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Cold-active enzymes in food biotechnology: An updated mini review

Mohammed Kuddus

Department of Biochemistry, University of Hail, Hail, Saudi Arabia

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ABSTRACT

Cold-active enzymes and their anticipated application in various industries including food industry attracted attention of worldwide scientific community. Cold-active enzymes, also known as psychrophilic enzymes, possess high catalytic activity at low and moderate temperatures. Due to low-temperature activity, these enzymes utilize less energy in biochemical reactions and also stabilize fragile compounds in the reaction medium. The source of cold-active enzymes is basically psychrophilic/psychrotrophic microorganisms which are found in cold environments. In comparison to mesophilic and thermophilic enzymes, till date, very few cold-active enzymes are known and least explored so far in the food industry. This review contains latest development and innovation in cold-active enzymes along with their applications in food biotechnology.

1. INTRODUCTION

The role of enzymes is well known in the production of foods since ancient times [1]. One of the most common examples is the production of beverages using an industrial enzyme. Nowadays, the finding of novel enzymes for its commercial applications in the food industry is a challenge for the food scientists and biotechnologists. However, biotechnology is emerged as an advance tool for food industries. This technology is providing new products, improving nutritional value, lowering production costs, improving food processing and also deal with waste problems, food safety problems, and packaging issues. In future also this is going to play an important role in food producing and processing industries. Nowadays, production of almost all commercial foods or its ingredients includes enzymes or enzyme-catalyzed reactions. Some common examples of enzymes application include production of alcoholic beverages, fruit juices, syrups, sweeteners, chocolates, infant foods, egg and bakery products, cheese and dairy products, candy, flavor development, and meat tenderization.

Enzymes have many advantages in food production and processing. The leading one is the replacement of old chemical-based technology with eco-friendly enzymes that provide biodegradable products along with environmental care. Moreover, enzyme-catalyzed processes produce less waste products (byproducts) due to the specific action of enzymes in comparison to chemical catalysts. Although plants, animals, and microbes produce most of the food enzymes, the enzymes produced by microbial sources are more advantageous than their equivalents from plant and animal sources due to the following reasons: (1) Low production cost, (2) more predictable and controllable enzyme contents of microbes, (3) easy availability of raw materials with constant composition for their cultivation, and (4) microbes comprises less injurious constituents in comparison to plant and animal tissues. In food industries, there are numerous possible applications of cold-active enzymes along with their producing organisms. Some common specific microbial enzymes used in food industries include α-amylase, β-amylase, β-glucanase, glucose isomerase and oxidase, aminopeptidase, amyloglucosidase, catalase, cellulase, pectinase, xylanase, cyclodextrin, glucanotransferase, transglutaminase, glucoamylase, hemicellulase, invertase, lactase, lipase, and protease [2-6].

Since past few years, it has been recognized that the cold-adapted enzymes along with its producing microorganisms deals vast prospective at commercial and biotechnological level [7-16]. In the coming days, it is expected that probably the significance of cold-active enzymes will be more than the thermostable enzymes. The important characteristics of psychrophilic or cold-active enzymes that fascinated its scope in biotechnology are; (1) they are cost effective as less amount of enzyme is required, (2) they are able to catalyze reaction without additional thermal aid, and (3) they can be inactivated selectively by mild heat input [17]. The application of cold-active enzymes can be beneficial not only for low energy requirement and their high specific activity but also due to their informal inactivation by relatively low heat. Furthermore, during the food processing adverse chemical reactions and bacterial contamination may be reduces at low temperature [18]. Nowadays, specific catalytic activity of cold-active enzymes is under consideration of global scientific community and need to explore at industrial level.

