JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

COLD-ACTIVE MICROBIAL CELLULASE: NOVEL APPROACH TO UNDERSTAND MECHANISM AND ITS APPLICATIONS IN FOOD AND BEVERAGES INDUSTRY

Ghazala Yunus[1] and *Mohammed Kuddus*[2*]

Address(es):
[1]Department of Basic Science, College of Preparatory Year, University of Hail, Hail-2440, KSA.
[2]Department of Biochemistry, College of Medicine, University of Hail, Hail-2440, KSA.

*Corresponding author: mkuddus@gmail.com

doi: 10.15414/jmbfs.2021.10.4.524-530

ARTICLE INFO

Received 6. 1. 2020
Revised 24. 9. 2020
Accepted 24. 9. 2020
Published 1. 2. 2021

Regular article

OPEN ACCESS

ABSTRACT

Cellulose, a carbohydrate mainly found in plant cell walls, is most abundant biopolymer on the Earth. Biodegradation of cellulose is carried out by a group of enzymes knows as cellulases. These enzymes play important roles in food processing and used in food industry. In the present era, cold-active enzymes are preferred instead of meso- and thermos-philic counterparts due to less energy requirement for their optimal activity and easy inactivation. The present study includes evaluation of cold-active cellulase from *Pseudoalteromonas haloplanktis* for its industrial applications in comparison to mesophilic and thermophilic cellulases, through molecular docking method. The binding energy of cold-active cellulase with the substrate cellulose was -126.60 KCals/mole. However, the energy for thermo- and mesophilic cellulase found to be -93.29 and -75.54 KCals/mole, respectively. The results concluded that cold-active cellulase has more efficacy compared to its counterparts and may be used in food processing industry at commercial level.

Keywords: Cellulase; cellulose; cold-active enzymes; food industry, *Pseudoalteromonas haloplanktis*, psychrophile

INTRODUCTION

Cellulose is an essential constituent of the primary cell wall of green plants, many forms of algae and the oomycetes (Klemm, *et al.* 2005). It is one of the most abundant natural biopolymer on the Earth and most dominating renewable agricultural waste with great potential for bioconversion to value-added bioproducts (Sadhu and Maiti 2013). Cellulases are the enzymes that hydrolyze β-1,4 linkages of cellulose chains and help in cellulose degradation. Cellulose degrading microorganisms produce all three types of cellulase components that include endo-1,4-β-D-glucanase (EC 3.2.1.4), exo-1,4-β-D-glucanase (EC3.2.1.91) and β-glucosidase (EC 3.2.1.21); and produced by bacteria, fungi, protozoans, plants, and animals (Zhang and Zhang 2013). These enzyme components either work separately in an enzyme catalyzed reaction or act synergistically in the form of a complex enzyme for complete cellulose hydrolysis. Cellulases produced by microorganisms have attracted worldwide attention because of their diverse applications in various industries including food and beverage industry. The major industrial applications of cellulases are as an additive in detergents, in food industry, textile industry, pulp and paper industry as well as in agriculture industry for controlling plant pathogens. The details of cellulase applications in various industries are presented in table 1 (Behera *et al.* 2017; Jayasekara and Ratnayake 2019). In food industry, cellulase is widely used in the coffee processing and wine making due to its cellulolytic activity (Anoop Kumar *et al.* 2018; Jayasekara and Ratnayake 2019). Cellulase degrades the skin of the grape and along with it removes tannins and the unpleasant aroma (Claus and Mojsov, 2018). Cellulase also used in reducing food spoilage and extraction of fruit juices along with other enzymes by working synergistically (Kuhad et al. 2011). Now cellulases account for a significant share of the world's industrial enzyme market and expected to further increase due to application in pretreated cellulose hydrolysis and formation of bioethanol and other bio-based products at commercial level (Zhang and Zhang 2013; Sadhu and Maiti 2013). It is estimated that the global demand for enzymes will increase upto 4.6 percent through 2020 to $7.2 billion. Food and beverages will remain the largest market for enzymes by value, with gains driven by increasing consumption of products containing enzymes. United Nation Department of Economic and Social Affairs (UNDESA) estimates that food demand is expected to increase by 70 % by 2050 due to increasing world population to 9.1 billion (http://www.un.org/waterforlifedecade/food_security.shtml). Food enzymes can resolve the shortage of quality food supply globally through increased food production and improvement in the quality such as flavor, texture and nutritional value (Neidleman 1984; Raveendran *et al.* 2018).

Table 1 Function and use of cellulases in various industry (modified from Behera *et al.* 2017).

Industry	Function	Application/Use	References
Food industry	Degradation of cell wall constituents; reducing viscosity of fruit juice, texture conservation	Fruits juice extraction, food colouring agent, Sensory properties modification of fruits, vegetables and oil, decreasing food spoilage	(Bhat 2000)
Beer and wine	Hydrolysis of plant cell wall polysaccharides, Modification of aromatic residues	Improvement in skin maceration and colour extraction of grapes, quality, stability and clarification and aroma of wines	(Galante *et al.* 1998)
Animal feed	Pretreatment of agricultural silage and grain feed for partial hydrolysis of lignocellulosic materials	Improvement in the nutritional quality of animal feed; increasing weight of broiler chickens; decreasing pathogenic bacteria accumulation in large intestine	(Cowan 1996; Godfrey and West 1996)
Textile and laundry	Break off the small fibre ends on the cotton fabric, thereby loosening the dye after washing, prevention or permanent	Bio-stoning of denim fabrics: bio-polishing of non-denim fabrics, defibrillation of lyocell containing fabrics and bio finishing, production of high quality and environmentally	(Kirk *et al.* 2002)

