

A Study on Aquatic Proteases and Their Probiotic Significance

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Abstract – *P. acidilactici* strains are commonly dispersed in plant material for fermentation, including silage, cereal mashes and pickles, potato mashes, barley malt, dried leaves, and hay. It is usually used in cheese and yoghurts as a helpful microbe, and is believed to maintain a healthy microflora balance in the digestive system. *Pediococci* cells are circular and arranged in tetrads, but the liquid colonies often reveal pairs. Like cocci of lactic acid, *Pediococcus* splits between two planes of symmetry. They are facultative anaerobes non-motile, and non-sporulating. Standard gut ecology has 10:1 anaerobic to aerobic bacterial ratio and *P. acidilactici* has the advantage of being an anaerobic facultative bacteria. The present paper identifies enzyme groups that are of probiotic and industrial significance.

Keywords: Enzyme, *P. Acidilactici*, Bacteria

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INTRODUCTION

Later renamed *Pediococcus cerevisiae* as *P. acidilactici* was the first lactic acid bacteria to be produced as a pure starter culture in the United States. A starter culture is an inoculum of beneficial micro-organisms applied to food items like beef, milk and vegetables to enhance the consistency and protection of the food. Starter cultures provide accelerated production of lactic acid from fermentation of added sugars to sausage resulting in a decreased pH that retards the growth of most spoilage producing microorganisms and improves the final product's taste and texture.

Pediococcus imparts desirable qualities to cheese which indicate that if they possess the ability to use the specific sugar, they might be good dairy starters. *Pediococci* is used to prepare and protect sausages, fruits, cheeses and soya products. It also applies to some wines and some types of beer butter / butter scotch flavouring.

Pediococcus produces pediocins that demonstrate antibacterial action against both spoilage and pathogenic organisms including *Listeria monocytogenes*, *Clostridium perfringens* and certain types of *Salmonella* and *Lactobacillus*. They were well known for pediocin / bacteriocin and were thus used in veterinary practise as probiotics despite the lack of evidence to support their clinical effects. However, *Pediococcus* vancomycin-resistant strains have been identified in patients with septicemia but their virulence has not been further investigated

making it difficult to differentiate between healthy probiotic strain and clinical isolate.

And if it is considered an opportunistic pathogen, it is unlikely that it causes any infection in healthy people. Vancomycin tests have shown that *P. acidilactici* as MIC has 3.5 mg / ml which is far greater than its trough value (5-10 µg / mL). Reports of the use of *Pediococcus acidilactici* as probiotics are incomplete and fragmentary.

Studies of this strain's in-vitro probiotic properties are positive. It possessed all the qualities needed in vitro. They considered the strain to be abundant in multiple proteolytic activities. It also had local galactosidase, antioxidant activity, and also developed lactic acid, thus giving the strain both probiotic and industrial value.

Microbial extracellular enzymes are mainly involved in holding the cells intact by supplying the required supply of amino acids as dietary support. Due to its use in various biotechnological processes, such as detergents, textiles, dairy manufacturing, oil refining, surfactant production and chiral pharmaceutical synthesis, the isolation of extracellular enzyme-producing microorganisms has gained considerable interest.

Supplying enzymes is also one of the attribute and possible mechanism of action for probiotic bacteria and their analysis in probiotic bacteria is of great importance. To date, focus has been given only to LAB intracellular peptidases but only to a small

number of general peptidases with high specificity viz. Purified and characterised to date amino peptidases, dipeptidases and tripeptidases. When compared to end peptidases, exopeptidases are less studied. These enzymes are isolated and characterised in order to understand their physiological and industrial function.

PROTEASES

Proteases are a group of enzymes that hydrolyze protein peptide bonds and break them down into either polypeptide or free amino acids. Protease is present in all lifestyles viz. Plants, animals and micro-organisms are important to the development and division of cells. Microorganisms provide an excellent source of enzymes due to their extensive biochemical complexity, susceptibility to genetic modification, rapid development, restricted cultivation space need, etc.

In comparison to its precision of operation, the vast variety of proteases has drawn worldwide interest in attempts to leverage their uses in the dairy, meat, detergent and leather industries. In addition to their commercial function, proteases are essential in physiological processes such as blood clotting, programmed cell death, tissue differentiation and control of various enzymatic cascades of metabolic reactions involving the breakdown of fats, carbohydrates etc.

The interest in using proteolytic enzymes as tools for the production of therapeutic agents is renaissance. They are decaying enzymes of essential significance and they can be used in a variety of industries to produce improvements in the flavour, form, quality of the commodity and in the processing of waste.

Proteases catalyse both the structural and decaying roles. These enzymes vary in properties such as the specificity of the substrates, the active site, the catalytic function, the optima pH and temperature, the stability profile etc. Several microorganisms such as influenza, protozoa, bacteria, yeast, and fungi have proteases.

Protease genes from many bacteria, fungi and viruses were also cloned and sequenced with the primary target of gene amplification overproduction of the enzyme, delineation of the function of the enzyme in pathogenicity and modification of enzyme properties to accommodate its commercial application(s). Microorganisms degrade proteins, and use the degradation products as nutrients for their development. The degradation is initiated by microorganism-secreted endoproteases accompanied by exopeptidases at the extra- or intracellular sites.

PROBIOTIC FUNCTION OF PROTEASES

Fermented functional foods are attractive because of the combination health and environmental advantages. While proteolytic enzymes aren't very precise, during fermentation a great variety of peptides are published. Bioactive peptides, formed by enzymatic protein hydrolysis in vivo or in vitro, have physiological effect. When delivered orally, they also play a major role in biological processes such as respiratory, neurological, gastrointestinal and immune systems.