**Corresponding Author*
Mohammed Kuddus, Department of Biochemistry, University of Hail, Hail Saudi Arabia.
Email: mkuddus@gmail.com

2. SOURCE OF COLD-ACTIVE ENZYMES

The literatures suggested that cold-active enzymes are found in both prokaryotes and eukaryotes. However, most of the cold-active enzymes are obtained from microorganisms and fishes living in Arctic zones [15]. Most of our planet Earth has cold regions as it is surrounded by deep oceans that always bear temperature near about 3°C [19]. Psychrophiles are those extremophiles which are inhabitants of cold places [17]. Psychrophilic organisms are further categorized, on the basis of growth temperature, into psychrophiles and psychrotrophs/psychrotolerants. The optimum growth temperature for the psychrophiles is below 15°C; however, for psychrotrophs it is about 20-25°C [20]. These organisms are located in the cold regions of the Earth including polar zones, high mountains, glaciers, and deep oceans along with exteriors of flora and fauna surviving in cold atmospheres [21-27]. Psychrophilic microorganisms, including bacteria (e.g. *Pseudoalteromonas, Psychrobacter, Polaromonas, Psychroflexus, Polaribacter,* and *Pseudomonas*), archaea (e.g. *Methanogenium* and *Methanococcoides*), yeasts (e.g. *Candida* and *Cryptococcus*), fungi (e.g. *Penicillium* and *Cladosporium*), and microalgae (e.g. *Chlamydomonas* and *Chloromonas*) are basically located in soils, waters, plants, and animals of cold regions [10-11,13,15-16]. The isolation of these microorganisms and potential applications of cold-active enzymes are already described in many published literatures [10,13,15-16]. These psychrophiles are able to produce cold-active enzymes, namely, amylase, cellulase, pectinase, protease, and lipase that are able to degrade starch, cellulose, pectin, protein, and lipid, respectively [8-11,13-16]. Some of the latest cold-active enzymes and its producing microorganisms are presented in Table 1. The details of recently isolated enzymes and their mechanism are described by Santiago *et al.* [28].

3. COLD-ACTIVE ENZYMES IN FOOD TECHNOLOGY

There are tremendous scope of cold-active enzymes in food industry and biotechnology [7]. Some of the important applications are in milk, juice, meat, and baking industries. Cold-active β-galactosidase is responsible for the decreasing of lactose amount in milk processing industry. Lactose, a disaccharide sugar, is accountable for lactose intolerance throughout the world. Pectinases are used during juice extraction process in the fruit juice industry that reduces the viscosity and refine final product. In the meat processing industry, cold-active proteases are used for meat tenderization process. Some enzymes including proteases, amylases, and xylanases are helpful in baking processes to reduce the dough fermentation time along with retention of aromas and moisture levels. Other cold enzymes may also be used as substitutes to mesophilic and thermophilic enzymes in many industries [7]. Advantages of cold-adapted enzymes over mesophilic and thermophilic enzymes are described in various publications [15,17,29,30]. Nowadays, psychrophilic enzymes are mostly used in meat tenderization, food processing, flavoring, baking, brewing, cheese production, and in animal feed. Due to specific characteristic of cold-active enzymes, we can conclude that cold-active enzymes have much more to contribute in the field of food biotechnology. Even though the cold-active enzymes have high specific activity but small half-life and low thermal stability are the major drawback that limits utilization of such enzymes at industrial level. To increase thermal and solvent stability of cold-active enzymes, different methods of enzyme immobilization are recommended by various researchers [31]. Along with immobilization, different molecular tactics, for example, protein engineering, recombinant DNA technology and metagenomic approach could also be used to cope the commercial expectations and development of unique cold-active enzymes. Hence, instead of traditional methods of cold-active enzyme production (Fig. 1), these novel approaches could also contribute significant role in food industries.