	removal of fuzz formation and pilling, Bio-polishing of cotton and non-denim fabrics, defibrillation of lyocell	friendly washing powders. Production of high quality fabrics	
Pulp and paper	Mechanical pulping, bio-modification of fibres, removing of ink coating and toners from paper	Increase tensile strength, reduce energy consumption, manufacturing of soft papers such as paper towels and sanitary papers	(Kuhad *et al.* 2010; 2010a)
Agriculture industry	To solubilize plant and fungal cell walls, fungal growth	Plant and fungal protoplasts formation, soil fertility, plant growth	(Beguin and Aubert 1994)
R&D industries	In the process of conjugation and fusion of genes; Expression of heterologous proteins and enzymes	Immobilisation and fusion of proteins, enzymes and antibodies; Production of high levels of proteins, enzymes and antibodies	(Bayer *et al.* 1995)
Biofuel industry	Conversion of cellulosic material to glucose and other fermentable sugars	Single cell protein formation and fermentation products such as ethanol	(Kuhad *et al.* 2011)
Pharmacy industries	Digestion of cellulose fiber	Cellulose, hemicellulose and beta-glucan breakdown by hydrolysis, digestin production	(Gupta *et al.* 2013)
Waste management	Degradation of cellulosic wastes	Environmental pollution control	(Kuhad *et al.* 2010b)

It is well documented that most of the commercial enzymes, including cellulases, used in various industries are derived from mesophilic or thermophilic microorganisms. However, in food and beverages industries application of cold-active enzymes are always preferred due to the following economic and environmental benefits: (1) high specific activity at low temperatures, (2) reduction in energyconsumptiom, (3) preservation of compounds that are volatile in nature, (4) contamination inhibition, (5) easy inactivation of enzyme by relatively low heat, (6) minimizes undesirable chemical reactions, and (7) decrease the cost of required heat treatments (Adapa *et al*. 2014; Kuddus and Ramteke 2012; Ramya and Pulicherla 2014). Thus, cold active enzymes from psychrophiles became hot topics, especially in food industry. Due to the enormous applications of cold active cellulase enzyme in different industries, it is required to understand its structural and functional characteristics along with mechanism of cellulose hydrolysis (Zhang and Zhang 2013). In the present study, a well-documented Antarctic microorganism *Pseudoalteromonas haloplanktis* is considered as an ideal psychrophilic organism used for cellulose degradation (Violot *et al*. 2005). The study includes investigation of cellulase protein sequences from thermophilic, mesophilic and psychrophilic microorganisms for characterization of their conservation at the level of amino acid and find out enzyme homology. Also, for understanding of enzyme interaction with cellulose substrate, docking studies have been carried out that explain activity and stability of psychrophilic cellulase compared to its thermophilic and mesophilic equivalents.

METHODOLOGY

Reclamation of cellulase sequences and multiple sequence alignment

In this study, sequences of cellulase enzyme were extracted from three different habitats such as psychrophilic, mesophilic and thermophilic. Based on potential industrial importance. Total 38 full-length cellulase sequences including 25 from bacteria, 11 from fungi and 2 from actinomycetes were selected from NCBI (www.ncbi.nlm.nih.gov) in FASTA format. *In silico* analysis and multiple sequence alignment for all three categories of cellulases were performed by the modified method of Ramya and Pulicherla (2014).

Phylogenetic investigation and dendrogram construction

Sequences of cellulase from bacteria, fungi and actinomycetes were aligned by using Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo/). The approaches given by Ramya and Pulicherla (2014) was used for phylogenetic trees construction.

Crystal structures of cellulases

In the present work, most prominent source of microbial cellulase from three different habitats *viz*. thermophilic, mesophilic and psychrophilic were selected. The crystal structure of cellulase from *Rhodothermus marinus* (thermophile), *Bacillus* sp. (mesophile) and *Pseudoalteromonas haloplanktis* (Psychrophile) were extracted from Protein Data Bank (PDB) (Table 2). The preference was given to the crystal structures that were obtained by X-ray diffraction method.

Table 2 Reported crystalline structures of cellulase enzyme from different bacteria (2000 onwards)

Organism	PDB ID	Total residue (Amino acids)	UniProt Accession ID
Xanthomonas citri	5HOS	342	Q8PRD4
Xanthomonas citri	4W7U	332	Q8PRD3
Xanthomonas citri	4W7V	332	Q8PRD3
Xanthomonas citri	4W7W	332	Q8PRD3
Xanthomonas citri with one mutation	5HPC	377	Q8PRD3
Xanthomonas citri with triple mutation	5HNN	1011	Q8PRD3
Caldicellulosiruptor saccharolyticus	5ECU	555	A4XHB2
Bacillus sp.	5E09	537	D4P8C6
Bacillus sp.	5E0C	537	D4P8C6
uncultured bacterium	3WX5	488	W8PWF3
uncultured bacterium	4HTY	359	I6PLH5
uncultured bacterium	4HU0	359	I6PLH5
uncultured bacterium	3II1	535	A1E9A6
uncultured bacterium	3FW6	534	A1E9A6
Rhodothermus marinus	3B7M	864	O33897
Rhodothermus marinus	2BW8	454	O33897
Rhodothermus marinus	2BWA	454	O33897
Rhodothermus marinus	2BWC	454	O33897
Rhodothermus marinus	1H0B	512	O33897
Pseudoalteromonas haloplanktis	1TVN	586	O86099
Pseudoalteromonas haloplanktis	1TVP	586	O86099

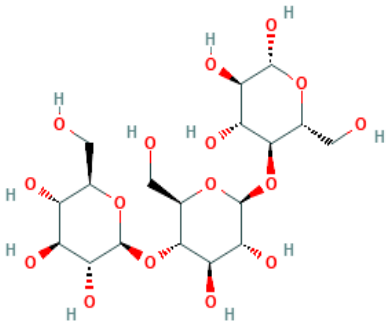
Analysis of active site

Active site residues of cellulase enzymes was retrieved from PDB database and by docking studied as describe by the method of Laurie and Jackson (2005).

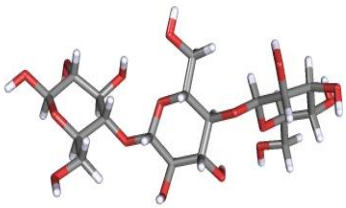
Molecular docking studies

Ligand preparation

The structures of cellulose unit (2D and 3D) was extracted from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Fig. 1). The structure was converted to .pdb format and optimized by means of ligand preparation using default settings in Molegro Virtual Docker (MVD-2010,4.2.0). In the structure, hydrogens were added through PyMol software and ligand was prepared for further docking studies (Ramya and Pulicherla 2014).



