Psychrophilic Microbial Enzymes Implications in Coming Biotechnological Processes

Anam Javed\textsuperscript{a*}, Javed Iqbal Qazi\textsuperscript{b}

\textsuperscript{a}PhD scholar, Department of Zoology, University of the Punjab, Lahore, Pakistan.
\textsuperscript{b}Professor of Zoology, Department of Zoology, University of the Punjab, Lahore, Pakistan.

\textsuperscript{a}Email: anam.javed09@yahoo.com
\textsuperscript{b}Email: qazi.zool@pu.edu.pk

Abstract

Psychrophilic microorganisms produce a variety of cold-active enzymes which are used as production accelerators at commercial level to cope increasing demands necessitating low temperature conditions. The psychrophilic enzymes are frequently employed in food processing, textile, detergents, feed stocks, bioremediation, cosmetics, paper and pharmaceutical industries. But being extermophilic in nature, psychrophiles have certain pH, ionic strength and temperature limitation. To overcome such issues, their molecular biology and beneficial genetic engineering approaches are current goals of researchers. In this regard, many successful studies have accomplished importance of cold-active enzymes at industrial level. This review summarizes applications of potential psychrozymes.

Keywords: Production accelerators; bioremediation; ionic strength; genetic engineering; cold-active enzyme.

1. Introduction

Microbial flora exist in different habitable locations all around the globe and according to their optimum temperature requirements; microbes are divided into two categories i.e. mesophiles and extermophiles. Among them, mesophiles have been frequently used in various biotechnological processes e.g., from baking to industrial feed stocks’ preparation since ascent era. Moreover, potential estimation and utilization of extermophiles are in deliberation to get biotechnological benefits in recent decades. Extermophiles are further diversified into; thermophiles and psychrophiles.
As in major working areas temperature remains high so thermophiles are easily adjustable there to serve in already running set ups parallel to other products production. With gradual scientific progress, now psychrophiles are center of attention to get biotechnological gains. Their enzymes are **significantly indulged in various processes to serve mankind.**

### 1.1 Psychrophiles

Psychrophiles are those extermophiles which are inhabitants of cold places. Their survival temperature range tolerance is below zero to even above 20°C [1-3]. They are commonly found in ice dust [4, 5], glaciers, in all Antarctic zones and in artificial cold systems like refrigerators and freezers either at domestic or industrial level [6-11]. However, based on the growth temperature, psychrophilic organisms are again sub-divided in to two categories such as psychrophiles (optimal growth temperature below 15°C) and psychrotrophs or psychrotolerants (breed at optimal temperature of around 20-25°C) [12]. Moreover, cold environment represents an enormous pool of potential microbiota, ranging from Gram negative bacteria (e.g. *Pseudoalteromonas, Moraxella, Psychrobacter, Poloromonas, Psychrophlexus, Polaribacter, Moritella, Vibrio* and *Pseudomonas*), to Gram-positive bacteria (e.g. *Arthrobacter, Bacillus* and *Micrococcus*), Archaea (e.g. *Methanogenium, Methanococcoides* and *Halorubrum*), Yeasts (*Candida* and *Cryptococcus*) and Fungi (*Penicillium* and *Cladosporium*). Communally all these organisms have revolutionized the cold biotechnology [13-17].

### 1.2 Industrial implication of psychrophiles

There is an increasing demand for the utilization of microbial biocatalysts in industrial application because of their withstanding nature at diverse robust processing conditions. Most of the enzymes which are in use in today’s industrial methods are sourced from mesophiles. No doubt mesophiles are of commercial importance but their enzymatic action is limited at peak of different factors like temperature, pH and ionic bonds strength [1]. Moreover, the constrain for cost-cutting lies in the heating or cooling steps of industrial processes and increase in the upturn of the products of enzymatic reaction strength [18] can give a much attention to the use of proteins isolated from cold loving microorganisms. Several psychozymes and their biotechnological applications are shown in Table 1.

