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An overview: Industrial application of amylolytic lactic acid bacteria

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Abstract
Gelatinization and liquefaction are performed on natural source ingredients that are complicated in starch used to make lactic acid before they are using an enzyme; glucose is converted to lactic acid by the Lactobacillus bacteria. By removing the two-step process, bacteria having both lactic acid and amylolytic -producing traits will be able to convert starchy biomass directly to lactic acid. Sadly, only a very small number of Bacteria producing amylolytic lactic acid include robust ability to produce high substrate concentrations, lactic acid. The review examines a number of amylolytic lactic acid bacteria that are used in food processing and other industrial settings to produce lactic acid from biomass and other industrial application of α-amylase and amylolytic lactic acid bacteria.

Keywords: Gelatinization, Lactic acid bacteria (LAB), amylase, amylolytic lactic acid bacteria, lactobacillus

1. Introduction

In the food processing industry, amylolytic lactic acid bacteria (ALAB) are used to make enzymes (such as α-amylase) and organic acids (such as lactic acid) that are used as food additives. The amylase uses of ALAB have been extended to different industries due to biotechnology advancements, including biopharmaceuticals, distillery and brewing (Bhanwar and Ganguli 2014; Hattingh *et al*. 2015) [1, 6]. In order to improve the dietary starch utilisation in newborns and young children, ALAB are also included in the preparation of cereals with a high energy density (ED) diets (Konkit & Kim, 2016) [2]. Several methods have been created to enhance the ED of breakfast cereals with the proper transformation from a semi-liquid to a fully liquid state occurs due to the reduction in the swelling of starch granules caused by either partial starch hydrolysis through dextrinization or ALAB. ALAB are usually thought to be safe for use in food and studies demonstrated how Fermenting pre-heated mixtures of rice and soybean, combined with either spray drying or high -pressure homogenization techniques, can lead to the production of highly digestible gruels. These gruels are initiated by utilizing ALAB as the initial culture for starch breakdown. Similarly, a specific strain of ALAB, originating from Ben-saalga in Burkina Faso and employed in the fermentation and starch hydrolysis of pre-cooked African pearl millet (*Pennisetum glaucum*) and groundnut slurry, has been developed for the nourishment of young children i.e. *Lactobacillus plantarum*.

2. Lactic acid bacteria (LAB)

In general, non-sporulating, catalase-negative, cytochrome-free and non-motile LAB are a broad category of microorganisms that re Gram positive. Both bacilli and cocci from *Lactobacillus*, *Enterococcus*, *Leuconostoc, Pediococcus*, *Streptococcus* etc. are included in them (Mazzoli *et al*. 2014) [3]. Commonly referred to as potent probiotics, lactic acid bacteria (LAB) have a variety of positive effects on the gastrointestinal (GI) tract and promote health. *Lactobacillus acidophilus, L. rhamnosus, L. plantarum, L. gasseri* and *L. casei* are among the probiotic *lactobacillus* species. Consuming LAB fermented foods may have the aforementioned impact since *lactobacilli* have been repeatedly linked to antitumorigenic, anticarcinogenic and antimutagenic actions. The microorganisms that bio-refineries are most likely to use are LAB, which can transform starchy waste biomasses into useful industrial products (Unban *et al*., 2020) [4]. A consequence of anaerobic glycolysis is lactic acid that is produced by LAB with high yield and productivity. Based on the final result of the fermentation, LAB is classified as either hetero-or homo-fermentative. Aldolase enzymes are present in homofermentative LAB, which primarily create lactic acid as an output.

The maximal output of lactic acid, however, is only 1.0 mol/mol or 0.5 g/g glucose moiety due to hetero-fermentative LAB's production of acetic acid, water, and ethanol in addition to lactic acid (Abdel-Rahman *et al*., 2013) [5]. According to reports (Hattingh *et al*. 2015) [6], metabolically engineered *Lactobacillus plantarum* can convert pentose to lactic acid via a homofermentative mechanism. Since they have a long history of usage in food and drink without having any negative health impacts on consumers, the majority of LAB is thought to be safe for industrial lactic acid synthesis. Important LAB strains that may be manipulated to produce D- or L-lactic acid, including as *L. plantarum, L. rhamnosus* and *L. acidophilus* have proven helpful in the commercial world. Certain LAB have amylase activity. The GI tracts of chickens, mammals such as pigs, rabbits, horses, and humans, including infants, all contain *Lactobacillus* amylase, which plays a significant role in the degradation of dietary starch into fermentable monosaccharides that can be readily assimilated by the body. The pH of the environment is lowered by lactic acid generated in the GI tract, which prevents the growth of harmful bacteria like *Escherichia coli*, *Salmonella and Staphylococcus.* Additionally, amylolytic LAB are now thought to be involved in the preparation of cereal-based high energy density diets for enhancing how well babies and kids use dietary starch.

