, and its sweetness, which can be up to 2000 times greater than table sugar with a low-caloric contribution (4 kcal/g) and no glycemic index. To meet the demand for sustainable and large-scale production of this sweetener, we aimed to transiently produce it in *Nicotiana benthamiana* plants using TMV-gate viral vectors adding the 5' UTR of the alcohol dehydrogenase (ADH) gene and the terminator of *Arabdidopsis thaliana*'s heat shock protein (HSP). Although these elements have individually shown to significantly increase protein expression, their combined effect had not been previously assessed for Brazzein production using TMV vectors. To analyze the efficiency of this new system, several construct/vector combinations have been built through successive molecular cloning to finally get stable *Agrobacterium tumefaciens* clones for Brazzein's expresión on *N. benthamiana* leaves. Despite challenges in quantifying protein abundance due to photosynthetic pigment interference, we observed characteristic patterns indicative of small proteins, suggesting potential Brazzein accumulation for clones containing the construct with additional regulatory elements, using both, conventional and TMV-gate vectors. These findings sugget that boosters (ADH's 5' UTR and HSP terminator) could enhance protein expression in both vector systems, as anticipated for conventional ones, but unexpectedly also for TMV-gate vectors.

BT-6

FORMULATION OF LOW-COST CULTURE MEDIA FOR THE IMMOBILIZATION OF *Pseudomonas* sp. P26 ON NATURAL SUPPORTS

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Pseudomonas sp. P26 is an environmental bacterium with desirable physiological and safety properties for environmental bioremediation processes. The objective of this study was to formulate low-cost culture media using industrial by-products for the immobilization of *Pseudomonas* sp. P26 (P26) on natural organic supports. The bacterial strain P26 was immobilized on peanut shells and walnut shells using a standard culture medium (LBm) and media formulated with industrial by-products (corn steep water –CSW– and crude glycerol) and K_2HPO_4 (as an inorganic biostimulant). The metabolic activity of the immobilized bacteria was quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. In the selected immobilized system, the number of viable cells and intracellular inorganic polyphosphate (polyP) content were quantified (using a spectrophotometric method to measure inorganic phosphate released by the acid hydrolysis of polyP), and electron microscopy analyses were performed. In the first trial, significantly higher metabolic activity values were observed in walnut shells compared to peanut shells, in the control medium LBm, both in the presence and absence of inorganic phosphate. In the second trial, the highest metabolic activity values were determined in the medium named M4 (formulated with 2.5% v/v CSW + 1% v/v glycerol) and in LBm, with and without inorganic phosphate. In media with added inorganic phosphate, no significantly higher metabolic activity values were observed compared to those without this substrate. Electron microscopy revealed biofilm formation on the walnut shells, few connections between bacterial cells, and moderate extracellular material production. PolyP accumulation under immobilized conditions was higher than in planktonic cultures. An immobilization system based on *Pseudomonas* sp. P26 cultivated in corn steep water and crude glycerol, using ground walnut shells as support, was developed. The immobilized system is a potential candidate for future studies on the removal of aromatic compounds from petroleum.

BT-7

ISOLATION OF CONSTITUTIVE PROMOTERS FOR THE EXPRESSION OF A FLAVIN MONOOXYGENASE UNDER MICROAEROBIC CONDITIONS: A THERAPEUTIC APPROACH FOR TRIMETHYLAMINURIA

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