(a)



(b)

Figure 1 Structure of cellulose unit, 2D (a) and 3D (b)

Preparation of receptors (proteins) and docking studies

For docking studies, X-ray crystal structure of thermophilic (3B7M), mesophilic (5E09) and psychrophilic (1TVN) cellulases were retrieved from PDB and molecular structure was optimized through MVD and HYPERCHEM software (Adam 2008). The docking was performed by MVD software and best-docked ligand was determined based on MoleDockScore (Ramya and Pulicherla 2014).

Parameters for docking search algorithms

Parameters for algorithm and scoring functions for docking was used as per the method described by Srikanth et al. (2017).

RESULTS

Reclamation of cellulase sequences and multiple sequence alignment

On the basis documentation and as a maximum enzyme producer, total 38 cellulase sequences from different organisms including bacteria, fungi and actinomycetes were extracted from NCBI. The selection was also based on different habitats of organisms which includes thermophiles, mesophiles and psychrophiles and their potential applications at industrial level as mentioned in table 1 (Behera *et al.* 2017). The details of organisms, their accession numbers and number of amino acids found in enzymes are presented in Table 3. The result of multiple sequence alignment (Clustal Omega) shows major amino acids that are involved in active site (Fig. 2).

Table 3 Organisms producing cellulases and their accession number

Sl. No.	Microorganism	Accession number	Length (Amino acids)
BACTERIA			
1	*Acidothermus cellulolyticus*	ABK51910.1	469
2	*Anoxybacillus flavithermus*	GAC90965.1	355
3	*Anoxybacillus* sp.	EPZ39606.1	361
4	*Bacillus coagulans*	AEH52529.1	352
5	*Bacillus* sp.	BAB19360.1	824
6	*Bacillus* sp. BG-CS10	ADD62401.1	569
7	*Bacillus* sp. HY2-3	AAV34758.1	499
8	*Bacillus* sp. NBL420	AAK73277.1	440
9	*Bacillus* sp. WRD-2	AAX54913.1	499
10	*Cellulomonas fimi*	AEE44521.1	567
11	*Clostridium acetobutylicum*	KHD37670.1	878
12	*Clostridium autoethanogenum*	ALU36597.1	319
13	*Clostridium cellulovorans*	ADL50682.1	404
14	*Eubacterium cellulosolvens*	EIM57752.1	775
15	*Eubacterium* sp.	OLA09410.1	342
16	*Fibrobacter succinogenes*	ABU45500.1	910
17	*Methanosarcina thermophila*	BAW29155.1	357
18	*Microbispora rosea*	SIR96958.1	510
19	*Microbispora* sp.	ETK32165.1	536
20	*Pseudoalteromonas haloplanktis*	ADI47126.1	493
21	*Pseudomonas* sp.	AEM45646.1	467
22	*Pseudomonas stutzeri*	AFN77157.1	348
23	*Rhodothermus marinus*	AAB65594.1	260
24	*Ruminococcus albus*	ADU21423.1	644
25	*Ruminococcus flavefaciens*	AAB19708.1	455
FUNGI			
26	*Aspergillus flavus*	KOC18344.1	569
27	*Aspergillus niger*	CAA03658.1	331
28	*Fusarium solani*	CDL74337.1	82
29	*Humicola grisea*	BAA12676.1	388
30	*Humicola insolens*	CAA53631.1	388
31	*Mucor ambiguus*	GAN11144.1	499
32	*Neurospora crassa*	EAA30637.1	422
33	*Penicillium brasilianum*	OOQ91129.1	374
34	*Thermothelomyces thermophila*	AEO58455.1	337
35	*Trichoderma gamsii*	KUF02176.1	422
36	*Trichoderma harzianum*	KKP03485.1	419
ACTINOMYCETES			
37	*Streptomyces azureus*	GAP48400.1	535
38	*Streptomyces* sp.	EGE40950.1	359

```
Bacillus          ----------MALIQLSFK-SRALML-------QTSVNVLLPVGTNAVDFTPSDDF
Rhodothermus        MNNSSNNHKRKDFKVASLSLALLLGCSTMANAAVEKLTVSGNQILAGGENTSFAGPSLFW
Pseudoalteromonas   ---------------------------------AVEKLTVSGNQILAGGENTSFAGPSLFW
                                      .* * :*  * *:    **  :

Bacillus          S---YDSDP-----------FPVLY----LLHGATDDH---SAWL----RLSSIERYA
Rhodothermus        SNTGWGAEKFYTAETVAKAKTEFNATLIRAAIGHGTSTGGSLNFDWEGNMSRLDTVVNAA
Pseudoalteromonas   SNTGWGAEKFYTAETVAKAKTEFNATLIRAAIGHGTSTGGSLNFDWEGNMSRLDTVVNAA
                  *  :.::          * .    : **:: .     *    **.:: . *

Bacillus          EEKKLAVIMPNADMSAYTDMVHGHRYWTYISQELPAFLKATFPISQHRE-DTFAAGLSMG
Rhodothermus        IAEDMYVIIDFHSHEAHTDQATAVRFFEDVATKYGQYDNVIYEIYNEPLQISWVN--DIK
Pseudoalteromonas   IAEDMYVIIDFHSHEAHTDQATAVRFFEDVATKYGQYDNVIYEIYNEPLQISWVN--DIK
                 :.: **:  . .*:** . . *:: :: :  : :. : * :.   ::.  .:

Bacillus          GYGAF---KLALRQPERFVAA--VSLSGAVDM--------REVGQP--------------
Rhodothermus        PYAETVIDKIRAIDPDNLIVVGTPTWSQDVDVASQNPIDRANIAYTLHFYAGTHGQSYRN
Pseudoalteromonas   PYAETVIDKIRAIDPDNLIVVGTPTWSQDVDVASQNPIDRANIAYTLHFYAGTHGQSYRN
                  *.    *:  :*:.::..  : *  **:       ::.

Bacillus          ---------ESLF-----------------------------------------------
Rhodothermus        KAQTALDNGIALFATEWGTVNADGNGGVNINETDAWMAFFKTNNISHANWALNDKNEGAS
Pseudoalteromonas   KAQTALDNGIALFATEWGTVNADGNGGVNINETDAWMAFFKTNNISHANWALNDKNEGAS
                     :**

Bacillus          ---------------------VNAFGEGAKIAGTDHDLFHLV---KQL------------
Rhodothermus        LFTPGGSWNSLTSSGSKVKEIIQGWGGGSSNVDLDSDGDGVSDSLDQCNNTPAGTTVDSI
Pseudoalteromonas   LFTPGGSWNSLTSSGSKVKEIIQGWGG--------------------------------
                        ::.:*

Bacillus          ----------------------------------------AGSEGAKPAL-------
Rhodothermus        GCAVTDSDADGISDNVDQCPNTPVGETVNNVGCVVEVVEPQSDADNDGVNDDIDQCPDTP
Pseudoalteromonas   ------------------------------------------------------------


Bacillus          ------FQACGTDDFLYEDNVRFRDYARKVNANLTYEEGPGG----------------HE
Rhodothermus        AGTSVDTNGCSVV--SSTDCNGINAYPNWV--NKDYSGGPFTHNNTDDKMQYQGNAYSAN
Pseudoalteromonas   ------------------------------------------------------------


Bacillus          WAYWDRMIAHALDWLPLKTTN
Rhodothermus        W-YTNSLPGSDASWTLLYTCN
Pseudoalteromonas   ---------------------
```