3. Amylase

According to Sundaram *et al*. (2014) [7], the majority of hydrolase enzymes are amylases, which disrupt the glycosidic linkages entirely. Found in starch molecules to create oligosaccharides and dextrins. According to Kumar (2011) [8], amylases are a part of the family of enzymes known as glycoside hydrolases 13 (GH-13). Amylases, also known as glycoside hydrolases, hydrolyze -1, 4-glycosidic bonds in starch. In 1833, Anselme Payen made the initial discovery of amylase, as documented by Sharma *et al*. (2015) [9]. Amylases are classified into two main categories: exo-amylases, which target the non-reducing end of starch, and endo-amylases, which hydrolyze the glycosidic bonds within starch molecules, as indicated by Kamon *et al*. (2015) [10]. Amylase, a crucial enzyme in biotechnology, is predominantly sourced from microorganisms and possesses various industrial applications, as highlighted by Gopinath *et al*. (2017) [11].

3.1 α-amylase

The scientific name of α-amylase is 1, 4-glucan-4-glucanohydrolase, and the enzyme commission number is EC 3.2.1.1. Higher animals, plants, and microbes all secrete it (Lee and deMan 2018) [12]. A metalloenzyme, calcium metal is employed as a co-factor, which is essential for its correct operation (Sundarram *et al*. 2014) [7]. Endo-amylase that prevents hydrolysis of the terminal glucose and -1, 6-linkages and cleaves the -d-(1,4) glycosidic linkage (Pan *et al*. 2017) [13]. Starch serves as α-amylase's substrate. Temperature and other factors have a significant impact on enzyme activity. At pH 7.0, α-amylase functions best (Sundarram *et al*. 2014) [7]. Three-dimensional structure of α-amylase aids in substrate binding and contributes to its high level of activity specificity (Ofen *et al*. 2015) [14]. According to Sahni and Goel (2015) [15], the oligosaccharide chain that makes up α-amylase has 512 amino acids and a molecular weight of 57.6 kDa (Farooq *et al*. 2021) [16].

3.2 Industrial Application of α-Amylase

3.2.1 Starch conversion: The starch business depends on amylase for a variety of purposes. During the process of starch liquefaction, starch hydrolysis is carried out with the participation of the enzyme α-amylase. The end result of starch saccharification is glucose and fructose syrups (Radelof and Beck 2014). The starch conversion process comprises three distinct steps: (1) gelatinization, (2) liquefaction, and (3) saccharification. The first process, known as (1) Gelatinization refers to the process of dissolving starch granules in water to form a thick suspension. The water now includes both amylose and amylopectin as a result of this dissolving (unban *et al*., 2020) [6]. In the second phase of liquefaction, the starch solution's viscosity decreases due to the partial breakdown of starch residues into shorter dextrin chains. The third and final phase, called saccharification, involves further hydrolysis, resulting in the production of glucose syrup, fructose syrup, and maltose. This reaction is facilitated by glucoamylase, an exo-amylase that targets the -1-4-glycosidic bonds in the non-reducing portion. Fructose syrup, a valuable product used as an artificial sweetener in the beverage industry, can be easily obtained by isomerizing high glucose syrup with the assistance of glucose isomerase, as detailed by Farooq *et al*. in 2021 [3].