The latest example of novel cold-active α-amylase (AmyA1) isolated from Antarctic psychrotolerant fungus (*Geomyces pannorum*) and its application in baking industry is reported by He *et al.* [32]. The study revealed that AmyA1 would have a great potential in traditional baking and food industry. AmyA1 was also immobilized on magnetic nanoparticles to improve its stability for possible industrial

Table 1: Production of some important cold-active enzymes by psychrophilic/psychrotolerant microorganisms (published from 2005 and onwards)

Cold-active enzymes	Source	Reference
α-amylase	*Microbacterium foliorum* GA2	[40]
α-amylase	*Bacillus cereus* GA6	[41]
β-glucosidase	*Exiguobacterium antarcticum* B7	[42]
Alkaline protease	*Stenotrophomonas maltophilia*	[43]
Chitinase	*Bacillus cereus* GA6	[44]
Esterase	*Pseudomonas mandelii*	[45]
Esterase	*Monascus ruber* M7	[46]
Esterase	*Psychrobacter pacificensis*	[47]
Esterase	*Streptomyces coelicolor A3*	[48]
Lipase	*Pseudomonas* sp.	[49]
Lipase	*Pseudomonas* sp. TK-3	[50]
Metalloprotease	*Curtobacterium luteum*	[39]
Protease	*Pseudoalteromonas haloplanktis*	[51]
Protease	*Pseudoalteromonas* sp.	[52]
Protease	*Aspergillus ustus*	[53]
Protease	*Clostridium* sp.	[54]
Protease	*Pedobacter cryoconitis*	[55]
Protease	*Bacillus cereus*	[56]
Protease	*Chryseobacterium* sp.	[33]
Pullulanase	*Exiguobacterium* sp. SH3	[57]
Transglutaminase	*Euphausia superba*	[58]

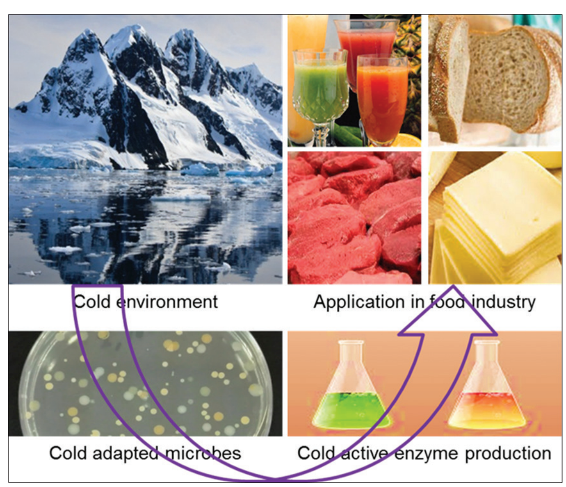

Fig. 1: Outline of cold-active enzyme production and applications

applications [32]. In another study, production of the cold-active protease was reported from a novel *Chryseobacterium* sp. Along with low-temperature activity; this protease was tolerant to several organic solvents and surfactants. Furthermore, it increases meat tenderization process that could be used in food processing industry at low temperature [33]. Cold-active esterase obtained from the marine Arctic metagenomics libraries showed high activity under the influence of high salt concentrations. Due to salt tolerance property at low temperature, this esterase could be a highly valuable candidate for cheese ripening processes [34]. Lipases, another important enzyme in food processing, are isolated by many psychrophilic microbes [35]. Recently, the gene encoding lipase was isolated from *Aeromicrobium* sp. and cloned using *Escherichia coli*. The recombinant lipase showed high catalytic activity and stability at low temperatures that have potential value in industrial applications as well as in food additive [36]. Pectin, a complex heteropolysaccharide, is one of the main constituents of higher plant cells that creates complications during the extraction and clarification of fruit juice. Polygalacturonase, a pectin-degrading enzyme, is commonly used for the treatment of pectin compounds in fruit processing industries [37]. Ramya and Pulicherla [38] recommended applications of cold-active polygalacturonase from *Pseudoalteromonas haloplanktis* in various food industries. On the basis of *in silico* analysis, they proposed that cold active polygalacturonase would be a better choice in comparison to its meso and thermo counterparts [38]. Some of the important cold-active enzyme and their applications are summarized in Table 2.