The most promising approach to producing these peptides is the development of in situ ACE-inhibitory peptides in dairy material. Bioactivities of peptides produced as a result of the action of microbial enzymes on milk proteins include hypotensive, immunomodulatory, anticancer, hypocholesterolaemic, antimicrobial, antibiotic, and mineral-binding. Enzymatic peptide synthesis was frequently used for pharmaceutical and nutritional synthesis. Probiotics can produce bioactive peptides, actually.

Esterase's/Lipases

Lipases are considered the third largest group, based on gross amount of revenue. Esterase (carboxyl esterase, EC 3.1.1.1) and lipase (EC 3.1.1.3) are commonly referred to as lipolytic enzymes that catalyse the cleavage and form ester bonds. Esterases catalyse also the hydrolysis of short-chain triglycerides or esters (10 carbon atoms) ester bonds. The main difference between esterases and lipases is their ability to function on surface display as lipases show interfacial activation while esterases do not. Esterases and lipases, from bacteria to higher eukaryotes, are commonly found in different species.

Owing to its distinctive characteristics viz. Large range of non-natural substrate sensitivity, high organic solvent stability and high enantioselectivity (for some), esterases are useful for a number of industrial applications such as fat and oil manufacturing additives, laundry detergents, fine chemicals and pharmaceutical synthesis, paper making and cosmetics. Because of their possible application in scientific and medicinal biotechnology and also for the elucidation of their unexplained metabolic activity, these enzymes were thus the focus of studies.

During cheese ripening, the release of free fatty acids (FFAs) due to lipolytic activity contributes to flavour growth. The enzymes are mainly extracellular, but the enzymes are also reported to be intracellular and cell dependent. In the dairy and food processing industries in particular, they are used widely for the hydrolysis of milk fat for the intention of improving the taste or speeding the

ripening process in cheese and cheese related products.

The probiotic value of the lipases/esterase's

As fat levels determine the dietary, physical, chemical and sensory characteristics of food, this contributes to increased interest in functional foods. Generation of short chain fatty acid is an important probiotic trait. Howeveryric acid plays a part in the prevention of colorectal cancer (Pouillart, 1998). They illustrate preventive action against cardiovascular disorders by reducing serum cholesterol levels and triglycerides.

Hydrolysis peptidoglycana

Peptidoglycan (PG) is the major component of Gram positive bacteria's cell wall and is thus important for bacterial survival as it retains cell structure and protects the integrity of the cells. Peptidoglycan hydrolases (PGH) or autolysins cleave peptidoglycan (PG) strands or cross-links of the producer strain resulting in PG mesh and thus cell lysis being killed. Autolysis is a complex mechanism containing multiple enzymes that are known as peptidoglycan hydrolases by their common name.

Peptidoglycan hydrolases (PGH) represent a wide group of enzymes of distinct, but related, origin. They are categorised according to their catalytic domain behaviour, viz. I — N-acetyl-glucosaminidases (hydrolysis — 1,4-bonds between alternating N-acetylmuramic acid and N-acetyl glucosamine residues of the glycan chain, (ii) — N-acetylmuramidases (hydrolysis of N-acetylmuramyl, 1,4-- Nacetylglucosamine bonds), (iii) N-acetylmuramoyl-L-alanine amidase or amidase (cleavability of the amide bond between the lactic acid side chain of MurNAc and L-Ala stem peptide) and (iiii)

PGH in Bacteria with Lactic Acid

Since LAB is used in dairy fermentation as the starting cultures, their lysis is of particular importance. Lysis contributes to cytoplasmic peptidases, lipase, and other enzymes released.

PGH plays a significant role in various processes, such as the division of daughter cells, cell-wall turnover, autolysis, sporulation and germination, the development of biofilms, the resuscitation of inactive cells and genetic allolysis. These enzymes are being researched with a view to extending their use for numerous applications. Some *Lactobacillus* species probiotics have been found to interact with immune system receptors in gastrointestinal tract via PGH-dependent processes such as autolysis and/or cell wall turnover which release muramyl-peptides or PGH fragments.

PGH Probiotic Qualities

As these are important for a variety of bacterial processes and host-microbe interactions, the functional role of peptidoglycan degrading enzymes is being increasingly documented. PGH's most significant probiotic attribute includes impact on immune response, especially down regulation of inflammatory response through activation of anti-inflammatory pathways regulated through immunocompetent cells and secreted cytokines.

Inactivation of PGH resulted in altered immunomodulatory effects. These enzymes also serve as a molecule for cell adhesion as Acm2 from the mucin, an extracellular portion of the intestinal mucosal layer, recognised by probiotic *Lactobacillus plantarum*.

PGHs also have antimicrobial properties, and are therefore antagonistic to pathogens. Many human and animal infections include some enterococcal strains as Gram positive bacteria. When infections caused by these bacteria develop antibiotic resistance, they become unmanageable.

Peptidoglycan hydrolases tend to be a novel alternative to antibiotics and are effective. Even in animal models the efficacy of certain members was tested and shown in vitro and in vivo. *Pediococcus acidilactici* being a probiotic strain requires extensive knowledge of these enzymes in order to develop autolysis-resistant strains under stress and to better understand the role of released muramyl peptides as probiotic immunomodulators and antipathogenic enzymes.

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