1.3.3 - Number of students undertaking project work/field work/ internships

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From : Principal
Secretary, G.B.

Number of students undertaking project work/field work/ internships

Programme name	Program Code	List of students undertaking project work/field work/internship
M.Sc Botany	BOT PG	1.Deblina Saha 2.Payel Roy 3.Rejaul Khan 4. Swati Karmakar 5.Sujata Chatterjee 6.Biswajit Pal 7.Tanushree Patra 8.Anwesha Sadhukhan 9.Dibya Mondal 10.Keya Dhara 11.Parthasarathi Mondal 12.Payel Goswami 13.Piyali Kar 14.Poulami Saha 15. Susmita Roy
Zoology	ZOOH	1. Bony Chettey 2. Ayesha Khatun 3. Annisha Chowdhury 4. Imajuddin Bayen 5. Barnali Modak 6. Piyali Dutta

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Project / Field Work Related Document

Botany

ROLE OF COLD SHOCK PROTEINS IN PSYCHROPHILES: A PROTEOMICS APPROACH

ROLE OF COLD SHOCK PROTEINS IN PSYCHROPHILES: A PROTEOMIC APPROACH

DISSERTATION REPORT
IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF
M.Sc. IN BOTANY

BY

PAYEL ROY (UID NO. 19173013008, Reg No. 00008 of 2019-20)

UNDER THE GUIDANCE OF
DR. SABYASACHI CHATTERJEE
ASSISTANT PROFESSOR

PG DEPERTMENT OF BOTANY
RAMANANDA COLLEGE
BISHNUPUR, BANKURA

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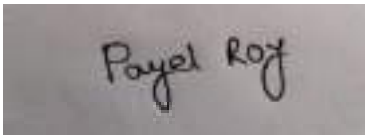
DECLARATION

I, **Payel Roy**, student of **M.Sc Botany** under **Department of Botany** of **Ramananda College(Bankura University)**, Bishnupur, Bankura, hereby declare that all the information furnished in this dissertation project is based on my review of research papers.

This dissertation does not, to the best knowledge, contain part of my review work which has been submitted for the award of my degree either of this college or any other college without proper citation.

Date – 15.08.2021



Payel Roy

UID-19173013008, Reg No. 00008 of 2019-20

M.Sc in Botany, Ramananda College

ACKNOWLEDGEMENT

I would like to acknowledge God for abundant blessings in my life that have allowed us to be where I am today.

I express my deep sense of gratitude and profound to my supervisor Prof. Sabyasachi Chatterjee (HOD, UG Department of Botany, Ramananda College) who helped and encouraged me for this work with great patience, motivation, enthusiasm and immense care. I am grateful to him for his valuable guidance and suggestions for this work.

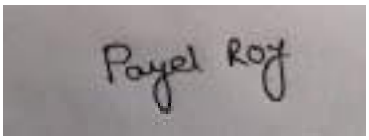
I would like to express my special thanks of gratitude to my teacher Dr. Ajit Datta (HOD, PG Department of Botany, Ramananda College) as well as our Principle Dr. Swapna Ghorai, who gave me the golden opportunity to do this wonderful project, which also helped me in doing a lot of Research and I came to know about so many new things, I am really thankful to them.

I am extremely grateful to my parent for their love, prayers, carrying sacrifices for education and preparing me for my future.

I would like to express my special heartily thanks to my supervisor Mr. Sourav Singha (SACT, Department of Microbiology, Bankura Sammilani College) who helped me at all stages of my review work. His suggestions and instructions contribute vast towards the completion of this review work.

And now, thanks to my friends, whose loves and friendships have provided the inspiration to strive harder and a foundation of stability both inside and outside of college, my thanks for the wonderful memories and those yet to be made.

In the accomplishment of this project successfully, many people have best owned upon their blessings and the heart pledged support, this time I am utilizing to thank all the people who have been concerned with this Review Work.



Payel Roy

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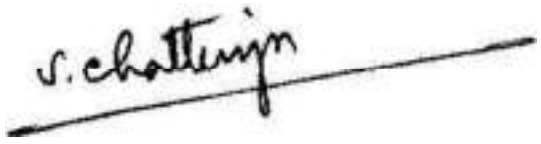
CERTIFICATE

This is to certify that the dissertation project entitled **"Role of Cold Shock Proteins in Psychrophiles: a proteomics approach"** has been carried out by Payel Roy (UID: 19173013008, Reg No. 00008 of 2019-20) under my guidance and supervision. To the best of my knowledge, the present work is the review of her original investigations and study done in the Depertment of Botany, Ramananda College. No part of the dissertation has ever been submitted anywhere for any other degree.

The dissertation is fit for submission and the partial fulfilment of the conditions for the award of degree in M.Sc in Botany.



Date-15.08.2021

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Dr. Sabyasachi Cahatterjee

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HOD, UG section of Botany Department

Ramananada College, Bishnupur

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Introduction:

Bacteria are generally dived into thermophiles, psychrophiles and mesophiles on the basis of temperature Ranges in which they can grow. Microorganisms, which are able to grow at low temperature have been known as Psychrophiles. Cold adapted microorganisms can grow at 0°C and their optimum and maximum temperature for growth are ≤15 and ≤ 20°C respectively (Robinson, 2001; Gounot, 1986). Psychrotolerant microbes have an optimum growth temperature between 20-40°C, but are also capable of growth at O°C (Morita, 1975). Cold environment represents an enormous full of potential microorganisms ranging from Gram negative bacteria, Gram Positive Bacteria, archaea, yeasts and fungi. These cold adapted microorganisms have proven to be more economical and eco-friendly when compared with microorganisms operating at normal or higher temperatures. Psychrophiles produce cold evolved enzymes that are partially able to cope with the reduction in chemical reaction rates induced by low temperatures (D'Amico *et al*. 2002). Cold active enzyme might offer novel opportunities for biotechnological exploitation based on their high catalytic activity in low temperature, unusual specificities and low thermo stability (Russell, 2000).

Psychrophiles have many useful biotechnological applications. For this, Psychrophiles have become increasingly studied in recent years, of the microorganisms most isolated and studied from cold environment, the majority are Bacteria (Margesin and Miteva, 2011). The range of species within a particularly cold habitat reflects many kind of parameters (for example, primary nutrient, ability to withstand desiccation, pH, salinity) to which an organism must adapt (Blaise *et al*. 2004).

This review aims to cover topics to highlight psychrophilic bacteria and their Cold Active Enzymes. It focused some of these following: (1) An introduction about Psychrophilic bacteria and their habitat (2) habitats and their biodiversity (3) examples of some this type of bacteria (4) some physiological activities with adaptation mechanism (5) bioinformatical analysis of cold adapted protein, (6) comparative proteome analysis of mesophiles vs psychrophiles and (7) a glimpse at some biotechnological uses of psychrophiles.

A common thread of all sections are showing how little we know about psychrophiles. A goal of this review is to raise awareness about psychrophiles that are having great potential and their characterization will enhance our basic knowledge of microbial physiology, enzyme structures and helps in developing industrial applications.

Habitats and biodiversity:

Ecological limiting factors, like water availability, pressure, salinity, nutrient, UV irradiation and temperature are all characteristics of cold environment. In some terrestrial habitats, these stresses dictate that psychrophilic organisms develop most effectively in protected niches (Cary SC, McDonald IR *et al*. 2010). The major region of the low temperature environment is represented by the deep sea (90% of the ocean volume), followed by snow (35% of land surface), glaciers (10% of land surface), sea ice (13% of the earth's surface) and finally permafrost (24% of land surface). Other cold environments are cold soils, cold-water lakes, caves and cold deserts. These earth dominant environments are successfully colonised by the communities of psychrophilic bacteria, algae, yeasts, archaea, insects and fishes, that are able to grow and even maintain metabolic activity at sub-zero temperatures. Soils of alpine regions undergo dramatic temporal changes in their microclimatic properties, suggesting that the bacteria encounter uncommon shifting in selection gradients (Meyer *et al*. 2004). Psychrophilic microorganisms have been studied by culture-dependent and culture-independent methods in permafrost as well as the microbial long-term survival in permafrost has been revealed. There is evidence that bacteria are able to survive in permafrost that is 500,000 years old (Gilichinsky *et al*. 2008; Steven *et al*. 2007, 2009; Johnson *et al.* 2007).

