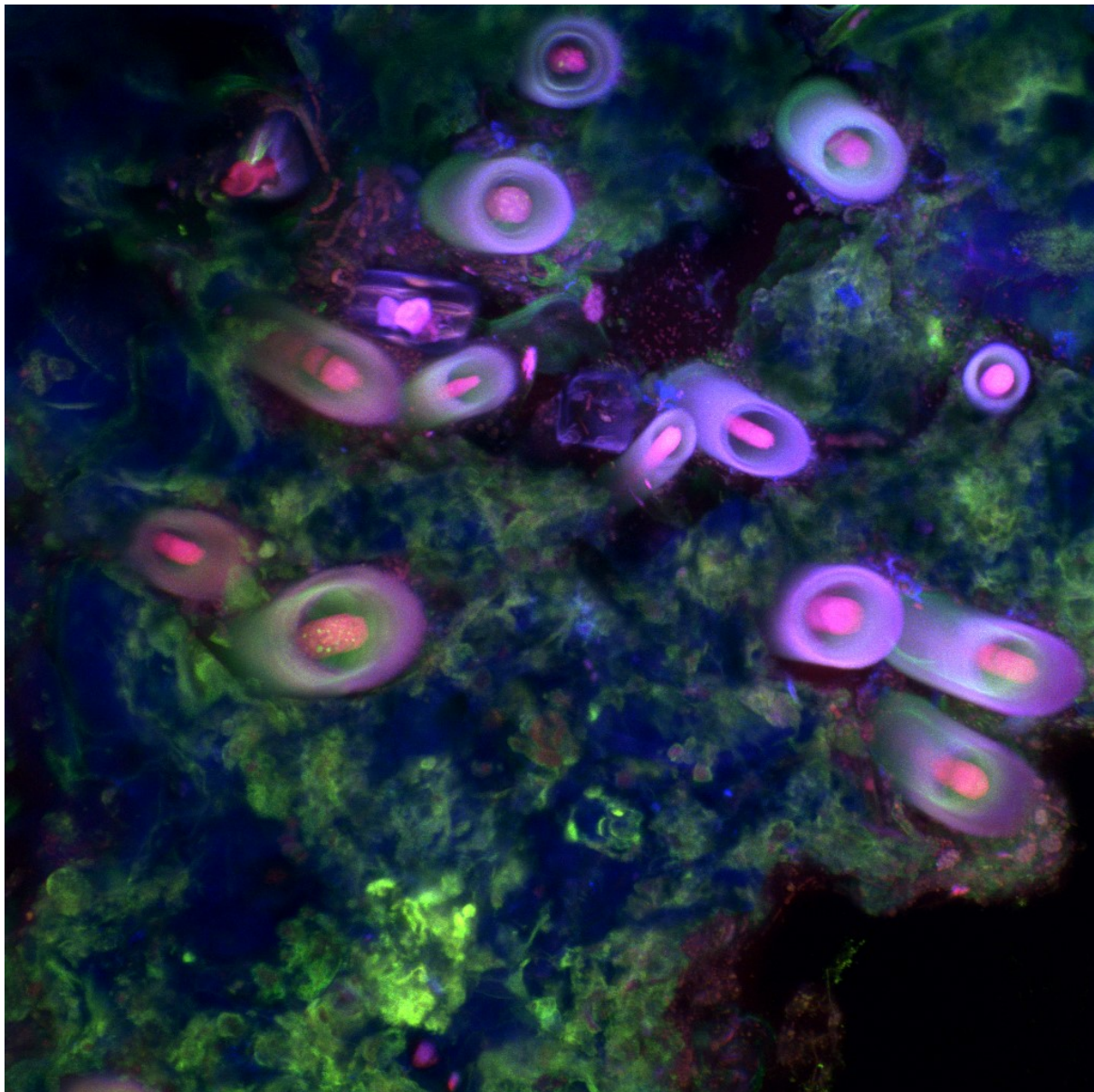




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with 100 % DP at the end of the assay. Besides, a temporary delay of four weeks on the onset of typical symptoms were visualized for these treatments and at least one week of delay for all the remaining isolates tested simultaneously, and slower rates of disease progression. In this way, we can conclude that the isolated actinobacteria have the ability to protect soybean plants from the tested fungal phytopathogen, achieving in some cases improvements in plant growth. To confirm the ability to improve crop yield, further work in field trials will be required.