Figure 2 Multiple sequence alignment of cellulase sequences obtained from meso, thermo and psychrophiles.

Phylogenetic tree and dendrogram

The selected protein sequences obtained from bacteria, fungi and actinomycetes were aligned with the Clustal Omega program. The dendrogram was constructed by using most acceptable and commonly used maximum likelihood method through MEGA software. The result shows that all the organisms appeared in three different clusters. In addition, the bacteria *Rhodothermus marinus* and *Microbispora* sp; and *Clostridium acetobutylicum* and *Eubacterium cellulosolvens* displayed different clusters. The organisms *Penicillium brasilianum*, *Fusarium solani*, *Humicola grisea*, *Humicola insolens*, *Aspergillus niger*, *Thermothelomyces thermophila*, *Trichoderma gamsii*, *Trichoderma harzianum* (among fungi), *Pseudomonas* sp., *Pseudomonas stutzeri*, *Acidothermus cellulolyticus* and *Microbispora rosea* (among bacteria) showed another clusters with similar sequence level. Other bacteria *viz*. *Clostridium autoethanogenum*, *Eubacterium* sp., *Methanosarcina thermophila*, *Anoxybacillus flavithermus*, *Anoxybacillus* sp. and *Bacillus coagulans* showed a different group of cluster. Also *Fibrobacter succinogenes*, *Pseudoalteromonas haloplanktis*, *Bacillus* sp., *Bacillus* sp. HY2-3, *Bacillus* sp. NBL420, *Bacillus* sp. WRD-2 and *Streptomyces* sp. showed sequence similarity and form another cluster. Other organisms including *Aspergillus flavus*, *Clostridium* cellulovorans, *Streptomyces azureus*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Bacillus* sp. BG-CS10 and *Cellulomonas fimi* showed a different group of cluster (Fig. 3).

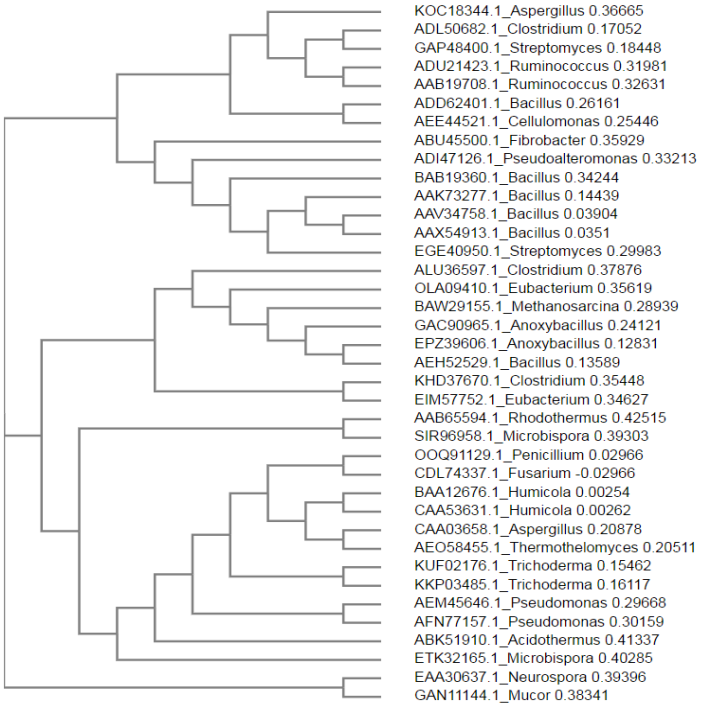


Figure 3 Phylogenetic tree of cellulase sequences

Validation of active sites

Protein sequences related to thermophile (3B7M), mesophile (5E09) and psychrophile (1TVN) were extracted from PDB (Fig. 4, 5 and 6). All the three modeled cellulase enzymes *viz*. thermophilic from *Rhodothermus marinus*, mesophilic from *Bacillus* sp. and psychrophilic from *Pseudoalteromonas haloplanktis* were also confirmed for their active site residues by using blind docking method. Active site residues of thermophilic, mesophilic and psychrophilic cellulases are presented in table 4 and also represented in Figure 7, 8 and 9, respectively.

Table 4 Amino acid residues for active site of cellulase enzyme from thermophilic, mesophilic and psychrophilic microorganisms.