In bacterial family, there is many important members of the sea ice habitat, including many unique taxa. Heterotrophic gas-vacuolate bacteria, not reported in other marine habitats, have been discovered in and near sea ice. Among those cold-adapted bacteria, the genus *Colwellia* provides an unusual case. Members of this genus produce extracellular enzymes that capable of degrading high molecular weight organic compounds. These traits make *Colwellia* species important to nutrients and carbon cycling wherever they occur in the cold marine environment, from contaminated sediments to ice formations as analogs for possible habitats on other planets and moons (e.g Mars and Europa).

Representatives of the family Vibrionaceae are among the most commonly reported bacteria to populate almost all extreme environments. Nevertheless, a wide range of phylogenetic diversity within the genera *Alcaligenes, Colwellia, Achromobacteria, Cytophaga, Altermonas, Bacillus, arthrobacter, Aquaspirillum, Bacteroides, Flavobacterium, Brevibacterium, Methanogenium, Clostridium, Gelidibacter, Moritella, Phormidium, Methanococcoides, Methanosarcina, Polaribacter, Microbacterium, Micrococcus, Octadecabacter, Shewanella, Photobacterium, Vibrio, Polaromonas, Pseudomonas, Psychroserpens and Psychrobacter* have been found to be psychrophilic across the domain Bacteria (Hamdan, 2018).

In general, in deep sea habitats fungi are relatively rare compared to bacteria. Fungal isolates reported in frozen environments belong mainly to the genera *Penicillium, Rhodotorula, Alternaria, Ustilago, Cladosporium, Aureobasidium, Ulocladium, Valsa, Verticillium and Geomyces.*

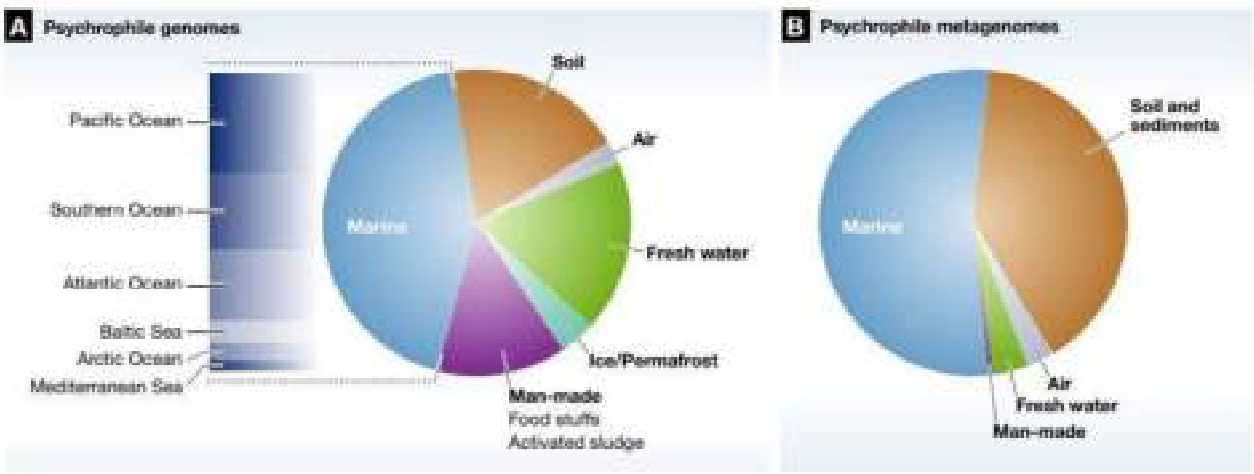


Figure 1: Distribution of psychrophile genomes and metagenomes in different cold ecosystems (Pieter De Maayer, Dominique Anderson, Craig Cary & Don A Cowan, 2014)

(A) Pie chart of the relative proportions of sequenced psychrophile genomes per ecological niche. Psychrophile genome statistics were determined by key word search against the GOLD database. The geographic distribution of marine genomes is given in the chart.
(B) Pie chart of the relative proportions of psychrophile metagenomes derived from different ecological niches. The psychrophile metagenomes include all datasets submitted to the MG-RAST database for which temperature data are available (lower than 15^0C).

Biotechnological Applications of Psychrophiles:

Most of the enzymes from psychrophiles are cold active and heat labile. In biotechnology, these specific traits are responsible for the 3 main advantages of cold shock enzymes: (a) as a result of their cold activity: they remain efficient at ambient temperature or tap water, therefore during a process avoid heating, either at industrial or domestic levels. (b) as a result of high activity: to reach a given activity, a lower concentration of

the enzyme catalyst is required. (c) as a result of heat lability: after a process by moderate heat input, they can be efficiently and sometimes selectively inactivated. Besides these traits, enzymes from organism's endemic to cold environments can be a valuable source of new catalysts possessing useful enzymological characteristics.

1. In Food Processing Industry:

Psychrophilic microorganisms have a huge range of applications in food industry, also in dairy industry. Psychrophilic milk coagulation enzymes have the advantages of controlled casein coagulation for maintaining the quality of whey resulting from cheese industry which can be used in other processes. By pasteurization, the enzyme activity in whey can be destroyed. In the market of developed countries, the commercial microbial rennet available with the brand names Marzyme, Rennilase 50TL. and Modilase are products of cold active microorganisms. Another interesting application of cold shock enzymes is in the form of Beta-galactosidase. Lactose hydrolysis in whey and milk to glucose and galactosidase results in increased digestibility, solubility and sweetness of milk. Beta-galactosidase acquire from mesophilic strains of *Kluvermyces* and *Aspergillus* strains are active at relatively higher temperatures i.e. 30-40°C, and the milk has to be processed in conventional methods for at least four hours for complete hydrolysis of lactose. During the process these conditions increase the chances of microbial contamination. At 5-10°C, with the use of thermolabile Beta-galactosidase hydrolysis of lactose can be carried out in about 16-24 hours. Using the cold active Beta-galactosidase 70-80% of products yields can be obtained, which is much higher in comparison to the processes obtained using enzyme from mesophilic organisms. The commercial cold active neutral protease is mainly obtained from *Bacillus subtilis* and being marketed under the commercial name eutrase. The enzyme is known to increase the flavour intensity with reduction in the ripening time from 4 to I mon. Psychrophilic microorganisms are able to produce various enzymes of industrial importance. Neutral proteases from psychrophilic bacteria are being used in cheese maturation. Polymer degrading enzymes such as amylases, pullul anases, xylanases, and proteases are employed in food processing. Proteases with low optimum temperature and high pH are being marketed under the commercial names Savinase, Maxaca, and Opticlean.

2. Source of Natural Pigments:

Carotenoids are present in various microorganisms and they play an important role in protecting the photo synthetic machinery of the organism from photo oxidation. Several bacteria of antarctic origin can also produce pigments and mainly belong to the *Flectobacillus, Pseudomonas, and Micrococcus*. As there is growing tendency to use natural pigments, bacterial pigments of different hues and colours may prove to be handy and renewable source for food processing industry.

3. Lipids as Food Additives:

Microbial lipids containing polyunsaturated fatty acids (PUFA's) are recommended to increase nutritional value of food products and as additives in cosmetics and as starting substrates for the preparation of pharmaceuticals. In marine microorganisms, polyunsaturated fatty acids are commonly found. These organisms produce PUFA's in response to low temperature of marine habitats. Lipids extracted from psychrophilic antarctica bacteria and marine algae mainly consist of C18 and C16 unsaturated fatty acids. *Anadymene stellata*, a marine alga, can synthesized 16-22 carbon containing unsaturated fatty acids possessing as much as four conjugated double bonds. In chloroplast and endoplasmic reticulum of these eukaryotic microorganisms, the synthesis and modification of fatty acids mainly occurs. A group of psychrophilic sea ice derived bacterial strains are known to produce polyunsaturated fatty acids such as arachidonic acid and eieosapentaenoic acid. Bacteria of Flavobacteriacea family known to synthesize a range of volatile fatty acid containing lipids in addition to algae.

4. Hydrolysate of Biomass as Feed Stock:

In *Laminaria sp*, the extra cellular production of decomposing enzymes was partly characterised in marine bacterial isolates belonging to the genera *Alteromonas sp, Flavobacterium sp, Pseudomomonas sp, Moraxella sp.* These enzymes have a highly active against many marine polysaccharides such as cellulose, alginate, fucoidan. In marine bacterial populations hydrolytic activity is a common trait. At a depth of 4500m sea water bacteria and cyanobacteria participate in the biodegradation of Phyto detritus between 2^0C to 15^0C temperature. Most of the psychrophilic micro algae has been listed from Antarctica and other chilling habitats, cause of their inexpensive growth requirements substrate comprising solar light and other inorganic compounds attend in marine waters can be used for biochemical production like carotenoids, protein, vitamins, foods, pigments polysaccharides. Hydrolytic activity of microorganisms may help in manufacturing liquid fuel and SCP after hydrolysis of vast amounts of sea weeds and aquatic plant biomass.