The documented data of psychrophiles revealed the capability to degrade an extensive variety of polymeric substances and the producer of various enzymes like amylases, cellulases, pectinases, α- Galactosidase, oxidases, protease and lipase etc. Due to the potential properties, cold-active enzymes have led to accelerate the concern in investment of considerable finance and global effort in the research and expansion for exploring more number of industrially important cold active enzymes in the fields of food commerce (such as pectinase, α-galactosidase), biopolishing and stone washing of textile items and detergent formulation industries (e.g., lipases, amylases, and cellulases). Moreover these psychozymes effectively take part in bioremediation (such as oxidases) and for biotransformations (methylases and aminotransferases) [52] as well as in biomedical applications [8]. This review covers the microbial cold adapted enzymes and their biotechnologically significant industrial implications.
Table 1: Major psychrophilic enzymes and their biotechnological potential

<table>
<thead>
<tr>
<th>Cold-active enzyme</th>
<th>Biotechnological implementation</th>
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<tbody>
<tr>
<td>Metalloprotease</td>
<td>Food, detergents, molecular biology [19]</td>
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<tr>
<td>Serine peptidase</td>
<td>Food, detergents, molecular biology [20]</td>
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<tr>
<td>Lipase</td>
<td>Food, detergents, cosmetics [21]</td>
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<td>Alkaline phosphatase</td>
<td>Molecular biology [22]</td>
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<tr>
<td>Alcohol dehydrogenase</td>
<td>Asymmetric Chemical synthesis [23]</td>
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<tr>
<td>3-Isopropylmalate dehydrogenase</td>
<td>Asymmetric Chemical synthesis [24]</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Biotransformation, biosensor, lactose removal from milk [25]</td>
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<tr>
<td>Valine dehydrogenase</td>
<td>Biotransformation [26]</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>Dairy industries (e.g. enhancing standards of ice-cream and whey) [27]</td>
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<tr>
<td>RNA polymerase</td>
<td>Molecular biology [28]</td>
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<tr>
<td>DNA polymerase</td>
<td>Molecular biology [29]</td>
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<tr>
<td>DNA ligase</td>
<td>Molecular biology [30]</td>
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<tr>
<td>Uracil-DNA glycosylase</td>
<td>Molecular biology [31]</td>
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<tr>
<td>Restriction Endonuclease UnbI</td>
<td>Molecular biology [32]</td>
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<tr>
<td>Triose phosphate isomerase</td>
<td>Biotransformation [33]</td>
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<tr>
<td>Chitobiase</td>
<td>Food, health products [34]</td>
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<tr>
<td>Chitinase A</td>
<td>Food, health products [35]</td>
</tr>
<tr>
<td>Cellulase</td>
<td>Animal feed, textiles, detergents [36]</td>
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<tr>
<td>Polygalacturonase (pectinase)</td>
<td>Preparation of cheese, wine and fruit nectar [37]</td>
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<tr>
<td>Pectate lyase</td>
<td>Cheese ripening, fruit juice and wine industry [38]</td>
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<tr>
<td>Nitrile hydase</td>
<td>Low-temperature acrylamide synthesis [39]</td>
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<tr>
<td>Pullulanase</td>
<td>Pullulan hydrolysis [40]</td>
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<tr>
<td>Xylanase</td>
<td>Dough rising, protoplast formation, wine and beverages production [41]</td>
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<tr>
<td>Alanine racemase</td>
<td>Food storage, Antibacterial agent [42]</td>
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<tr>
<td>α-Amylase</td>
<td>Detergents, Dough fermentation, desizing denim jeans, pulp bleach [43]</td>
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<tr>
<td>Glucoamylase</td>
<td>Starch hydrolysis [44]</td>
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<tr>
<td>β-Lactamase</td>
<td>Antibiotic degradation [45]</td>
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<tr>
<td>Phosphoglycerate kinase Catalase</td>
<td>Biotransformation Dairy, water treatment in paper, food, textile, semiconductor industries [46, 47]</td>
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<td>Aspartate carbamoyl- transferase</td>
<td>Biotransformation [48]</td>
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<td>Chlamysin (lysozyme-like)</td>
<td>Antibacterial agent, food preservation [49]</td>
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<tr>
<td>Isocitrate lyase</td>
<td>Biotransformation [50]</td>
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<tr>
<td>Malate synthase</td>
<td>Biotransformation [51]</td>
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2. Food technology