Source	Active site residues
Thermophilic *Rhodothermus marinus*	HIS75, LEU76, LYS77, LEU120, CYS122, ASP239, ILE241, ASP260, LYS329
Mesophilic *Bacillus* sp.	ASN84, CYS99, THR104, GLN148, GLN155, ASN157, ASN174, PHE175, ASN200
Psychrophilic *Pseudoalteromonas haloplanktis*	TYR27, ASP30, THR31, GLN32, ARG48, PRO235, ALA236, GLY237, ASP238, GLY239, THR240

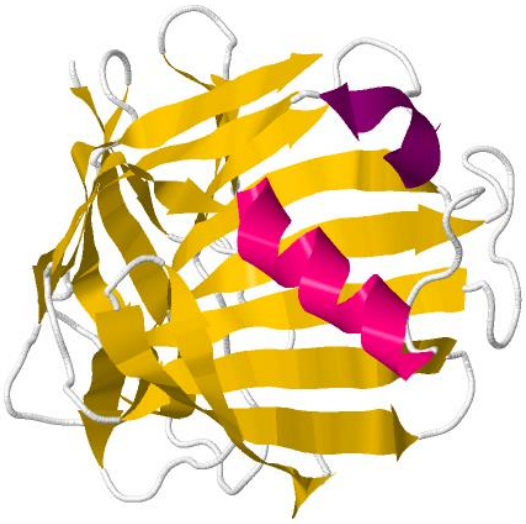


Figure 4 Modeled cellulase enzyme from thermophilic *Rhodothermus marinus* (3B7M)

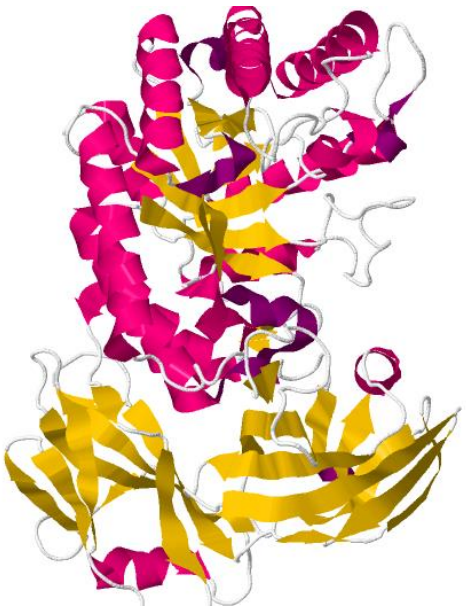


Figure 5 Modeled cellulase enzyme from mesophilic *Bacillus* sp. (5E09)

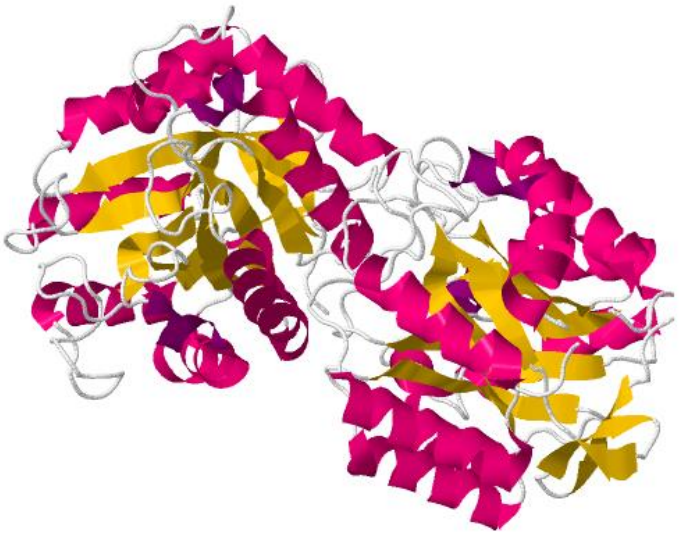


Figure 6 Modeled cellulase enzyme from Psychrophilic *Pseudoalteromonas haloplanktis* (1TVN)

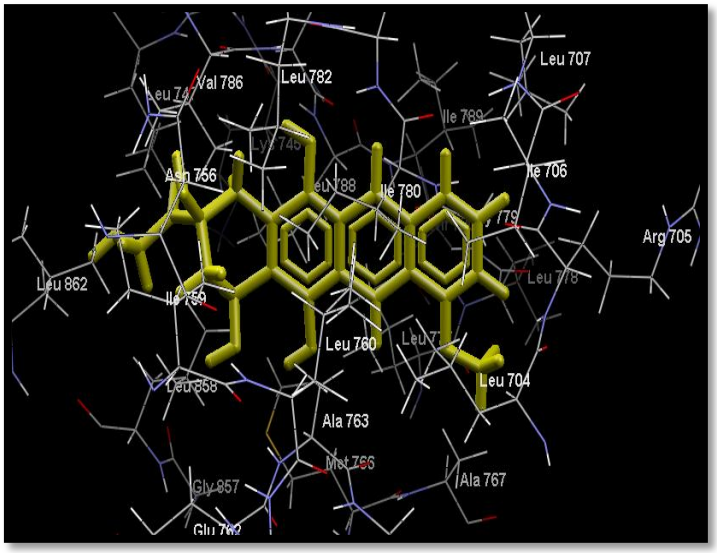


Figure 7 Enzyme cellulase from thermophilic *Rhodothermus marinus* docked with cellulose (in mesh)