5. Detergents:

Globally, 30%-40% of psychrozymes are used at industrial level. At domestic level, psychrozymes based detergents are employed for mechanical and financial input reduction, to shield texture and in brightening clothes. Subtilisin, alkali serine protease collected from *Bacillus* species, known for best washing.

PHYSIOLOGICAL ADAPTATION OF PSYCHROPHILES:

In growth temperature physiological adaptations can be identified by comparing the properties of microorganisms that grow naturally at different temperatures. Compared with protein adaptations where insight can be gained by comparing the properties of proteins between thermophiles and psychrophiles, physiological adaptation is more complicated owing to the greater number of factors that can impact the complex variety of components in a cell and ultimately cause an adaptive response. Physiology of cells is dictated by its regulation of gene expression and genomic complement of genes. Depending on the environment, a large number of abiotic (e.g., oxygen, pH, nutrient flux, salinity), biotic (e.g., antibiotics, predation by grazers and viruses, cell-cell interactions) and broader ecological factors (e.g., particle attached versus free living, sea ice versus seawater) can greatly influence the selection and growth properties of microorganisms. Most of the diversity of microorganisms, colonising in Earth's biosphere, is widespread in the cold. Very few microorganisms can successfully colonise both high and low temperature extremes have developed. Methanogens, members of *Archaea,* the only group known to have individual species that spread the growth temperature range from sub-zero to 122^0 C (Saunders *et al*. 2003, Reid *et al*. 2006, Cavicchioli 2006, Taki *et al*. 2008).

There are limited chances to compare the adaptive traits of thermophiles and psychrophiles that belongs to the same of family. Therefore, physiological adaptations knowledge has been obtained by examining the response of individual microorganisms to different growth temperature. Global expression studies (e.g., transcriptomics, proteomics) linked to knowledge of straight physiological measurements (e.g., growth rate, solute composition, modification of nucleic acids temperature and nutrient perturbation of morphology, rates of macro molecular synthesis, membrane lipid composition) have demonstrate particularly valuable for determining the mechanisms of psychrophile adaptation (Cavicchioli 2006).

	PROTEIN NAME	ORGANISM	PDB ENTRY	AMINO ACID	ACCESSION NUMBER
1	Phosphoheptosa isomerase	*Colwellia psychrerythraea 344*	5BY2	260	OUR77399
2	Alpha amylase	*Pseudoalteromonas haloplanctis*	1G94	448	IG9H-A
3	Thioesterase	*Arthrobacter sp.*	1Q4S	151	IQ4U-B
4	subtilisin	*Bacillus subtilis*	2GK0	381	SNY73755
5	Beta-galactosidase bga	*Halorubrum lascusprofundi ATCC 49239*	6LVW	700	B9LW38
6	Aliphatic amidase	*Nesterenkonia sp.*	5JQN	263	ACS35546
7	Isocitrate dehydrogenase	*Desulfotalea psychrophila*	4AOV	402	WP-011188023
8	Aspartate carbamoyltransferase regulatory chain	*Moriteua profunda*	2BE7	153	2BE7-F
9	Competence atimwating peptide type 2	*Streptococcus pheumoniae*	6COV	41	C0T07865
10	Adenylate kinase	*Marinibacillus marinus*	3FB4	216	AAT90907
11	Tyrosine phosphatase	*Shewanella sp.*	1V73	336	2ZBMLA
12	Endonuclease 1	*Vibrio cholerae*	2G7F	227	AEU11429
13	Lipase	*Photobacterium sp. M37*	2ORY	340	AAS78630
14	Superoxide dismutase	*Allivibrio salmonicida*	2W7W	194	OAH83634
15	Pseudoalteromonas arctica PAMC 21717	*Pseudoalteromonas arctica*	5YLF	347	5YL7-A
16	Cellulase	*Pseudoalteromonas haloplanctis*	1TVN	376	WP-058429549
17	S-formylglutathione hydrolase	*Pseudoalteromonas haloplanctis*	3LS2	278	WP-036968767
18	Phosphoglycerate kinase	*Pseudomonas sp.*	6I06	387	WP-030137856
19	Cytochrome c552	*colwellia psychrerythraea*	4O1W	606	OUR80884
20	Beta galactosidase	*Marinominas sp.*	6Y2K	657	ABR70937
21	BA42 protein	*Bizionia argentinensis JUB59*	2LT2	145	2LT2-A
22	Beta-lactamase	*Pseudomonas fluorescens*	2QZ6	381	KJH87413
23	Deoxyribose-phosphate aldolase	*Colwellia psychrerythraea*	5C2X	256	KGJ89957
24	3-phosphoshikimate 1-carboxyvinyltransferase	*Colwellia psychrerythraea 34H*	5XWB	426	AAZ27668
25	Triosephosphate isomerase	*Moriteua marina*	1AW2	256	AAA88910
26	Leucine dehyalogenase	*Sporosarcina psychrophila*	3VPX	364	BAMO5529
27	Fumarylacetoacetate hydrolase	*Exiguobacterium antarcticum*	6IYM	352	K0A9N9
28	ATP phosphori bosyltransferase	*Psychrobacter arcticus*	5M8H	231	WP-011281160

29	Haloalkane dehyalogenase	*Phychrobacter cryohalolentis K5*	6F9O	309	6F90-A
30	Inorganic pyrophosphatase	*Shewanella S AS-11*	6LL7	308	

Table 1: Some Psychrophiles with their Cold Adapted proteins, PDB entry, amino acid and accession number.

Membrane function: The fluidity of the membrane is essential for its structural integrity and cellular functioning (Deming, 2002). The most important impacts of low temperature depend on membrane fluidity and the organisms that grow at the biotic thermal range, have evolved a range of mechanisms to change membrane fluidity (Chintalapati *et al.,* 2004). It is observed that extensive differences exist in the physiologies of Gram-positive and Gram-negative bacteria and archaea, particularly in their cell membrane compositions and responses to temperature changes. Psychrophile membrane adaptations include increased polyunsaturated to saturated fatty acid ratios in membrane phospholipids, changes in lipid class composition, reduced size and charge of lipid head groups, which affects phospholipid packing and conversion of trans- to cis-isomeric fatty acids and have been extensively reviewed (Casanueva *et al*., 2010 & Deming, 2002). Recent transcriptome analysis corroborate earlier physiological work and have shown that exposure to cold temperatures induces a rapid up-regulation of genes involved in membrane biogenesis, such as fatty acid and LPS biosynthesis, glycosyltransferases, peptidoglycan biosynthesis and outer membrane proteins (Gao *et al.,* 2006). Comparative genomic studies have also revealed that genes involved in cell membrane biogenesis are over represented in the genomes of psychrophilic microorganisms. Proteomic and transcriptomic studies have shown that general membrane transport proteins are also up regulated, which serves as a counteractive measure against the lower diffusion rates across the cellular membranes experienced at chilled temperature (Cacace et al., 2010). In particular the up regulation of peptide transporters facilitates cold and hyperosmotic stress acclimatization by enhancing the uptake of nutrients, compatible solutes and recycling of membrane peptides for peptidoglycan biosynthesis (Durack *et al.,* 2013). Carotenoid pigments represent another class of membrane fluidity modulators. Both polar and non-polar carotenoid pigments are produced by various Antarctic bacteria and have been postulated to buffer membrane fluidity and assist in maintaining homeo viscosity during temperature fluctuations (Rodrigues DF, Tiedje JM, 2008). Wax esters are also believed to play an important role in cold-adjusted membrane fluidity. In *Psychrobacter urativorans*, they may account for up to 14% of the cell lipid content, and in P. arcticus, the wax ester synthase is constitutively expressed, regardless of the growth temperature (Ayala-del-Rio *et al*. 2010).