The potent involvement of cold-adapted enzymes in the food trade is significant. For example, in the milk industry, β-galactosidase is introduced at low temperature for lactose reduction [53] accountable for severe intolerances to the milk sugar in world’s around two thirds of the population. Moreover, this psychrophilic β-galactosidase can also be used for conversion of cheese byproduct, to quickly fermentable glucose and galactose [54]; whereas commercial addition of pectinases improves the fruit juice extraction, reduction of the viscosity and helps to elucidate the end product; another group of enzymes, proteases facilitate in meat tenderization; moreover, for bakery items enzymes like amylases, proteases and xylanases can be used to optimize the dough fermentation period along with the dough and the crumb quality enhancement, in addition to the retention of aromas and moisture grades. These psychrozymes act directly on starch, gluten and hemicellulases in the flour. Their other important feature is easy activation and this prevents the extended catalysis from altering the crumb which otherwise may become too sticky. That is why, available rheological data indicates efficiency of psychrozymes in the baking industry [55-60]. In addition to that a considerable range of cold active enzymes are regarded as ideal to mesophilic ones in brewing and wine industries, cheese manufacturing and animal feed, and so on [61].

3. Textile

Economically, a cold-adapted cellulase is of great importance in textile industry for biopolishing of fabric and stone-washing of jeans. In fabric production, the pretreatment with it inhibits protrudance of cotton fibers, pill formation and enhances texture durability and appearance of cloth even after several washes [61].
4. Detergents

At industrial level, 30%–40% of psychrozymes are consumed around the globe. The leading one is subtilisin (an alkaline serine protease usually obtained from \textit{Bacillus} species). Whereas at the domestic level, the recent practice is to utilize psychrozymes based detergents are employed for mechanical and financial input reduction as well as to shield texture and brightness of the garments. As an outcome, cold-active subtilisins are well known for best washing at moderate tap water temperatures. In this regard, the first psychrozyme subtilisins isolated from Antarctic \textit{Bacillus} species \[\text{[62, 63]}\]. However, they lack temperature stability for their shelf-life. Therefore, in current practice, engineered subtilisins are added in cold-active detergents that exhibit storage and alkaline stability along with cold-activity \[\text{[11]}\]. Moreover, the addition of other psychrophilic enzymes in surfactants like proteases, lipases, α-amylases and cellulases, as additives causes a decrease in energy input and wear and tear reduction. Meanwhile possible drawback is their instability as component of produce and their poor shelf life yet. Thus, still a channelized effort is required to improve the stability psychrozymes along with maintained good catalytic competence at colder temperatures \[\text{[63]}\]. In this account, one more improvement has mentioned in a recent report that another enzyme group of estrases from Psychrophilic \textit{Rhodococcus sp.} can be employed in detergents for effective stain removal \[\text{[64]}\].

5. Paper pulp

Various psychrophilic enzymes are currently used in paper industry. The major commercial contributors are xylanases which are capable to stand with the high temperature (55–70°C) and alkaline pH of the pulp proper biobleaching \[\text{[65, 66]}\]. Moreover, thermo-alkaliphilic or even thermoacidophilic xylanases may also serve as bioconversion agents in hot water and steam explosion, alkaline, solvent or acidic treatments \[\text{[67, 68]}\]. Moreover, cold active enzymes are involved in the catalysis of wood and other lignocellulosic matter to carbon dioxide, water, and humic substances. Such enzymatic action on cellulose, hemicelluloses, and lignin is required basically in paper and pulp industry. Psychrophilic cellulases are employed in biotechnological processes at commercial level to perform microbial delignification, and to serve as biosensors for pulp fiber surfaces analysis \[\text{[69]}\].

