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ROLE OF MOUTH BACTERIAL GLYCOSIDASES IN HUMAN AROMA PERCEPTION

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Abstract.

Taste perception is a complex mechanism generated by instructions from gustation and olfaction along with oral and nasal somatosensory inputs. This process includes the integration of different modalities such as aroma, taste, trigeminal sensations, texture and visual. There is a relationship between how we perceive taste and oral microbiota diversity. Flavor perception is different among individuals and this dissimilar sensibility could be explained by the oral microbiota composition. Glycoside-derived aroma compounds are mainly produced by bacterial glycosidase enzymes in the mouth. These bacterial families are mainly represented as Prevotella, Streptococcus, Veillonella, Neisseria, and Haemophilus. Qualitative distinctions among oral bacteria may bring to different metabolizations of aroma compounds and their precursors, thus leading to different retronasal olfactory responses. There is little research focused on studying the oral microbial communities associated with β-glucosidase activity and the release of volatiles from flavor precursors. Aim of the present project is to show the ability of mouth bacterial glycosidase to release aroma from food products. For that, specific oral glycosidases will be recombinantly produced in Escherichia coli, purified, characterized and applied within food products. New insights gained on oral microbial enzymes will be useful for the design of molecular tools optimized for aroma compound production or enhancing the flavor intensity of specific food products, as well as for certain food manufactures to optimize and modulate food processing.

Introduction.

Glycosidases, also known as glycoside hydrolases (EC 3.2.1.-) are a type of hydrolase enzyme that catalyze the hydrolysis of glycosidic bonds, which link carbohydrate molecules [1]. These enzymes have a wide range of specificity and can hydrolyze different types of carbohydrates, such as monosaccharides, disaccharides, and polysaccharides. Different microorganisms are a rich source of glycosidases, particularly fungi and bacteria. Many species of Aspergillus, Penicillium, Trichoderma, and Rhizopus produce a wide range of glycosidases that can be used in the food and beverage industry [2].

Flavor perception plays a major role in this organoleptic experience. It is a multimodal perception corresponding to functional integration of information from the chemical senses: olfaction, gustation, and nasal and oral somatosensory inputs. There is a relationship between how we perceive taste and oral microbiota diversity. Flavor perception is different among individuals and this dissimilar sensibility could be explained by the oral microbiota composition. Qualitative distinctions among oral bacteria may bring to different metabolizations of aroma compounds and their precursors, thus leading to different retronasal olfactory responses [3]. There is little research focused on studying the oral microbial communities associated with β-glucosidase activity and the release of volatiles from flavor precursors.

Methods.

Sequences of three glycosidases were obtained from Protein Data Bank. These glycosidases are GH1 family beta-glucosidase [Prevotella sp.], Beta-glucosidase [Streptococcus pneumoniae], Putative alpha-galactosidase/6-phospho-beta-glucosidase [Klebsiella pneumoniae subsp.]. E. coli BL21 strains were used as hosts for gene cloning and expression. The pET22-b plasmid (Navagen, USA) carrying the N-terminal pelB signal sequence was used as an expression vector. The transformed bacteria were inoculated on selective agar medium (LB/agar + ampicillin 100 $mg.L^{-1}$) and were placed for incubation at 37°C overnight. Then culture with bacteria was cultivated in Luria-Bertani (LB) for 6 hours at 37°C. For purification proteins were sonicated. HisTrap HP column for histidine-tagged proteins was used to purify recombinant enzymes. HisTrap HP column was equilibrated with a phosphate buffer (50 mM, NaCl 300 mM, pH 8.0). Recombinant proteins were eluted by an increasing linear gradient of phosphate buffer with imidazole from 0 to 500 mM at a flow rate of 1 ml/min. Purity of proteins had been verified by SDS-PAGE. All purified proteins were dialyzed in 2L of 50mM Tris-HCl buffer pH8.0 using a Dialysis Membrane of 12-14kDa. And then protein concentration was measured by Nanodrop, calculating the Beer-Lambert law. Enzymatic activity was determined on a spectrophotometer by measuring absorbance at the range of 405-420 nm which corresponds to the 4-Nitrophenyl-β-D-glucopyranoside (P-NPG) absorbance wavelength. Prior to crystallization, the enzyme solution was concentrated to ~15 mg/mL with a centrifugal concentrator (Falcon®) in a 20 mM Tris buffer, pH 8.0. Crystals were grown in drop vapor diffusion plates. Commercial kits such as Wizard Classic and JBS were used as initial conditions. The plates are incubated at 20°C.

Results.

Current study demonstrates successful purification and characterization of β-glucosidase from oral bacteria, thus providing a better understanding of its role in the taste perception, and establishing a basis for further research. Good conditions for production of glucosidases were established (37 °C, 6h, 1μM IPTG). The purified enzyme beta-glucosidase [Prevotella sp.] was able to liberate β-galactose and p-nitrophenol, with the concomitant appearance of a yellow color. A specific activity of 6,048 sec^{-1} was quantified for 4-nitrophenyl β-d-glucopyranoside.

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