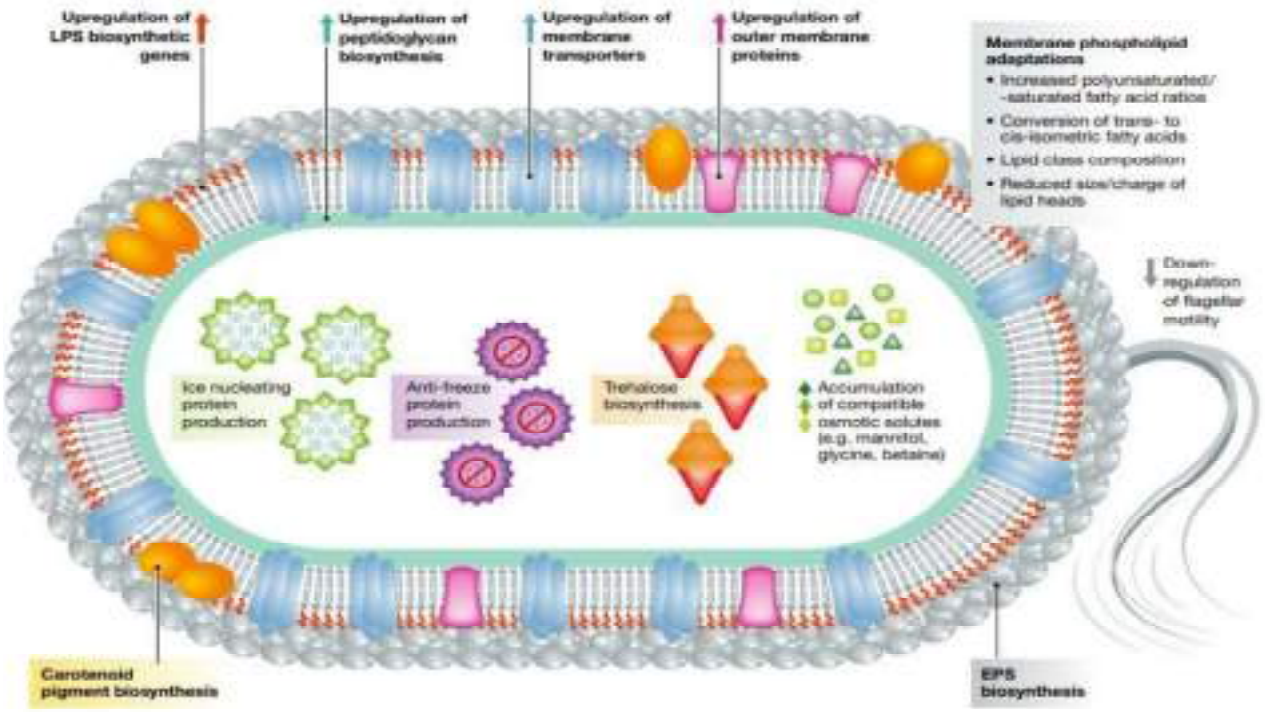
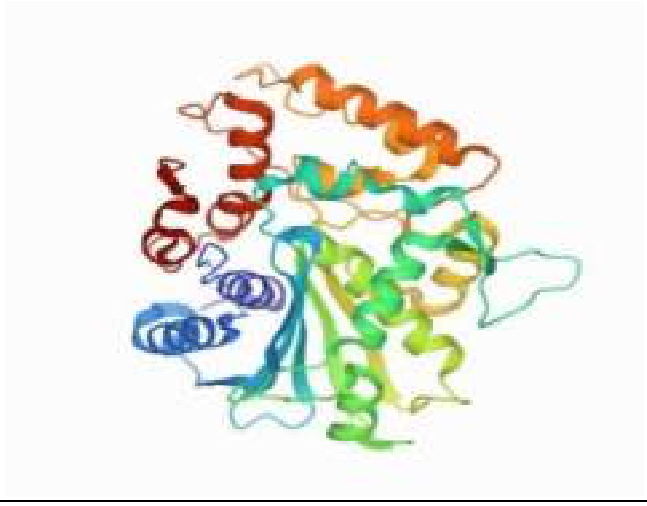
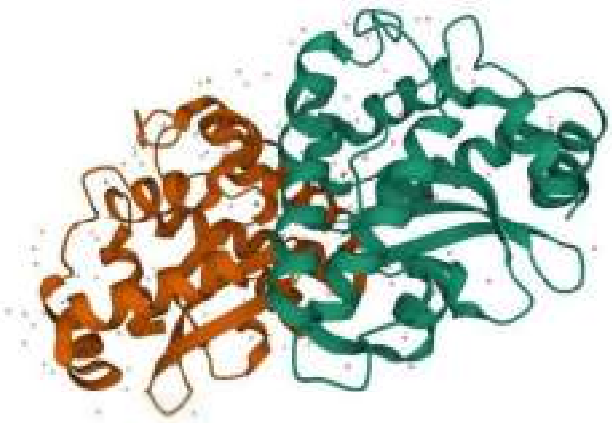
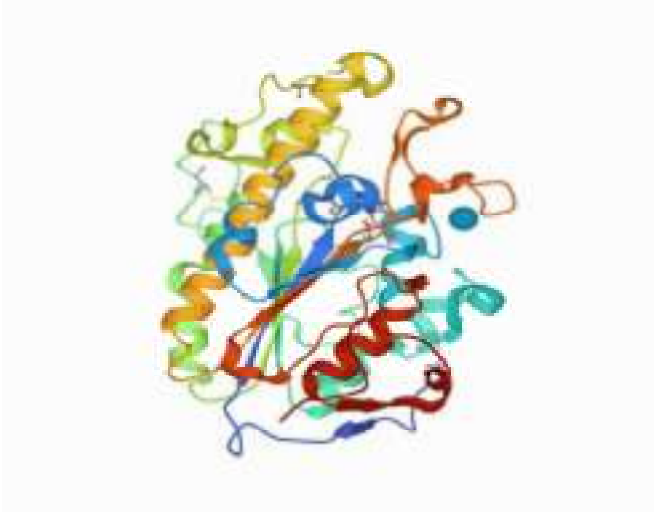
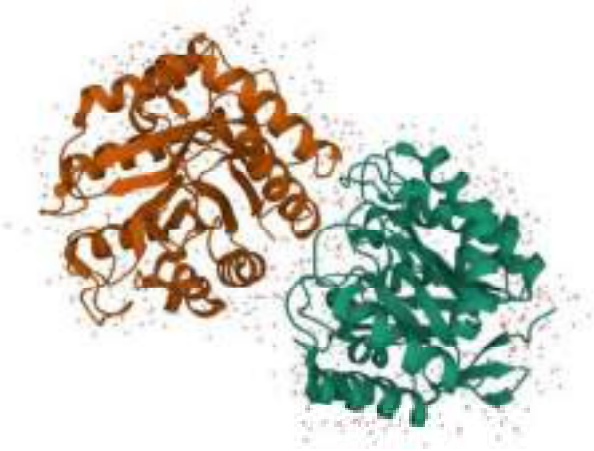
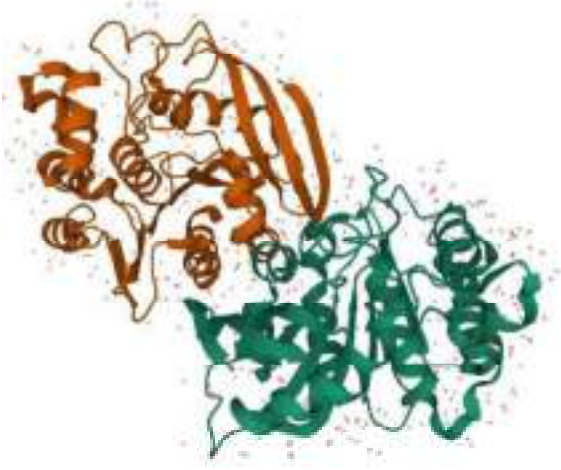
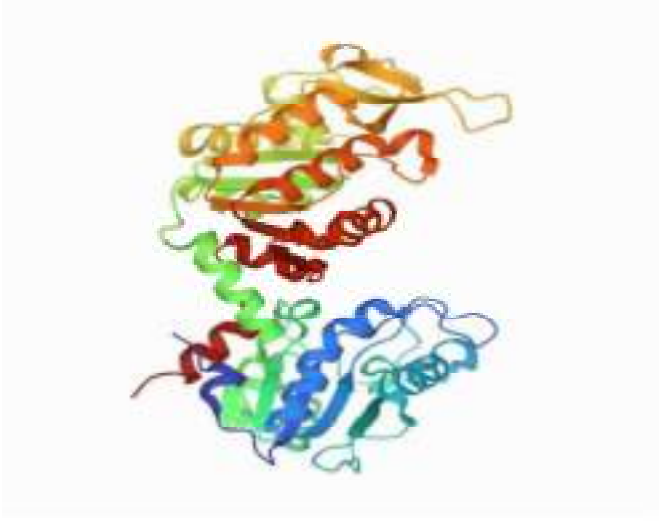


Figure 2: Common physiological adaptations in a psychrophilic prokaryote (Pieter De Maayer, Dominique Anderson, Craig Cary & Don A Cowan, 2014)