3.2.2 Food industry: The process of making cakes, beer, digestive aids, fruit juices and starch syrups are only a few of the processed food industries' many uses for amylases. The baking industry has widely employed α-amylases, which can be incorporated into bread dough to assist in the breakdown of starch from the flour into smaller dextrin molecules. Subsequently, yeast utilizes these dextrins as a substrate during fermentation. When α-amylase is added to the dough, the fermentative rate is accelerated and the dough's thickness is decreased, which improves the product's volume and texture. Furthermore, it results in a higher sugar content within the dough, which not only enriches the bread's taste and enhances the crust's color and toasting attributes but also contributes to preventing bread from becoming stale during the baking process and prolonging the softness and shelf life of baked goods (sources 29 and 86). Currently, the baking industry commercially utilizes a thermos table maltogenic amylase derived from *Bacillus stearothermophilus* (source 86). In addition, amylases find application in the clarification of beer, fruit juices, and animal feed before processing to increase the digestibility of fibers (as noted by Souza *et al*. in 2010) [17].

3.2.3 Paper industry: In the paper industry, α-amylase plays a crucial role in the hydrolysis of high molecular weight starch present in coated paper, resulting in a reduction of viscosity (Sahni and Goel, 2015) [15]. Coated paper is utilized to enhance paper properties such as smoothness, strength, and overall quality, consequently improving the caliber of written documents. The process involves breaking down the starch polymer, which has inherently high viscosity, using either a batch fermentation or continuous fermentation method. Besides its effectiveness as a paper coating agent, starch also serves as an exceptional sizing agent, contributing to paper quality by enhancing erasability, stiffness, and strength.

Commercially available microbial amylases used in the paper industry include brands like Termamyl®, amylase G9995® (Enzyme Biosystems, USA), Fungamyl, BAN® (Novozymes, Denmark), and Amizyme® (PMP Fermentation Products, Peoria, USA) (de Souza *et al*., 2010) [17].

4. Amylolytic lactic acid bacteria

According to Bhanwar and Ganguli (2014) [1], the genera *Streptoccocus, Lactobacillus, Pediococcu, Lactococcus*

Weissella and *Carnobacterium* are where most amylolytic LAB (ALAB) are found. As opposed to chemical starch hydrolysis, ALAB's starch-modifying enzymes (amylases) have a wider range of uses in the starch processing industries (Petrova *et al*. 2013, Smerilli *et al*. 2015) [18, 19]. According to Yousif *et al*. (2010) [20] and Turpin *et al*. (2011) [21], ALAB are mostly found in amylaceous fermented foods, such as fermented meals made from manioc (cassava) (damayanti *et al*., 2020) [22], sorghum, maize, rice, millet, taro etc. Fermented cereals, drinks with a maize base like Pozol (Daiz-Ruiz *et al*. 2003) [23], beer malt (Zeng *et al*., 2023) [24], and the probiotic beverage Boza (Petrova *et al*. 2013) [18] are other sources of ALAB. ALAB are also discovered in plant and animal digestive systems. The first *Lactobacillus fermentum*'s amylolytic strains were discovered in Benin's mawe, ogi, and sourdough (petkova *et al*. 2020) [26]. Similar to this, *Lactobacillus paracasei* B41, a recently discovered ALAB strain, was the first amylolytic member of the *Lb. casei* group (Petrova *et al*. 2012; Bhanwar and Ganguli 2014) [25, 1]. Since amylaceous foods have a high starch content, ALAB can rapidly transform these compounds into monosaccharides and disaccharides, rendering them readily available for lactic acid fermentation (Guyot *et al*. 2000) [28]. ALAB are mostly present in amylaceous foods. Inducing amylography and viscosity properties in starch using ALAB can alter their microstructure (Demiate *et al*. 2000) [27]. Amylolytic *L. fermentum* and *L. plantarum* strains have recently been identified in a variety of traditional amylaceous fermented foods from Nigeria, according to Sanni *et al*. (2002) [28]. Furthermore, Nakamura and recognised *Lb. amylophilus* as the first ALAB.