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FUNCTIONAL FERMENTED BEVERAGES ENRICHED IN SELENO-AMINO ACIDS AND SELENO-NANOPARTICLES

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Selenium (Se) is an essential micronutrient for human health, which is found as selenocysteine (SeCys) in the active site of Se-dependent enzymes involved in the response to oxidative stress and in thyroid functions. The main inorganic Se forms, selenite, and selenate are toxic. Some lactic acid bacteria (LAB) can reduce Se salts into seleno-nanoparticles (SeNPs) and seleno-amino acids, which are non-toxic and highly bioavailable forms. In several European countries, as well as in Argentina, Se intake is below the recommended dietary intake (RDI). Se-enrichment of foods is an attractive strategy to increase its ingestion. We aimed to formulate a fermented fruit juice-milk beverage (FJMB) bio-enriched in Se. The fruit-origin strains *Fructobacillus tropaeoli* CRL 2034 and *Levilactobacillus brevis* CRL 2051 were grown with or without 5 mg/L of Se prior to co-inoculation (1% of each strain) in the FJMB and were incubated 14 h at 30°C. The survival of the strains under storage conditions (6°C, 52 days) and after digestion [using an *in vitro* gastrointestinal system (GIS)] was analyzed. The strains grew (up to 8.6 U log each) and acidified FJMB, reaching a final pH of 4.6. Sugar metabolism and organic acid production were similar for control and selenized cells (RP-HPLC), while mannitol production by selenized cells of the *Fructobacillus* was lower (0.18 ± 0.03) than control cells. The studied strains could not degrade the proteins present in the FJMB (SDS-PAGE). Selenized cells increased the beverage total Se concentration (ICP-MS, 84.9 ± 4.5 µg/L) and biotransformed selenite into SeCys (39.1 ± 0.4 µg/L) and SeMet (6.1 ± 0.1 µg/L) as detected by LC-ICP-MS. Moreover, SEM images of the fermented FJMB revealed the presence of SeNPs attached to the cell surface of both strains. Interestingly, microbial resistance at the end of the shelf life was greater (between 0.5 and 0.7 U log) for selenized than non-selenized cells. However, no differences were observed in the sugar and organic acid concentrations between treated and non-treated cells, and a lower (0.29 ± 0.04 g/L) mannitol production was detected at 28-day incubation by the treated strains. After GIS digestion, a decrease in the cell counts of *F. tropaeoli* and *L. brevis* (1.60 and 0.80 U log, respectively) was observed. Interestingly, 64.3 ± 3.3 µg total Se/L partly as SeCys (25.8 ± 2.3 µg/L) and SeMet (2.4 ± 0.2 µg/L) were found in the FJMB supernatant after intestinal digestion, highlighting the bioaccessibility of these compounds. Remarkably, 250 mL of the FJMB could cover 64% of the Se RDI (25 µg/day), from which 28% is composed of seleno-amino acids. Our results suggest that selenized cells of *F. tropaeoli* CRL 2034 and *L. brevis* CRL 2051 could be used for formulating functional Se-enriched beverages to improve this micronutrient intake in humans.

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BIOCATALYTIC CHARACTERIZATION OF THREE BACTERIAL BAEYER-VILLIGER MONOOXYGENASES

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Baeyer-Villiger monoxygenases (BVMOs) are flavoenzymes that catalyze the insertion of one atom of oxygen from molecular oxygen into the substrate while the other one is reduced to water. Ketones are the typical BVMO substrates, therefore their oxidation produces esters or lactones in an environmentally friendly reaction. We detected seven putative type I BVMOs in *Bradyrhizobium diazoefficiens* USDA 110 by genome mining. We cloned and functionally expressed in *Escherichia coli* three of these flavoenzymes, which we named BVMO2, BVMO4, and BVMO5. Each of these sequences belongs to a different group of an inferred phylogenetic tree of type I BVMOs. The aim of this work was to characterize new BVMOs in order to expand the set of this type of biocatalysts available for synthetic applications. First, we assessed their biocatalytic potential in whole-cell systems by challenging them with several ketones as candidate substrates. We found out that these enzymes oxidize linear, aromatic, cyclic, and bicyclic ketones but with different preferences. Then, we purified the recombinant BVMO2, BVMO4, and BVMO5 to homogeneity and characterized them *in vitro*. We determined the molar absorption coefficient of each of these enzymes and investigated the dependence of their activities with the cofactor, temperature (from 20 to 45 °C) and pH (from 6.0 to 9.0) as well as their pH and temperature stability. We determined the steady-state kinetic parameters of the three recombinant flavoenzymes from *B. diazoefficiens* for phenylacetone and heptan-3-one by following the consumption of NADPH. We observed that the catalytic efficiency of BVMO2 was similar for both substrates, BVMO4 performed very well on phenylacetone under the established assay conditions, and both ketones were very good substrates for BVMO5. Thus, according to the substrate scope and selectivities obtained *in vivo* and the performance of each enzyme *in vitro*, we propose a complementary behavior among the three BVMOs. While BVMO2 oxidized ketones with variable structure, BVMO4 and BVMO5 showed a narrow substrate profile with a preference for linear ketones and with particular regioselectivities for a