Cryoprotectants and antifreeze proteins: Cellular freezing induces the formation of cytoplasmic ice crystals, resulting in cellular damage and osmotic imbalance (Klahn &, Hagemann, 2011). The accumulation of compatible solutes, such as betaine, mannitol, glycine, sucrose, results in the lowering of the cytoplasmic freezing point thereby providing protection against freezing, as well as against desiccation and hyper osmolality (Cowan DA, 2009) (Fig 2). Some psychrophiles produce antifreeze or ice-binding (AFP) proteins (Fig 2), which bind to and control ice crystal growth and recrystallization by lowering the freezing thermal hysteresis point (Celik Y, Drori R, Petraya-Braun N, Altan A, Barton T, Bar-Dolev M, Groisman A, Davies PL, Braslavsky I, 2013). Ice-nucleating (IN) proteins can prevent supercooling of water by facilitating ice crystal formation at temperatures close to melting point (Kawahara H, 2002). The cryoprotective mechanisms employed may differ depending on the environment and microbial community structure, as demonstrated by a metagenomic study of temperate lakes that revealed a predominance of isolates with high cytoplasmic osmolyte content, with negligible ice-association (IN/AFP) phenotypes, whereas half of the epiphytic isolates from a frost exposed chrysanthemum phyllosphere community showed IN activity (Wu *et al*. 2012). Exopolysaccharide (EPS) production represents another potential cryoprotection mechanism and high levels of EPS are produced by psychrophiles under cold conditions (Feng *et al*. 2014). The high polyhydroxyl content of EPS lowers the freezing point and ice nucleation temperature of water. EPS can trap water, nutrients and metal ions and facilitate surface adhesion, cellular aggregation and biofilm formation, and may also play a role in protecting extracellular enzymes against cold denaturation and autolysis (Nichols CA, Guezzenec J, Bowman JP, 2005). The exopolymeric substances of the psychrophilic diatom *Melosira arctica* and of cold-tolerant bacterium *Colwellia psychrerythraea* have been shown to cause alterations in the desalination and microstructure of growing ice, by increasing ice crystal disorder and pore density (Emert & Deming, 2011). It results the reduction in permeability of ice, which subsequently leads to salt retention. Biologically active EPS may therefore affect the colonization of organisms in the sea ice habitat by reducing ice growth due to increased salinity (Deming *et al*., 2011).

Structure of some Psychrophilic Protein with their PDB Entry:

	
Lipase (2ORY)	**Superoxide dismutase (2W7W)**
	
PAMC 21717 (5YLF)	**Cellulase (1TVN)**
	
S-formylglutathione hydrolase (3LS2)	**Phosphoglycerate kinase (6I06)**

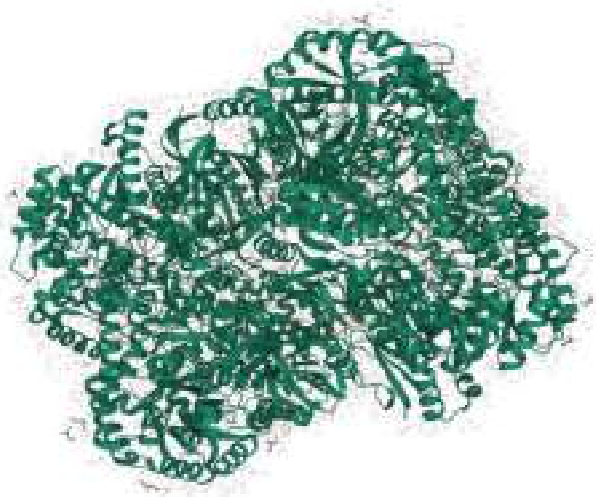
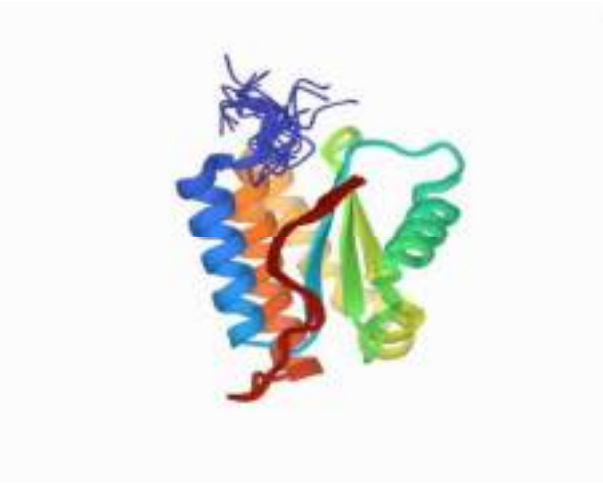
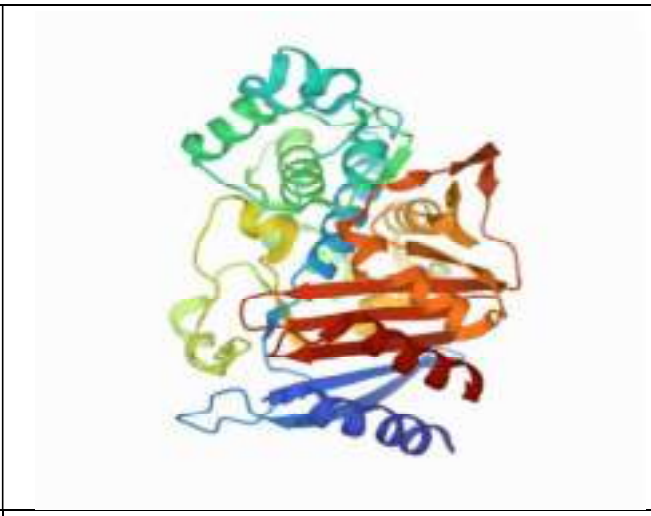
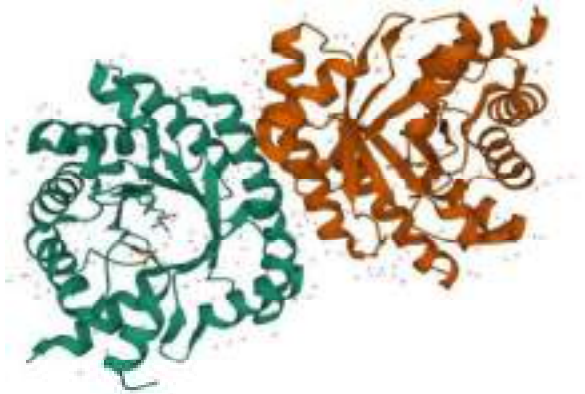
Cellular Mechanisms of Cold Adaptation:

Low temperature can hold up transcription and translation due to the expand stability of secondary structure. Prohibition or resolving inhibitory secondary structure of RNA can be obtained by RNA chaperones. Cold shock proteins are small proteins that bind RNA to conserve its single-stranded confirmation (Jones & Inouye 1994). Psychrophiles vary broadly in the number of *csp* genes in their genomes (Table 1). Csps contain a nucleic acid binding domain, known as Cold Shock Domain, and have more roles besides serving as RNA chaperons. Each cold shock domain containing proteins can synchronize the cold shock response or play vital role in subsequent growth at low temperature in mesophiles (Hebraud & Potier 1999). Accordingly, many of the Csps act as cold adaptive proteins in psychrophiles, because they are constitutively rather than transiently expressed at low temperature (D'Amico et al. 2006). Upregulation of *cspA* of *psychromonas arctica* was shown to expand cold resistance of *Escherichia coli* at low temperatures (Jung *et al*. 2010). One of three *Csps* seems to be essential in the low temperature growth of *Shewanella oneidensis* (Gao *et al*. 2006).

