Effect of *Arthrobacter* in Dairy Products

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Milk is naturally a rich source of many essential nutrients; therefore, it is quite a suitable medium for bacterial growth and serves as a reservoir for bacterial contamination. The genus *Arthrobacter* is a food-related bacterial group commonly present as a contaminant in milk and dairy products as primary and secondary microflora. *Arthrobacter* bacteria frequently demonstrate the nutritional versatility to degrade different compounds even in extreme environments. As a result of their metabolic diversity, *Arthrobacter* species have long been of interest to scientists for application in various industry and biotechnology sectors. In the dairy industry, strains from the *Arthrobacter* genus are part of the microflora of raw milk known as an indicator of hygiene quality. Although they cause spoilage, they are also regarded as important strains responsible for producing fermented milk products, especially cheeses. Several *Arthrobacter* spp. have reported their significance in the development of cheese color and flavor. Furthermore, based on the data obtained from previous studies about its thermostability, and thermoacidophilic and thermoresistant properties, the genus *Arthrobacter* promisingly provides advantages for use as a potential producer of β-galactosidases to fulfill commercial requirements as its enzymes allow dairy products to be treated under mild conditions. In light of these beneficial aspects derived from *Arthrobacter* spp. including pigmentation, flavor formation, and enzyme production, this bacterial genus is potentially important for the dairy industry.

Arthrobacter milk dairy

1. Introduction

Milk is a rich source of protein and a whole range of nutrients necessary for growth, including fats, carbohydrates, vitamins, minerals, and essential amino acids. Related to its proteins and peptides, milk also provides several physiological functions such as immunoglobulins, enzymes, growth factors, hormones, and antibacterial agents [1]. All milk obtained from a variety of animal sources has a nearly neutral pH and a high-water activity [2]. Due to the requirement of appropriate nutrition for health benefits and body growth and development as well as body maintenance and protection from diseases, milk becomes a vital source of nutrition for humans. With the rapid rise in population growth, increasing income, urbanization, and changes in consumption habits, in addition to the normal consumption of milk as fluid, global demand for milk-derived products is continuing to grow.

In addition to being an excellent source of human nutrition, milk has been referred to as a great environment for the survival and growth of a wide range of microorganisms because of its near-neutral pH, high water content, and complex biochemical compositions. As an agricultural product, milk is collected from domesticated animals, particularly cows. The number and types of microorganisms exhibited in milk are affected by the seasons, farm hygiene, feed, and efficiency of storage. The microorganism found in milk can be categorized into three groups depending on their involvements, namely, pathogenic, spoilage, and fermented-food-derived bacterial strains [3][4]. Some nutrients contained in milk can be used directly by all microorganisms, while others are provided following the types of microorganisms presented such as lactose, which is not utilized by many bacteria, and large molecules—e.g., proteins and lipids, which must be broken down by enzymes before allowing sustained microbial growth [5]. Changes in biochemical activities in microbial communities during nutrient utilization led to the different characteristics of milk depending on the period of these activities; in this way, microbes beneficially associate with milk and dairy product technology. With accumulating knowledge about the functionality of microorganisms, it became less complicated to apply them in technological processes.

Nowadays, microorganisms are widely used in the food industry to produce various types of products due to their desirable properties. In the dairy industry, the utilization of microorganisms has been of increasing interest since it is an important part of quality control as well as quality development. Due to the up-to-date technologies developed over the past few decades, a variety of dairy products are available. In addition to lactic acid bacteria that are commonly present in milk, many psychrotrophic bacteria are also a major component frequently consisting of *Actinobacteria*. The particular composition of milk microbiota directly influences the development of dairy products. Microorganisms can trigger the fermentation of milk causing the production of lactate as well as the different effects on the organoleptic properties of fermented resultants [6].