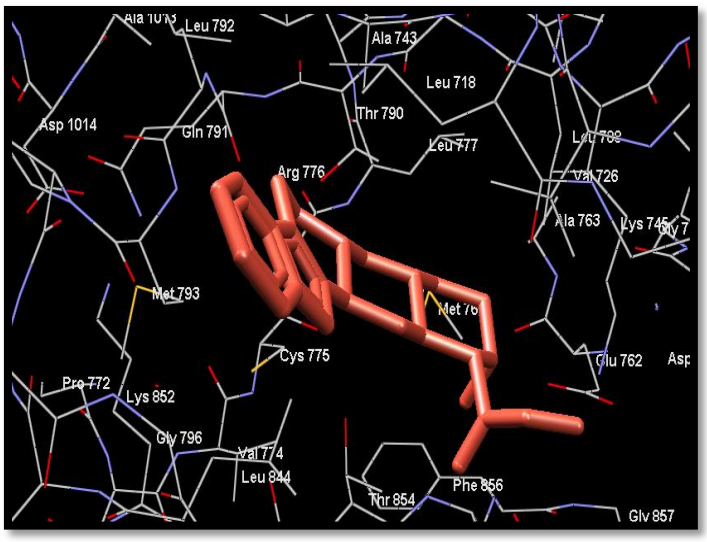


Figure 8 Enzyme cellulase from mesophilic *Bacillus* sp. docked with cellulose (in mesh)

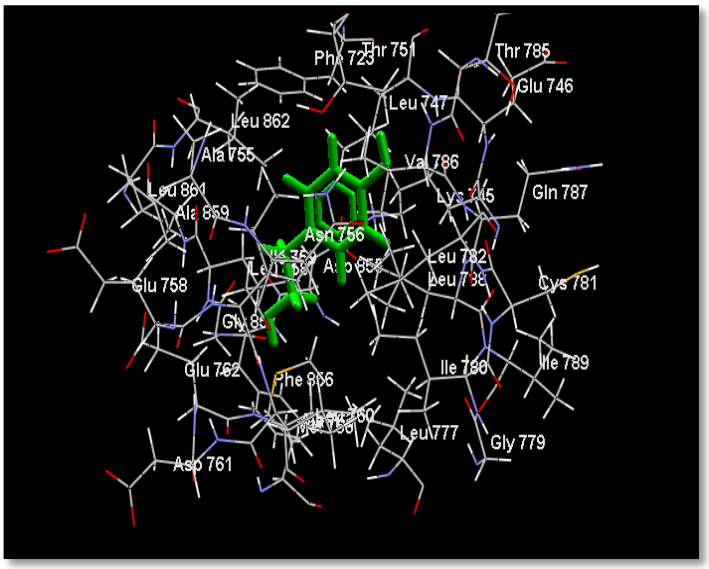


Figure 9 Enzyme cellulase from psychrophilic *Pseudoalteromonas haloplanktis* docked with cellulose (in mesh)

Molecular docking

The enzyme cellulase (receptor) from thermophilic, mesophilic and psychrophilic bacteria were used with the substrate cellulose (ligand) for further docking studies. The proteins were docked with cellulose unit and their binding energy were calculated as presented in table 5 (Figure 7, 8 and 9). The binding energy of the substrate cellulose for thermophilic, mesophilic and psychrophilic cellulase enzymes were -93.29, -75.54 and -126.60 Kcals/mole, respectively. The results concluded that based on different binding affinities of these enzymes, psychrophilic cellulase has shown to be most promising enzyme for substrate binding.

DISCUSSION

Cellulose is the major structural component of higher plants and a leading agricultural waste that can be hydrolyzed by microbial cellulase enzyme. Cellulose degradation by the enzyme cellulase involves hydrolysis of glycosidic bonds connecting the β-D-glucosyl residues of the cellulose. Cellulase has numerous industrial applications especially in food and beverage industries where it is widely used in coffee processing, wine making, degrading skin of the grape, reducing food spoilage and extraction of fruit juices (Behera *et al.* 2017). The enzymes used at industrial level are mostly isolated from mesophiles or thermophiles. Out of mesophilic and thermophilic enzymes, the second one is preferred due to its thermal stability at high temperature. However, maintaining low temperature (<15°C) play crucial role during in many food processing that maintain quality and propertied of final products (Molina *et al.* 2007; Ramya and Pulicherla 2014). Cold active enzymes play a significant role in many food industries as the process require mild condition to maintain the taste of products and to avoid spoilage of food materials (Hamid and Mohiddin 2018; Feller 2013; Gerday *et al.* 2000; Margesin and Schinner 1994; Russell 1998). In biofuel production, cold-active cellulase can produce ethanol from cellulose at low temperature resulting in saving production costs (Li *et al.* 2019). Cold-active enzymes has more flexibility in comparison to mesophilic and thermophilic enzymes (Adapa *et al.* 2014; Methe *et al.* 2005; Somero 2004). Along with cold-active enzymes, its producing microorganisms also possess significant characteristics such as modifications in the primary sequences of the proteins and greater number of flexible regions in order to tolerate the lower temperatures, in comparison to mesophiles and thermophiles.

In this study, cellulase enzymes from thermophilic, mesophilic and psychrophilic bacteria were selected by using bioinformatics tools for the study of similarity at the sequence level between these enzymes. The results of docking studies concluded that cold-active cellulase have strong affinity with the substrate cellulose and are energetically favorable in comparison to thermophilic and mesophilic cellulases. These binding is due to hydrogen bond formation among cellulose and amino acids of cellulase active site that are sufficient for strong bonding affinity (Patil *et al.* 2010). The cold-active cellulase possess reasonable low binding energy in comparison to its meso and thermo counterparts that clearly indicates higher efficiency of psychrophilic enzymes (Table 5). This finding would be encouraging for further research with respect to cold-active enzymes utilization in food and beverage processing among others. It is important to maintain low temperature in food processing rather than processing at high temperatures because low temperature treatments help in retaining nutritional value and taste and also avoid food spoilage which is very common problems in food processing industries (Nakagawa *et al.* 2004). The present *in silico* investigation about industrial enzymes concludes that cold-active enzymes, such as psychrophilic cellulase, have more efficient and beneficial than its counterparts mesophilic and thermophilic cellulases. Further research with different cold-active enzymes will be useful to evaluate and explore their potential at industrial level and their economic feasibility compared to thermophilic and normal mesophilic enzymes.