Table 2: Characteristics of selected bacterial and archaeal psychrophiles

Species And Strain	**Type**	**Phylogeny**	**Origin Of Strain**	***Csp* or *ctr* genes**	**Total genes**	**Genome Size (Mb)**
Cenarchaeum symbiosum A	Eurypsychrophilic archaeon	Crenarchaeota Marine Group I (or Thaumarchaeota) ,Cenarchaeales	Marine sponge symbiont, off California coast	1 *csp*	2,006	2.05
Colwellia psychrerythraea 34H	Stenopsychroplilic bacterium	Proteobacteria, Gammaproteobacterial, Alteromonadales	Artic marine sediments, off Greenland	4 *csp*	5,066	5.37
Octadecabacter antarctisus 307	Stenopsychrophilic bacterium	Proteobacteria, Alphaproteobacteria, Rhodobacterals	Sea ice off Antarctica	3 *csp*	5,544	4.91
Photobacterium profundum SS9	Stenopsychrophilic bacterium	Proteobacteria, Gammaproteobacteria, Vibrionales	Sulu Trough deep-sea sediments	8 *csp*	5,754	6.40
Flavobacterium psychrophilum JIP02/86	Eurypsychrophilic bacterium	Bacteroidetes, Flavobacteria, Flavobacteriales	Fish pathogen	1 *csp*	2,505	2.86
Listeria monocytogenes LO28	Eurypsychrophilic bacterium	Firmicutes, Bacilli, Bacillales	Foodborne pathogen	2 *csp*	2,455	2.91
Methanococcoides burtonii DSM 6242	Eurypsychrophilic bacterium	Euryarchaeota, Methanomicrobia, Methanosarcinales	Ace Lake sediments, Antarctica	3 *ctr*	2,506	2.58
Halorubrum lacusprofundi ATCC49239	Eurypsychrophilic bacterium	Eurychaeota, Halobacteria, Halobacteriales	Deep Lake sediments, Antarctica	3 *csp*	3,725	3.69
Exiguobacterium sibiricum 255-15	Eurypsychrophilic bacterium	Firmicutes, Bacilli, Bacillales	Permafrost, Siberia, Russia	6 *csp*	3,151	3.04
Polaribacter irgensii 23-P	Stenopsychrophilic bacterium	Bacteroidetes, Flavobacteria, Flavobacteriales	Subsurface seawater, off Antarctica	3 *csp*	2,602	2.75
Desulfotalea psychrophile LSv54	Eurypsychrophilic bacterium	Proteobacteria, Deltaproteobacteria, Desulfobacteralas	Arctic marine sediments, off Svalbard	7 *csp*	3,332	3.66
Psychroflexus torquis ATCC 700755	Stenopsychrophilic bacterium	Bacteroidetes, Flavobacteria, Flavobacteriales	Sea ice algal assemblage, Off Antarctica	2 *csp*	6,835	6.01

Structure of some Psychrophilic Protein with their PDB Entry:

	
Cytochrome c552 (4O1W)	**Beta-galactosidase (6Y2K)**
	
BA42 protein (2LT2)	**Beta-lactamase (2QZ6)**
	
Deoxyribose-phosphate aldolase (5C2X)	**Triosephosphate isomerase (1AW2)**

The proposed model of life under subzero conditions (*E.coli* cpn+, *p.arcticus 273-4* and *csd*A-*psy*C-1082):

CsdA-PsyC-1082 is the main component of the model proposed in this study (fig: 3). The next session is about- (a) a review about the mesophilic microorganism to be tested in the model.

(b) a review of psychrophilic microorganism that express the CsdA-PsyC-1082 protein.

(c) in Silico structural analysis of CsdA-PsyC-1082 protein by homology modelling.

'Omics' analysis okey cellular function and structures that fail at the parameter limit determined the range of growth for any given parameter. In specific low temperature membrane integrity and gene expression fail in mesophilic cells. Wild type E.coli cells grown at 15^0 C and at least 15 protein were convinced during the beginning of the lag phase to repair the cell from damages cause by drop I temperature and to repair the cellular psychology(Panoff *et al*.1998). chaperonins protein are responsible for refolding of other proteins acting in the final process of protein expression, are some the csp generated in the beginning of lag phase, and they are key determinant for *E.coli* growth at low temperatures(Ferrer *et al*.2003).

Escherichia coli grows best between 2pc and 49^0 C, with slower growth below 21^0 C and no growth below 7.5^0C(Strochhi *et al*.2006,Emanuele Kuhn,2012). A transgenic *E.coli* cpnt that received the chaperonin cpn60 and the co-chaperonin cpn10 from the Antarctic seawater psychrophiles *Oleispira antarctica* strain RB8 grow much faster than the wild type in a range of temperatures from 8^0 C to 18^0 C and grow at 4^0 C(Strochhi *et al*.2006). Cpn10 and Cpn60 from *Oleispira antarctica* show high protein refolding activities in vitro from 4^0C to 12^0C. The inclusion of these genes to the expression system of a mesophiles, which otherwise could have stopped growing at the limit of 7.5^0C, gave to the cells the volume to grow at 4^0C. The expression of these two genes in the mesophiles expand the expression level of 19 housekeeping proteins against cold-mediated inactivation by growing physical interactions (Strochhi *et al*.2006).

Psychrobacter arcticus 273-4, evolved in a chilling environment and its feedback to low temperature is completely different from *Escherichia coli. Psychrobacter arcticus* 273-4 overcome each of these extreme conditions and evolved a cellular psychology to inhabit this acute environment. Moreover, it is not the only psychrophile suited of growth at chilling temperatures. In *Psychrobacter* sp*.,* and *Arthrobacter* sp. Cell, bacterial metabolisms were detected at -15^0C. These species isolated from Lake Vostok accretion ice and at -10^0C in different isolates from permafrost (Christner, 2002, Bakermans *et al*. 2003). Their metabolic activity also discovers in a permafrost community at -20^0C, with a cellular doubling time of 160 days (Rivkina *et al*. 2000).

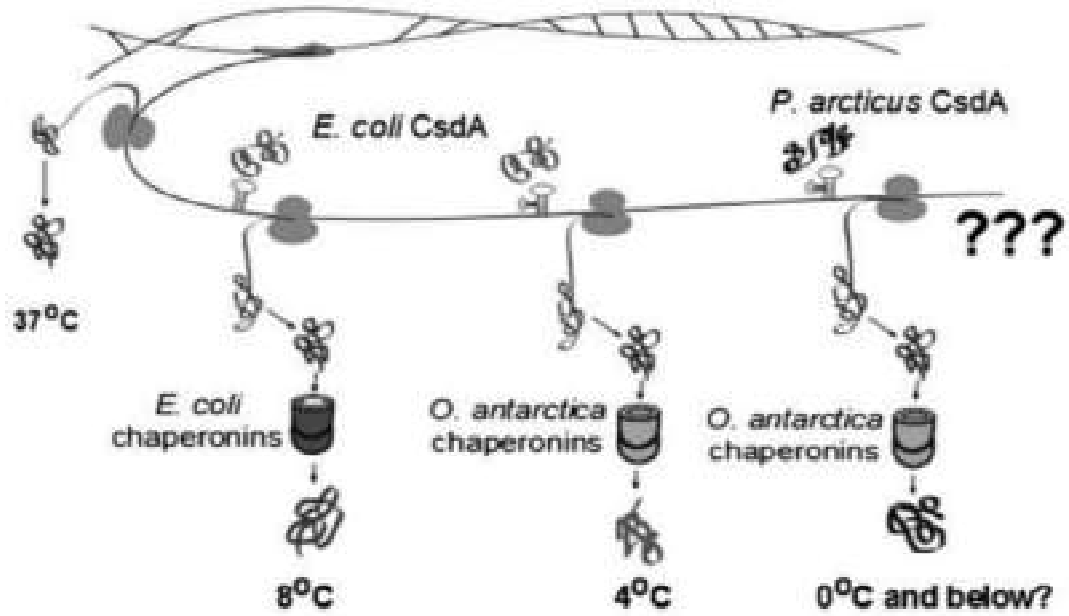


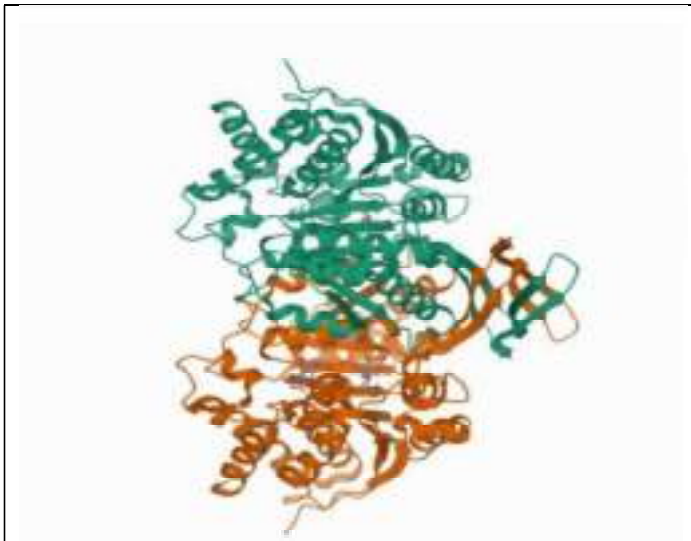
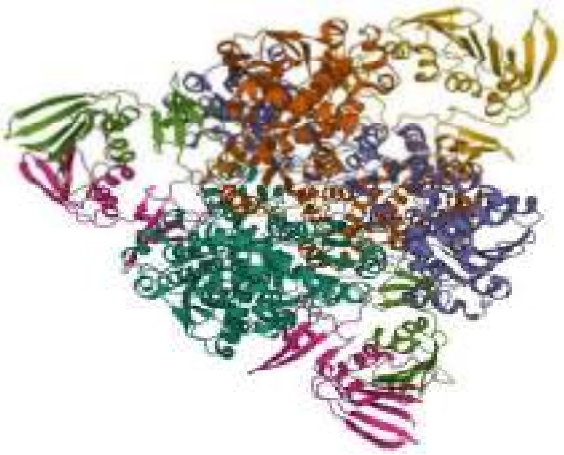
Figure 3: Schematic representation of the model suggest for the advance of psychrophilic protein expression in mesophilic organisms. Also, addition of the minimum growth temperature (Emanuele Kuhn, 2012)