Arthrobacter is a genus of bacteria that belongs to the ecologically and industrially important class *Actinobacteria* of the *Micrococaceae* family. This genus is an outstanding group of bacteria isolated from various sources and has been found to produce a great variety of pigment hues [7]. *Arthrobacter* strains are usually present as a contaminant in milk and play different roles in inducing diverse aspects of contaminated milk [8]. The genus *Arthrobacter* bacteria are widely distributed in nature even in various extreme environments as they are heterotrophic and do not require very attentive growth conditions. Bacteria of this genus are Gram-positive, catalase-positive, obligated aerobic, and non-spore-forming bacteria, with an optimum growth below 30 °C; moreover, several strains isolated from glacial environments are known as psychrophiles with an ability to grow well under below 0 °C [9][10]. At present, approximately 80 species of this genus have been described in the taxonomy [11]. The taxonomy of the genus *Arthrobacter* and related genera has been reclassified several times; therefore, some strains were originally recognized as belonging to the *Arthrobacter* genus but have been presently identified as other genera, and vice versa.

Protein, fat, and carbohydrates, represent significant macronutrients in milk, accounting for 3–5, 4–6, and 4–5% of the total milk content, respectively [12]. Nevertheless, genetic, environmental, physiological, and handling variables are generally regarded as factors affecting milk composition. The contamination of *Arthrobacter* in milk and dairy products is possibly encountered either earlier in raw milk or later in processed products and can cause negative or positive effects on the quality of milk and its products in terms of their nutrition. According to genome sequencing studies, certain strains of bacteria in the genus *Arthrobacter* exhibit the genes encoding proteolytic, lipolytic, and glycolytic pathways [13][14][15]. These revelations confirm the results of several studies related to such enzyme-producing *Arthrobacter* bacteria [16][17]. Proteolysis, lipolysis, and glycolysis are normally considered the important elements of biochemical change in milk during fermentation leading to the nutritional value of final products.

2. Proteolysis

Proteolysis is a hydrolysis reaction of peptide bonds in which proteins break down into smaller peptides and free amino acids. It is responsible for the metabolism of casein present in milk by the milk endogenous proteinase and other proteolytic enzymes of microorganisms present in milk; thus, leads to several biochemical reactions affecting nutritional and non-nutritional compounds—e.g., branched-chain and aromatic amino acids and sulfur-containing compounds. The branched-chain amino acids consist of leucine, isoleucine, and valine, whereas the aromatic amino acids include tryptophan, phenylalanine, and tyrosine [18]. The breakdown of amino acids via different pathways, namely decarboxylation and deamination, results in several compounds. Branched-amino acids can be decarboxylated using aminotransferases into amines, whereas dehydrogenases in deamination will convert these amino acids into carboxylic acids and ammonia [19]. Aromatic amino acids can also undergo decarboxylation and result in α-keto acids such as tryptophan to indole-3-pyruvate, tyrosine to hydroxybenzaldehyde, and phenylalanine to benzaldehyde [20].

Protein degradation generally occurs through extracellular enzymes synthesized by microorganisms during their growth or through intracellular enzymes produced up-on lysis. The synthesized proteinases expectedly involve casein degradation while those produced upon lysis tend to be peptidases and probably do not affect the degradation of casein but rather the breaking down of peptides originated by hydrolysis of proteinases [21]. By genome sequencing techniques, certain strains of *Arthrobacter* bacteria—e.g., *A. arilaitensis*, *A. aurescens*, and *A. chlorophenolicus*, demonstrate a number of genes encoding putative proteins with proteolytic functions [13][22].

The generation of peptides during microbial fermentation possibly produces the biologically active substances derived from milk protein. Milk-derived bioactive peptides are usually encrypted and inactive forms within the primary structure of milk protein and they are activated by proteolysis of casein namely α-, β-, γ- and κ-casein [23]. Numerous health-beneficial effects have been attributed to milk-derived bioactive peptides such as anti-hypertensive, hypolipidemic, anti-inflammatory, anti-oxidative, anti-microbial, and anti-osteoporotic effects [24][25][26]. In regards to health-promoting effects, milk-derived bioactive peptides are supposedly considered potential ingredients of functional foods.