6. Pharmacy

In recent era, dehalogenases in pharmaceutical industries are indulged for the formation of optically pure drug intermediates including hydroxylalkanoic and haloalkanoic acids \[\text{[70]}\]. In this regard, over the last 20 years, the marine environment has been recognized as a potential reservoir of psychrozymes and metabolites \[\text{[71]}\]. Oceans contain a vast range of halogens; especially marine algae are the best organohalogen producers \[\text{[72, 73]}\] and the halogenated terpenes which are obtained from them, exhibit antimicrobial and anticancer properties \[\text{[74, 75]}\]. Such compounds are composed of vanadium bromoperoxidase and their leading sources are species e.g., \textit{Corallina}, \textit{Laurencia} and \textit{Plocamium} sp. \[\text{[70]}\]. In another recent study, it has been explored that cold active enzymes producer isolates of caves show good antimicrobial activity and their enzymes are of great pharmaceutical importance. They also exhibit hydrolytic and hemolytic activities at low temperatures \[\text{[76]}\]. Similarly, among the Antarctic psychrophiles, \textit{Shewanella} and \textit{Colwellia} are ideal producers of PUFA, such as
eicosapentaenoic acid or docosahexaenoic acid. The traditional source for long-chain polyunsaturated fatty acids (LPUFA) is effective to fight against numerous disorders like atherosclerosis, diabetes and hypertension. But their purification from fish oil comes with some problems at the risk of large scale production. Moreover, it is expected that a number of economically important fishes are likely to decline in the future. Whereas, oils of algal origin demand comparatively higher level of technology and budget than of psychrophilic ones who also have low lipid profile. Bacterial PUFA are component of some membranous phospholipids, that is why, they are considered as ideal producers of pure PUFA [8].

7. Environmental application

For ecological balance recovery, microbial bioremediation is major contributor since log time [77]. But in temperate zones, steep weather and thermal variations lessen the microbial decomposition of organic pollutants. As in 2014, Bowman and Deming [78] reported that psychrophilic microbes have alkane hydroxylase genes which enable them to degrade alkanes and crude oils in to harmless compounds. However, bioaugmentation and inoculation of pollutants with specific psychrophiles in mixed cultures are expected to enhance the biodegradation of recalcitrant substrates with great catalysis [9, 79]. However, the optimization of such psychrophiles is under process yet. For this purpose, European Union has selected contaminated waste water to estimate the required potential of psychrophiles for a vast range of toxicants along with various biopolymers degradation [77, 80, and 81]. Specifically, the quality of psychrophilic yeasts to catalyze a broad range of phenols and other hydrocarbons is demanding biotechnological perspective [82, 83]. Yeasts isolates from contaminated glacial origins decompose a considerable amount of various hydrocarbons at 10 °C [84]. Likewise, in 2005, Bergauer and coworkers [85] screened a set of psychrophilic yeasts inhabitant of Alpine zones degrade phenol and related monoaromatic compounds at 10 °C. Moreover, they can also assimilate few non- or low volatile aromatic substances which is a strain-related feature. Recently, it has noticed that cold active basidiomycetous yeasts fully decomposed up to 12.5–15 mM phenol at 10 °C under fed-batch cultivation which immobilization may improve yield [83, 86].

8. Biocatalyst

Catabolic activity of psychrophilic enzymes is under consideration now days and need optimization to get proper benefit [11]. The significant properties responsible for the three chief privileges of psychrozymes in biotechnology are as under:

- They are cost effective as less amount of enzyme is required to meet activation energy requirement.
- Psychrozymes are proficient without additional thermal aid.
- Due to thermal lability, their selective inactivation can be achieved with less heat input.
- The endemic psychrophiles can be a valuable new source of frequently required catalysts e.g., lipases from the yeast Candida antarctica or by the xylanase from the bacterium Pseudoalteromonas haloplanktis [13, 61, 87-92].