Table 2: Some of the cold-active enzymes produced by psychrophilic/psychrotolerant microorganisms and their applications in food industry (published from 2005 and onwards)

Cold-active enzymes	Source	Possible application in food industry	Reference
α-amylase	*Geomyces pannorum*	Baking and food industry	[32]
α-amylase, glucoamylase	Various microbes	Cheese ripening Single-cell protein from shellfish waste	[29]
β-galactosidase	*Paracoccus* sp.	Lactose hydrolysis in milk	[59]
β-galactosidase	*Arthrobacter* sp. 20B	Dough fermentation Bakery products	[60]
β-galactosidase	*Arthrobacter psychrolactophilus*	Conversion of cheese byproduct to glucose and galactose	[61]
β-galactosidase	*Arthrobacter* sp. 20B	Production of low-lactose milk Synthesis of galactooligosaccharides	[62]
Chitinase	Various microbes	Meat tenderizing	[63-64]
Esterase	-	Cheese flavor	[34]
Glycogen branching enzyme	*Rhizomucor miehei*	Wheat bread making	[65]
Laccase	Various microbes	Removal of lactose from milk Conversion of lactose in whey into glucose and galactose in dairy industry	[66-67]
Lipase	Various microbes	Protein polymerization and gelling in fish flesh Improvement in food texture flavor modification Production of fatty acids and interesterification of fats	[35]
Lipase	*Pseudoalteromonas haloplanktis* TAC125	Animal feed for the improvement of digestibility and assimilation	[68]
Lipase	*Aeromicrobium* sp. SCSIO 25071	Food additive	[36]
Pectin methylesterase	*Penicillium chrysogenum* F46	Food industry Fruit firming	[69]
Pectinase	Various microbes	Degradation of pectin in food processing	[70-71]
Phytase	*Erwinia carotovora*	Food processing	[72]
Polygalacturonase	*Cystofilobasidium capitatum* PPY-1	Degradation of pectin compounds	[37]
Polygalacturonase	*Pseudoalteromonas haloplanktis*	Pectin degradation	[38]
Polygalacturonase	*Achaetomium* sp. Xz8	Papaya juice clarification	[73]
Proteases	Various microbes	In beer, bakeries, and cheese industry Tenderization of meat Functional food ingredients in the form of soluble protein hydrolysates	[18]
Serine protease	*Chryseobacterium* sp.	Food processing industry	[33]
Xylanase	*Pseudoalteromonas haloplanktis* TAH3A, *Flavobacterium* sp. MSY-2	Xylan hydrolysis and improving bread quality	[74]
Xylanase	*Pseudoalteromonas haloplanktis* TAH3a	Baking industry Hydrolysis xylan to xylotriose and xylotetraose	[75]

4. FUTURE PROSPECTS

It is well documented that most of the cold-active enzymes reported till date have high catalytic capability at low and moderate temperatures in comparison to their homologous mesophilic enzymes. This specific property of cold-active enzymes make them valuable in industry and research due to their less requirements, reduced process times, save energy costs, easy inactivation by mild heat, and the loss of volatile compounds [18,39]. However, in the field of food biotechnology, it needed extra effort to cope various bottlenecks specifically high cost of enzyme isolation and purification, low stability of most cold-active enzymes and least explored cold-adapted microbes from the cold habitats. Application of recombinant DNA technology, in the expression of specific genes and production of greater amount of recombinant enzymes, may be an essential and effective tool to achieve commercial requirement of the cold-active enzymes. It can be concluded that cold-active enzymes have much more to contribute in the field of food industry due to its high specific activity at low temperature. More studies are required to screen psychrophilic microbial strains from various cold habitats and apply r-DNA technology along with protein engineering to upgrade their biotechnological potential and cope commercial expectations of cold-active enzymes in food biotechnology. In this review, only specific examples of cold-active enzymes along with their potential applications in the food industry are discussed in Table 2. There is rife literature on cold-active enzymes and their applications in food and other industries. However, very few psychrophilic enzymes are used in real industrial applications, especially in food processing. Further, it is expected that these enzymes, along with their producing microorganisms, will be an asset to various industries in the near future.

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