Table 5 Docking results of cellulase binding with cellulose ligand

Type	Protein	Ligand	Binding Energy	Affinity	Ranked score	Torsion
Thermophilic (*Rhodothermus marinus*)	Cellulase	Cellulose	-93.29	-20.54	-33.85	5
Mesophilic (*Bacillus* sp.)	Cellulase	Cellulose	-75.54	-19.00	-56.63	5
Psychrophilic (*Pseudoalteromonas haloplanktis*)	Cellulase	Cellulose	-126.60	-24.10	-44.00	5

CONCLUSION

Nowadays cold-active enzymes from cold adapted microorganisms referred as an important and valued component in different food and beverages industry. Due to their exclusive low temperatures activity; along with retention of volatile compounds, prevention of contamination and energy saving; make them very attractive for food scientist globally. The present study comprises *in silico* characterization of cellulases obtained from thermophilic, mesophilic and psychrophilic bacteria namely *Rhodothermus marinus*, *Bacillus* sp. and *Pseudoalteromonas haloplanktis*, respectively; and docking studies of enzyme with cellulose substrate was performed. This study may be considered as initial stage for additional *in vitro* research and in industrial applications. The reported cold-active cellulase is more effective than mesophilic and thermophilic cellulases. Additional investigation needed to explore cold-active cellulases for commercial application especially in food and beverages industries.

Conflict of interest: Nothing to declare.

REFERENCES

Adam, J., Kříž, Z., Prokop, M., Wimmerová, M., & Koča, J. (2008). *In silico* mutagenesis and docking studies of *Pseudomonas aeruginosa* PA-IIL lectin — Predicting binding modes and energies. *Journal of Chemical Information and Modeling*, 48(11), 2234–2242. https://doi.org/10.1021/ci8002107

Adapa, V., Ramya, L. N., Pulicherla, K. K., & Rao, K. R. S. S. (2014). Cold active pectinases: advancing the food industry to the next generation. *Applied Biochemistry and Biotechnology*, 172(5), 2324–2337. https://doi.org/10.1007/s12010-013-0685-1

Anoop Kumar, V., Suresh Chandra Kurup, R., Snishamol, C., & Nagendra Prabhu, G. (2018). Role of cellulases in food, feed, and beverage industries. *Green Bio-Processes*, 323–343. https://doi:10.1007/978-981-13-3263-0_17

Bayer, E. A., Morag, E., Wilchek, M., Lamed, R., Yaron, S., & Shoham, Y. (1995). Cellulosome domains for novel biotechnological application. *Carbohydrate Bioengineering, Proceedings of an International Conference*, 251–259. https://doi.org/10.1016/s0921-0423(06)80108-5

Beguin, P., & Aubert, J.P. (1994). The biological degradation of cellulose. *FEMS Microbiology Reviews*, 13(1), 25–58. https://doi.org/10.1111/j.1574-6976.1994.tb00033.x

Behera, B. C., Sethi, B. K., Mishra, R. R., Dutta, S. K., & Thatoi, H. N. (2017). Microbial cellulases – Diversity and biotechnology with reference to mangrove environment: A review. *Journal of Genetic Engineering and Biotechnology*, 15(1), 197–210. https://doi.org/10.1016/j.jgeb.2016.12.001

Bhat, M. K. (2000). Cellulases and related enzymes in biotechnology. *Biotechnology Advances*, 18(5), 355–383. https://doi.org/10.1016/s0734-9750(00)00041-0

Claus, H., & Mojsov, K. (2018). Enzymes for wine fermentation: Current and perspective applications. *Fermentation*, 4(3), 52. https://doi:10.3390/fermentation4030052

Cowan, W.D. (1996). Animal feed. *In*: Industrial enzymology. T. Godfrey and S. West, Eds. Macmillan Press, London, UK, 2nd edition, pp. 360–371.

Feller, G. (2013). Psychrophilic enzymes: from folding to function and biotechnology. *Scientifica*, 2013, 1–28. https://doi.org/10.1155/2013/512840

Galante, Y.M., De Conti, A. & Monteverdi, R. (1998). Application of trichoderma enzymes in food and feed industries. *In*: Harman, G.F., Kubicek, C.P. (eds). Trichoderma & Gliocladium – Enzymes, biological control and commercial applications. Vol. 2. London: Taylor & Francis, pp. 327-342.

Gerday, C., Aittaleb, M., Bentahir, M., Chessa, J.P., Claverie, P., Collins, T., … Feller, G. (2000). Cold-adapted enzymes: from fundamentals to biotechnology. *Trends in Biotechnology*, 18(3), 103–107. https://doi.org/10.1016/s0167-7799(99)01413-4

Godfrey, T. & West, S. (1996). Textiles. *In*: Industrial Enzymology, Macmillan Press, London, UK, 2nd edition, pp. 360–371.

Gupta, R., Mehta, G., Deswal, D., Sharma, S., Jain, K. K., Kuhad, R. C., & Singh, A. (2013). Cellulases and their biotechnological applications. *Biotechnology for Environmental Management and Resource Recovery*, 89–106. https://doi.org/10.1007/978-81-322-0876-1_6

Hamid, B., & Mohiddin, F. A. (2018). Cold-active enzymes in food processing. *Enzymes in Food Technology*, 383–400. https://doi:10.1007/978-981-13-1933-4_19

Jayasekara, S., & Ratnayake, R. (2019). Microbial cellulases: An overview and applications. *Cellulose*. https://doi:10.5772/intechopen.84531

Kirk, O., Borchert, T.V., & Fuglsang, C.C. (2002). Industrial enzyme applications. *Current Opinion in Biotechnology*, 13(4), 345–351. https://doi.org/10.1016/s0958-1669(02)00328-2

Klemm, D., Heublein, B., Fink, H.-P., & Bohn, A. (2005). Cellulose: fascinating biopolymer and sustainable raw material. *Angewandte Chemie International Edition*, 44(22), 3358–3393. https://doi.org/10.1002/anie.200460587

Kuddus, M., & Ramteke, P. W. (2012). Recent developments in production and biotechnological applications of cold-active microbial proteases. *Critical Reviews in Microbiology*, 38(4), 330–338. https://doi.org/10.3109/1040841x.2012.678477

Kuhad, R. C., Gupta, R., & Singh, A. (2011). Microbial cellulases and their industrial applications. *Enzyme Research*, 2011, 1–10. https://doi.org/10.4061/2011/280696

Kuhad, R. C., Gupta, R., Khasa, Y. P., & Singh, A. (2010). Bioethanol production from *Lantana camara* (red sage): Pretreatment, saccharification and fermentation. *Bioresource Technology*, 101(21), 8348–8354. https://doi.org/10.1016/j.biortech.2010.06.043

Kuhad, R.C., Gupta, R., & Khasa, Y.P. (2010b). Bioethanol production from lignocellulosic biomass: An overview. *In*: Lal B, Ed., Wealth from Waste, Teri Press, New Delhi, pp. 53-106.