3. Lipolysis

Milk fat is primarily composed of triglycerides with phospholipids, cholesterol, and other lipids present at low concentrations. Milk triglycerides can be degraded through lipolytic pathways by endogenous enzymes in milk and microbial enzymes secreted during fermentation. The lipolytic enzymes can hydrolyze substrates to generate different forms of fatty acids, i.e., short- and intermediate-chain and free fatty acids [19]. The formation of these lipid molecules through lipolysis mostly reflects in the ester bonds between triglycerides and fatty acids cleaved by the lipases [27]. Fatty acids constitute a significant energy source for the growth of aerobic microorganisms. Some *Arthrobacter* including strains isolated from dairy products encode many proteins with putative lipase or esterase activity [13].

In the human body, free fatty acids (FFAs) serve in the first instance as an essential energy substrate and have a role in biological processes. FFAs can affect the gene expression of macrophages, adipocytes, or endothelial cells. In addition, FFAs can modulate the production of chemokines and cytokines and the expression of genes coding for adhesion molecules, and give rise to pro-inflammatory and inflammation-pro-resolving lipid-derived species [28]. High concentrations of FFAs are associated with some physiological conditions, for example, insulin resistance, fatty liver disease, atherosclerosis, and myocardial dysfunction [29]. Unlike FFAs, short-chain fatty acids (SCFAs) and medium-chain fatty acids (MCFAs) have many health benefits. The bioactivities of SCFAs, especially three major components, i.e., acetate, propionate, and butyrate, have been widely investigated. SCFAs demonstrate anti-inflammatory, immunoregulatory, anti-obesity, anti-diabetes, anticancer, cardiovascular protective, hepatoprotective, and neuroprotective effects [30]. For the regulation of MCFAs, these molecules modulate glucose and lipid metabolism. The unique transport and rapid metabolism of MCFAs provide additional health benefits compared to long-chain fatty acids (LCFAs); therefore, the use of MFAs for treating metabolic and neurological disorders has been of increasing interest [31].

4. Glycolysis

Milk contains carbohydrates mainly in lactose form and trace amounts of monosaccharides and oligosaccharides. Lactose is a reducing disaccharide consisting of glucose and galactose. The metabolism of lactose to lactate is regularly metabolized by the glycolytic pathways, which are commonly associated with the fermentative metabolism in microorganisms. During fermentation, enzymes responsible for breaking down carbohydrates, which are synthesized by several bacteria contaminated in milk, hydrolyze lactose into its monomers, of which glucose will be further degraded. The principal products of lactose metabolism are L- or D-lactate, which are then oxidized into acetate and CO_2 while some microbial strains create other products such as alcohol [32].

Cheese ripening is a type of milk fermentation that is probably most relevant to bacteria belonging to the genus *Arthrobacter*. Generally, the process of glycolysis in the degradation of milk carbohydrates to lactate seems to be responsible for lactic acid bacteria since they are the dominant bacterial species found at the beginning of the ripening process [18]. *Arthrobacter* strains that are frequently isolated from ripened cheeses may partly display a role in converting lactose to lactate due to their occurrence in the middle and late stages of the ripening process. Cheese-isolated *Arthrobacter* strains are aerobic respiratory bacteria and thus produced most of their ATP by oxidative phosphorylation. According to the presence of lactate at the beginning of ripening and quinone-dependent lactate dehydrogenases, glycolysis by ripened-cheese *Arthrobacter* is importantly related to the utilization of lactate as a carbon source [13]. However, several short-chain components—i.e., acetate, acetoin, diacetyl, ethanol, and acetaldehyde—are produced from an intermediate in lactose metabolism, pyruvate, as it is a precursor of these compounds [20]. The identification of gene encoding for enzymes required for the catabolism of carbohydrates sufficiently confirms the ability to utilize lactose as a substrate for glycolytic pathways [13][15].

Lactose-hydrolyzed milk (low-lactose and lactose-free milk) has been used for the preparation of flavored milk, cheese, yogurt, and other dairy products that serve as an important alternative for people who are lactose intolerant. The hydrolysis of lactose in milk for food processing also prevents lactose crystallization in frozen and condensed milk products; thus, it helps in improving the quality of the products [33]. Moreover, the use of hydrolyzed milk in yogurt and cheese manufacture accelerates the acidification process, because lactose hydrolysis is normally the rate-limiting step of the process, which reduces the set time of yogurt and accelerates the development of structure and flavor in cheese [34]

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