Moreover, a wide range of the commercially valuable fatty acid esters, peptides, oligosaccharide derivatives and
other compounds are gained from substrates having poor aqueous solubility which may be enhanced by introducing less water demanding enzymes [93]. Further investigations have revealed that the hydration level is best managed by limiting the water activity. Here, a conflict arises because in case of less hydration, enzymatic effectiveness usually reduces and it has direct relation with reaction kinetics. That is why, more supporting data is required yet [94]. Psychrozymes might therefore have a possible benefit for implications in less aqueous conditions due to their greater flexibility and tolerance than of mesophilic and thermophilic enzymes. This will permit the use of reduced water content with the consequence of rising yield [61].

9. Industrial usage

The polar yeast *Candida antarctica*, is of well renowned economic importance. It is producer of two types of lipases, A and B. among them, lipase B is involved in wide range of applications related to pharmaceuticals, cosmetics food and animal feed processing [11, 95, 96]. Moreover, in 2013, Novak and coworkers [70] reported that cold active dehalogenases are of industrial importance to achieve protein stability and flexibility in produce. Recently, it has been reported that cold active proteases [97, 98] lipases [99-101] cellulases [102-103] amylases [104, 105] pectinases [106] have found their role into many commercial wings applications like in detergents, food, textiles, cosmetics, beverages and bakery [107].

10. Cosmetics

Psychrophilic bacteria produce various types of enzymes but among them carboxylesterases, lipases and esterases are frequently utilized in cosmetics production. They are capable of hydrolysis of simple partially soluble ester-containing molecules. Similarly, lipases are considered ideal for hydrophobic long-chain triglycerides which are attractive constituents in various surfactants and cosmetic items [108, 109]. Moreover, it has also been observed that cold-active lipases upgrades application results of various beauty products [71]. In current era, cosmetic industry of China is using these psychrophilic enzymes to improve quality of their products [110].

11. Molecular biology

In this regard, cold active alkaline phosphatases have major scope in molecular biology. They are employed for the prevention of recircularization by pre-cloning DNA vector. However, the phosphatase should be vigilantly removed after dephosphorylation to evade disturbances in the later steps. Furthermore, *E. coli* and calf intestinal alkaline phosphatase have found heat-stable and they just require surfactant addition for inactivation. It follows that heat-labile alkaline phosphatases are exceptional alternatives as they are inactivated by moderate heat exposure in same glass ware with minimum amount of nucleic acid [111]. Fifteen years later, a group of Antarctic *V. bouriotis* isolates were explored for alkaline phosphatase and used as gene donor in *E. coli* cloning [112]. This effort solved its crystal configuration [113] and opened a way for further improvement to elevate catalysis and heat-lability [114]. Moreover, now days, the heat-labile alkaline phosphatase is also obtained from the Arctic shrimp *Pandalus borealis* for commercial purpose. In addition, psychrophilic lipase from deep sea inhabitant bacterium, *Psychrobactor sp.* has been used as target for gene cloning to make its utilization feasible in
mesophilic conditions effectively [115]. There are two more heat labile psychrophilic enzymes which are available for molecular biology applications. They are shrimp nuclease and heat-labile uracil-DNA N-glycosylase both capable of contaminants removal and DNA helix degradation during PCR. The former is used as recombinant form in *Pichia pastoris* which the later one is obtained from Atlantic cod (*Gadus morhua*) and used as recombinant form in *E. coli*. Both psychrozymes can be easily inactivated by moderate thermal treatment [11, 116].