Genomic analysis showed that in the range of 38% and 84% of *Psychrobacter articus* 273-4 amino acid sequences display cold adaptation indicators like reduction in proline content, less hydrophobicity, higher Arg/Lys ratio or acidic residues (Ayaladel-Rio. *et al*.2010, Emanuele Kuhn, 2012). Transcriptome of *P.arcticus* was the first transcriptome reported at temperature below 0^0C (Bergholz *et al*.2009). Bergholz *et al*. 2009 analyzed and compared growth rate measurements and transcryptomes at -6^0C, 0^0C, 4^0C, 17^0C, 22^0C in acetate medium. *Psychrobacter arcticus* at 0^0C and -6^0C down regulated peptidyl-prolyl cis-isomerases, trigger factor and the major heat shock associated chaperones. Chaperones are associated with oxidative protein damage, iron-sulphur cluster biosynthesis, *clp*B chaperone homologues were upregulated (Bergholz *et al*. 2009).

Both psychrophiles and mesophiles, *Escherichia coli* and *Psychrobacter arcticus* 273-4, respectively harbor DEAD-box RNA helicase genes. *Escherichia coli* contains DEAD-box helicase (*csd*A, *rhl*B, *rhl*E, *dbp*A, and *srm*B) and *p*sychrobacter *arcticus* 273-4 contains two [*rhl*B (Psyc-0943), and *csd*A (Psyc-1082)] (IOST and Dreyfus 2006; Bergholz *et al*. 2009). DEAD-box RNA helicase participates in many cellular processes such as transport, processing and break of RNA or ribosome biogenesis, it is considered as multifunctional enzyme (Prud'homme-Genereux *et al*., 2004 & Phadtare 2011). In psychrophilic organisms, CsdA is revealed as a housekeeping protein, or can be considered a CAP. CsdA expression in mesophiles occurs and is benefit only for cold adaptive response as a Csp (Jones *et al*.1996, Panoff *et al*. 1998, Emanuele Kuhn 2012, Hebraud and Potier 1999, Srinivs and Ray 2006, Charollais *et al*. 2004). Experimental exploration reveal two possible activities of CsdA related to low temperature adaptation- (a) mRNA decay, where it's helicage activity is considered crucial for promoting degradataion of mRNA stabilized at 15^0C in *E.coli* and (b) mRNA and ribosome biogenesis (Phadtare 2011). Research has also recommended that CsdA may help 50S space assembly by modulating RNA structure (Iost and Dreyfus, 2006). Its unwinding movement may be required to facilitate structural transition within the RNA and may also allow proper binding of r-protein (Iost and Dreyfus 2006, Emanuele Kuhn 2012). At last, it indicate that CsdA prevents and resolves rRNA misfolding caused by lowering the temperature, providing assistant to rRNA to reach its operating conformation (Phadtare 2011, Emanuele Kuhn 2012).

Figure 4: Alignment of *P.arcticus* 273-4 Psyc_1082(YP_264368) with 1 protein from mesophile *E.coli* K12(EFK25707), 3 homolog psychrophile *Psychrobacter cryohalolentis* K5(YP_580642), *Psychrobacter sp.(ABQ94096), Methanocaldococcus burtonii* DSM 6242(ABE52831) and 1 thermophile *Methanocaldococcus jannaschii* DSM 2661(AAB98663). Yellow boxes refers 100% similarity between sequences and green boxes suggest half similarity.

Structure of some Psychrophilic Protein with their PDB Entry: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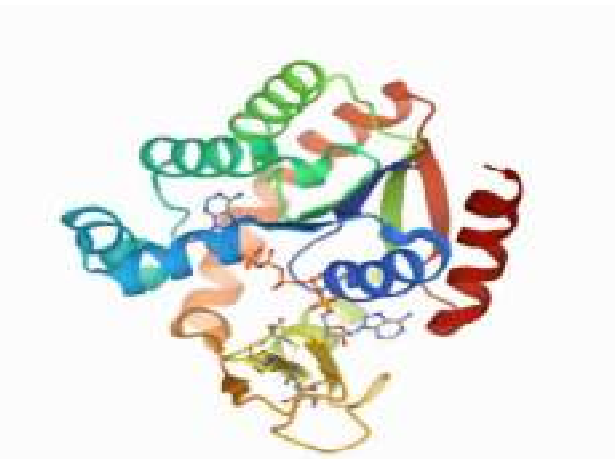
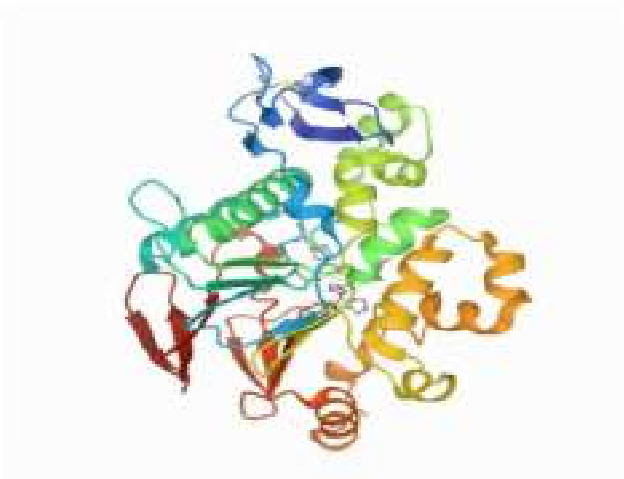
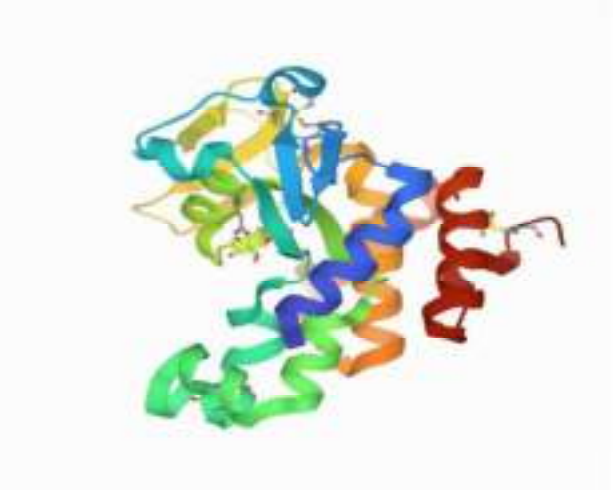
	
Competence atimwaiting peptide (6COV)	**Adenylate kinase (3FB4)**
	
Tyrosine phosphatase (1V73)	**Endonuclease 1 (2G7F)**

Mutant Analysis: In *Escherichia coli,* the deletion of *csd*A gene conducts to growth defects only at low temperatures, near 15^0C (Awano *et al*. 2007). The deletion of the *rhl*B gene in the mesophiles does not cause any deficiency in growth at the optimum temperature of a 37^0C (Awano *et al*. 2007). In *Psychrobacter arcticus* 273-4, a deletion of psyc-1082(*csdA)* resulted in decreased cellular growth rates above 4^0C (Bergholz *et al*. 2009). *P.arcticus* 274-4 with the psyc-0943 (*rhl*B) gene deleted did not result in growth at 4^0C or 17^0C, indicate that this gene plays an essential role in *Psychrobacter arcticus* 273-4 cell physiology at its optimum temperature (Bergholz *et al*. 2009). It has been exhibit in *E.coli* that the deletion of *csd*A leads to a severe deficit of free 50S subunit and accumulation of 40S particles that correspond to incomplete assembly of ribosomal large subunit (Emanuele Kuhn, 2012).