4.1 Industrial Application of Amylolytic Lactic Acid Bacteria

4.1.1 Starch based food processing: ALAB is a flexible enzyme utilised in the lactic acid generation process in the starch processing industry. It is more economically appealing because it directly combines the fermentation and saccharification processes by converting starch to lactic acid. In lactic acid fermentation, ALAB is used to preserve the nutritional value, longevity, consistency, fragrance, and flavour of finished goods. Additionally, it is employed in the production of a variety of fermented foods and drinks, including probiotic fermented meals and foods for hypersensitive youngsters. The extracellular amylases produced by ALAB are also employed as sourdough initiators to keep bread goods' authentic flavours and aromas. By inhibiting undesirable microorganisms and reducing the length of the amylopectin chain owing to the synthesis of oligosaccharides, this method enhances the properties of bread and lengthens the bread's shelf life. Beer, fruit juices, and smoothies may all have their starch removed using acidic -amylases. Due to their activity at 95 °C and pH 6.8, Ca+2 independent acid stable α-amylases are chosen over Ca+2 dependent amylases in the digestion of starch. ALAB is becoming more significant in biopharmaceutical applications, and it is anticipated that its use will increase.

4.1.2 ALAB in Grumble Made of Cereals: In many impoverished regions, grain-based porridge serves as a supplementary food source for young infants. Typically, the starchy flour is diluted with a significant volume of water to create a semi-liquid consistency suitable for feeding infants after cooking. This porridge exhibits a fluid texture, making it easy for young children to swallow, despite its relatively low fat content (5-10 g dry matter/100 ml). However, its energy content (20-40 kcal/100 g) falls below the recommended minimum of 84 kcal/100 g for children aged 9-11 months who are provided with two meals per day in addition to their regular breast milk intake (Bationo *et al*., 2019) [29]. Due to starch gelatinization, gruel becomes stiff with greater dry matter contents, making it inappropriate for young infants to eat. ALAB-based technologies have now been developed for the preparation of supplemental foods with a high calorie density that are semi-liquid, including gruel made from cereals or cereal-legume blends. By combining the generation of amylase and acidification using the same strain, utilizing ALAB as a functional initial culture could present a substitute for the traditional or industrial malt application. (Zeng *et al*., 2023) [24], improving fermentation control and producing products of higher quality. The capacity of ALAB (Lb. plantarum A6) to ferment rice-soybean mixes at high dry matter content, producing functional foods, has been examined. When this is combined with spray-drying, fermented flour may be used to make gruels that have a greater dry matter content (30-33%). In Burkina Faso, back slopping and ALAB were more effective than Lb. plantarum 6.1 at acidifying and partly hydrolyzing the starch in millet-groundnut slurry. High-energy density gruels with a liquid consistency were made possible by starch hydrolysis (84.7 4.4 and 80.4 23.8 kcal/100 g of gruel, respectively). In a research, ALAB strains, commercial probiotic bacteria strains, and co-cultures made of amylolytic and probiotic strains fermented gruels designed for school-age children made of rice flour and soy milk. The fibre from passion fruit had been added. The viscosity of the fermented products was less affected by the addition of passion fruit fiber than by the makeup of the bacterial culture.

4.1.3 Lacto-pickling ALAB: A key technology for the expansion of the amount of raw materials processed in the food industry is Lactic acid fermentation of vegetable items is employed as a preservation method in the production of both completed and partially processed products. ALAB are used to make lacto-juices, smoothies, and vegetable lacto-pickles. The procedure involves immersing the sliced and blanched roots in a solution of 2-10% NaCl and subsequently introducing them to the ALAB strain *Lb. plantarum* MTCC 1407 for a duration of 28 days. Sweet potatoes, which are high in anthocyanin or D-carotene pigments, were pickled by lactic acid fermentation (Panda *et al*. 2007) [30]. The final product had a pH between 2.9 and 3.0, titratable acidity between 2.9 and 3.7 g/kg, LA between 2.6 and 3.2 g/kg, and starch between 58 and 68 g/kg on a fresh weight basis. According to a sensory evaluation that considered attributes such as texture, taste, aroma, flavor, and the lingering taste, it was found that the lacto-pickle made from sweet potatoes was deemed acceptable.

5. Conclusion

While numerous microbiological sources can produce this enzyme effectively, only select strains of fungi and bacteria meet the criteria for industrial-scale production. α-Amylase has long played a pivotal role in starch-related industries. The search for novel microorganisms capable of amylase production is an ongoing endeavor. Recent studies by various authors have showcased successful methods for purifying α-amylase, enabling its application in pharmaceutical and therapeutic sectors where high-purity amylases are essential.

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