12. **Fuel energy**

For low cost fuel and energy production, psychrophilic enzymes implication is of great importance. Recently, it has been reported that hydrogen gas can be produced during whey biofermentation process by cold active enzymes [117, 118]. In this regard, majority studies hydrogen gas production during fermentation by employing pure sugars and biomass were addressed [119]. A high carbohydrate content of food waste makes it a probable feedstock for bio-production of hydrogen by psychrophilic microbes [120, 121]. It has been observed that a wide range of psychrophiles inhibit required activity at moderate to low temperatures and from commercial and scientific view, there is a direct relation between enzymatic globular protein structure and thermal stability. So they fulfill this criterion positively for biohydrogen production [16, 122, and 123]. Moreover, the effective biohydrogen production from whey powder by using different psychrophilic anaerobic bacteria like *Rahnella aquatilis, Carnobacterium maltaromaticum, Trichococcus collinsii* and *Clostridium algidixylanolyticum* has been achieved so far [118]. In addition to that, agriculture and farm wastes are abundant, cost effective and valuable renewable natural resources as biofuels and other commercial chemicals production [124]. Such lignocellulosic waste provides carbonic substrates for anaerobic catalysis. However, this may be affected by thermal variations of surroundings. In this regard, cold temperature is a common stress factor and directly alters enzymatic action. But with scientific progress, a number of reports indicated that psychrozymes have proved themselves for fermentation even in cold milieu [125-127]. So, for proper use of biotechnological potential of cold active agents should be analyzed and obtained for better service in fuel industry [128]. Psychrophilic fermentors considerably requires less energy input required for heating the bioreactor and even work in frozen zones for conversion of agricultural wastes in biogas [125].

Because cellulose and xylan are the most abundant polymers in plants, their microbial transformation is under active research. Similarly, undigested lignocellulose and bedding materials of dairy manure like cellulose containing straws are required to be decomposed to produce simpler hexoses and pentoses, while, lignin is the limiting step in the degradation of lignocellulosic substrates due to extremely recalcitrant in nature [129, 130]. The heterogeneity and composite constitution are a major task for the anaerobic break down of lignocellulosic biomass from plant material [131]. According to reported data, now methane production from cellulose, xylan, cellulose and xylan mixture, cow feces, and wheat straw is possible at psychrophilic state (20°C) by utilizing optimized inoculums [132].

13. **Genetic engineering**

To enhance microbial biotechnology potential for better industrial output, their genetic engineering is under
consideration yet. However, engineered psychrophilic activity having mesophilic enzymes have not been reported to date. The main hurdle is reason complexity of amino acid substitutions and bonding patterns leading to low temperature catabolism, need long period of optimization. As an outcome, engineered psychrozymes currently escape the computational capability. In this regard, laboratory microbial evolutionary analysis has been found successful for production of psychrozymes with or without heat-lability [133]. It is important to mention here, in many cases; due to psychrophilic enzymes structural complications, the after effects of selective random mutations cannot be analyzed yet. For this purpose, the likelihood to introduce a specific property based directed evolutionary study seems to be the best methodology to engineer enzyme cold-activity. Improved cold-activity by chemical alteration has also been reported but still the outcomes of chemical modification remain capricious [134, 135]. So, more research work and estimation is required to achieve better biotechnological gains.

14. Conclusion and future perspective

It can be concluded that psychrophiles have much more to contribute to the field of biotechnology [61]. That is why; the recent increasing interests on psychrophilic microorganisms not only focus on the genomic and proteomic study to establish the relationships but also on production of industrial important substitutions from psychrophiles. Although the psychrophilic enzymes have high specific activity but small half life makes a major drawback in the utilization of these enzymes at commercial aspect. The leading target is to such genetically modified and engineered psychrophilic strains which will be able to cope commercial expectations. Thus it is necessary to identify further key feature of these psychrophilic enzymes to evaluate and upgrade their biotechnological potential [8].

15. Recommendations

It is recommended that further research work is required to develop genetically modified psychrophiles with enhanced enzyme production at achievable temperature range for economical commercial manufacturing.

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