Kuhad, R.C., Mehta, G., Gupta, R., & Sharma, K. K. (2010a). Fed batch enzymatic saccharification of newspaper cellulosics improves the sugar content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae*. *Biomass and Bioenergy*, 34(8), 1189–1194. https://doi.org/10.1016/j.biombioe.2010.03.009

Laurie, A. T. R., & Jackson, R. M. (2005). Q-SiteFinder: an energy-based method for the prediction of protein-ligand binding sites. *Bioinformatics*, 21(9), 1908–1916. https://doi:10.1093/bioinformatics/bti315

Li, Y., Wang, Z., Zhou, Y., Zhu, G., & Lin, L. (2019). Enzymatic identification and functional sites study of a novel cold-active cellulase (MkCel5) from *Microbacterium kitamiensea*. *Biotechnology & Biotechnological Equipment*, 33(1), 739–747. https://doi:10.1080/13102818.2019.1612278

Margesin, R., & Schinner, F. (1994). Properties of cold-adapted microorganisms and their potential role in biotechnology. *Journal of Biotechnology*, 33(1), 1–14. https://doi.org/10.1016/0168-1656(94)90093-0

Methe, B. A., Nelson, K. E., Deming, J. W., Momen, B., Melamud, E., Zhang, X., … Fraser, C. M. (2005). The psychrophilic lifestyle as revealed by the genome sequence of *Colwellia psychrerythraea* 34H through genomic and proteomic analyses. *Proceedings of the National Academy of Sciences*, 102(31), 10913–10918. https://doi.org/10.1073/pnas.0504766102

Molina, A. M., Swiegers, J. H., Varela, C., Pretorius, I. S., & Agosin, E. (2007). Influence of wine fermentation temperature on the synthesis of yeast-derived volatile aroma compounds. *Applied Microbiology and Biotechnology*, 77(3), 675–687. https://doi.org/10.1007/s00253-007-1194-3

Nakagawa, T., Nagaoka, T., Taniguchi, S., Miyaji, T., & Tomizuka, N. (2004). Isolation and characterization of psychrophilic yeasts producing cold-adapted pectinolytic enzymes. *Letters in Applied Microbiology*, 38(5), 383–387. https://doi.org/10.1111/j.1472-765x.2004.01503.x

Neidleman, S. L. (1984). Applications of biocatalysis to biotechnology. *Biotechnology and Genetic Engineering Reviews*, 1(1), 1–38. https://doi.org/10.1080/02648725.1984.10647779

Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., & Varma, A. K. (2010). Optimized hydrophobic interactions and hydrogen bonding at the target-ligand interface leads the pathways of drug-designing. *PLoS ONE*, 5(8), e12029. https://doi.org/10.1371/journal.pone.0012029

Pulicherla, K. K., Ghosh, M., Kumar, P. S., & Rao, K. R. S. S. (2011). Psychrozymes- the next generation industrial enzymes. *Journal of Marine Science: Research & Development*, 01(01). https://doi.org/10.4172/2155-9910.1000102

Ramya, L. N., & Pulicherla, K. K. (2014). Molecular insights into cold-active polygalacturonase enzyme for its potential application in food processing. *Journal of Food Science and Technology*, 52(9), 5484–5496. https://doi.org/10.1007/s13197-014-1654-6

Raveendran, S., Parameswaran, B., Ummalyma, S. B., Abraham, A., Mathew, A. K., Madhavan, A., Rebello, S., & Pandey, A. (2018). Applications of microbial enzymes in food industry. *Food technology and biotechnology*, 56(1), 16–30. https://doi.org/10.17113/ftb.56.01.18.5491

Russell, N. J. (1998). Molecular adaptations in psychrophilic bacteria: Potential for biotechnological applications. *Advances in Biochemical Engineering/Biotechnology*, 1–21. https://doi.org/10.1007/bfb0102287

Sadhu, S. & Maiti, T.K. (2013). Cellulase production by bacteria: a review. *British Microbiology Research Journal*, 3(3), 235–258. https://doi.org/10.9734/bmrj/2013/2367

Somero, G. N. (2004). Adaptation of enzymes to temperature: searching for basic "strategies". *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 139(3), 321–333. https://doi.org/10.1016/j.cbpc.2004.05.003

Srikanth, J., Kavimani, S, Reddy, C.U.M. & Chitra, K. (2017). Comparative molecular docking study of phytoconstituents identified in *Morinda citrifolia* Linn on acetylcholinesterase and butyrylcholinesterase. *Journal of Chemical and Pharmaceutical Research*, 9(4),121-125.

Violot, S., Aghajari, N., Czjzek, M., Feller, G., Sonan, G. K., Gouet, P., … Receveur-Bréchot, V. (2005). Structure of a full-length psychrophilic cellulase from *Pseudoalteromonas haloplanktis* revealed by X-ray diffraction and small angle X-ray scattering. *Journal of Molecular Biology*, 348(5), 1211–1224. https://doi.org/10.1016/j.jmb.2005.03.026

Zhang, X.-Z., & Zhang, Y.-H. P. (2013). Cellulases: characteristics, sources, production, and applications. *Bioprocessing Technologies in Biorefinery for Sustainable Production of Fuels, Chemicals, and Polymers*, 131–146. https://doi.org/10.1002/9781118642047.ch8