Structural analysis: Protein synthesis and folding are the critical problems to overcome for life in cold and chilling environments, the secondary structure of RNA is stabilized via H-bonds, making translation difficult. RNA helicases are overexposed at low temperature in many psychrophile such as *Exiguobacterium sibiricum* (Rodrigues *et al*. 2008), *Sphingopyxis alaskensis (*Ting *et al* .2010),*Methanococcoides burtonii* (Lim *et al*. 2000), *Pseudoalteromonas haloplanktis* (Piette *et al*. 2010). These helicases can help unwind the RNA secondary structures and rearrange them for methodical translation in the cold. In enzymes, decreased stability and growing flexibility translate into greater entropy. Analysis of the amino acid sequences and structure of the enzymes of psychrophilic microorganisms, have given rise to the flexibility concept, that is a psychrophilic enzyme can exhibit growing catalytic activity at low temperature with limited loss of thermostability through adaptation for decreased numbers of stabilizing interactions between key amino acid residues (Grzymski *et al*. 2006). the thermodynamic effects of cold adaptation are a depletion in the temperature dependence of the maximum catalytic rate (Feller and Gerday,1997). The genome of *Psychrobacter arcticus* 273-4 shows a statistically significant shift in amino acid compared with mesophiles, to those known to favour flexibility at low temperatures for most cell functions, but particularly for those engaged in growth and reproduction (Ayala-del-Rio *et al*. 2010). To explore the structure of protein Psyc-1082, a tertiary structure prediction was conducted by homology modelling with the program RaptorX (Peng and Xu 2011, Emanuele Kuhn 2012). The secondary site of Psyc-1082 was aligned against 4 distantly related DEAD-box RNA helicase proteins with tertiary structure characterized by X-ray diffraction of the protein crystal. The DEAD-box RNA helicases from an archaeon, *Nethanococcus jannaschii* (1HV8), and 3 eukaryotes *Saccharomyces cerevisiae* (3I62), *Drosophila melanogaster*(2DB3) and *Homo sapiens* (3EX7) were extracted from and obtainable in PDB.

Adaptation of psychrophiles viewed through genome and global gene expression profiles:

Round about thirty bacterial and four archaeal genome sequences are available for psychrophilic microorganisms that were obtained from diverse cold samples, including sea sponge (symbionts), permafrost, Antarctic lakes, marine sediment, fish (pathogens), marshes and kimchi (Lauro *et al*. 2011). The dimension to overview global responses is greatly accelerating the ways in which knowledge is being acquired about adaptive mechanism in particular as researchers explain general characteristics of psychrophiles versus specific traits of individual psychrophiles. In addition to supply genomic blue print that elaborate the volume of psychrophilic microorganisms, genomes provide the basis for pointed and global functional studies (Transcriptomics and proteomics).

An analysis of *Psychrobacter arcticus* (growth temperature range from -10^0C to 28^0C) used transcriptomics to recognise differences in mRNA between five growth temperatures (-6^0C, 0^0C, 4^0C, 17^0C and 22^0C) (Bergholz *et al*. 2009, Emanuele Kuhn 2012), multiplex proteomics study of *M.burtonii* quantitated changes happening across seven growth temperature that span the organism's whole growth temperature range (-2^0C to 28^0C) (Williams *et al*. 2011)(fig. 5). In further study, by including growth temperature extremes as well as temperatures in between researchers were able to infer stressful versus non-stressful physiological states. The upregulation of oxidative stress proteins at both upper and lower temperature extremes described

the important, yet distinct, ways in which temperature induced oxidative stressed manifests in the cell. The review also revealed that protein profiles at temperature in which *M.burtonii* grew faster were identical to those at maximum growth temperature. These research works highlighted the extent to which this psychrophilic microorganism was heat stressed at these temperatures, which is compatible with a number of other studies recommend that psychrophilic microorganism growing at T_{opt} are likely to be heat stressed (Feller & Gerday 2003, Bakermans & Nealson 2004, Cavicchioli 2006, Williams *et al*. 2010, Good child *et al*. 2004).

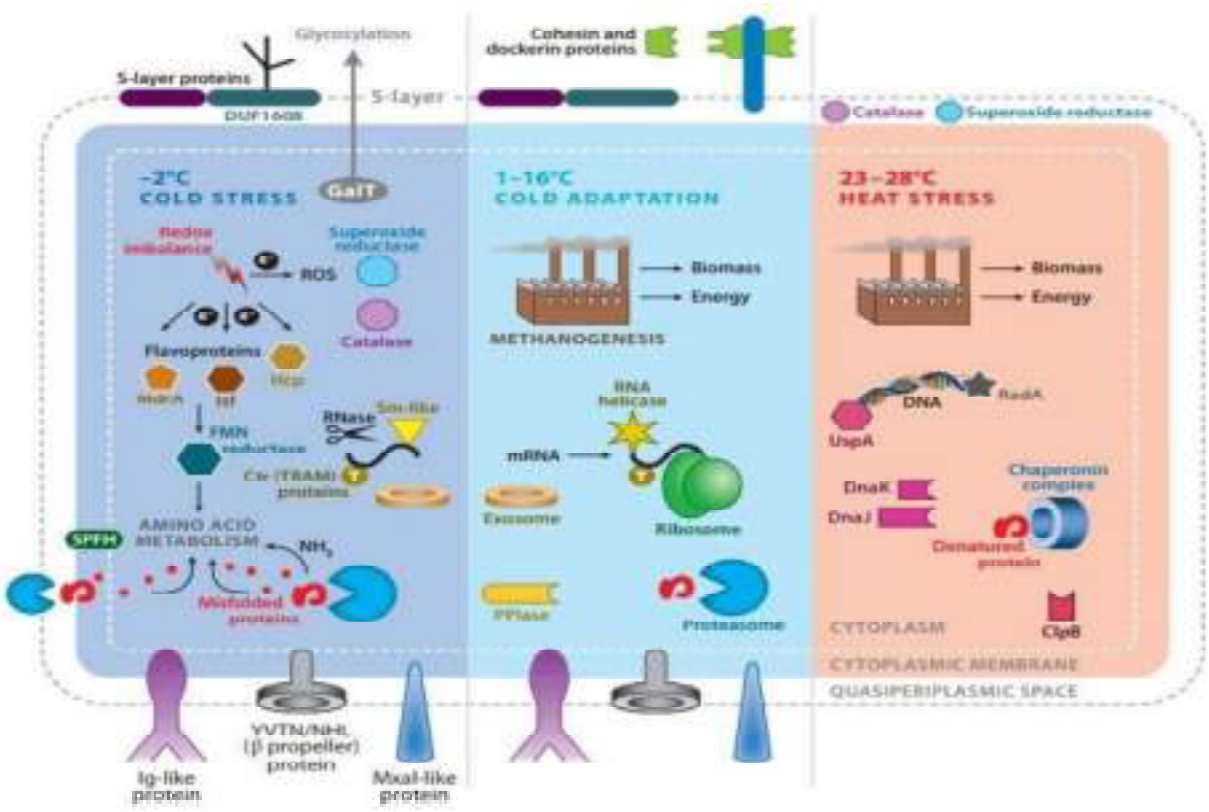


Figure 5: Temperature dependent physiological states in the Antarctic archaeon, *Methanococcoides burtonii.* Displayed the cellular process most influenced during cold stress(-2^{0}C), cold adaptation (1, 4, 10 and 16^{0}C) and heat stress(23 and 28^{0}C) states of the cell . Abbreviations : ClpB, chaperone; Dnaj/Dnak, chaperones; Ctr, cold responsive TRAM protein; DUF1608, S-layer protein containing domain of unknown function; FMN, flavin mononucleotide; e^-, electron; Hcp, hybrid-cluster protein; GalT, galactose-1-phosphate uridylyitransferase; MdrA, protein disulfide reductase; Isf, iron-sulfur flavoprotein; mRNA, messenger RNA; ROS, reactive oxygen species; RNase, ribonuclease; SPFH, degradation-related protein; Sm-like, RNA-binding protein homolog; YVTN/NHL, S-layer protein containing cell adhesion domain; UspA, universal stress protein A. Adapted with permission from Williams *et al*. 2011(society for applied Microbiology and Blackwell Publishing Ltd).

Mechanisms of Enzyme Adaptation to the Cold:

In low temperature environments, there is lack of kinetic energy to overcome enzyme activation barriers, causing in very slow rates of chemical reaction. Biochemical reaction in a mesophilic organism at 37^{0}C, a drop in temperature from 37^{0}C to 0^{0}C results in a twenty two to eighty fold reduction in enzyme activity. It is the major factor preventing growth at low temperature. To overcome this constraint organisms those are adopted to low temperatures have evolved several ways, including the energetically expensive way of enhanced enzyme production (Crawford & Powers 1992) and seasonal appearance of isoenzymes (Somero 1995). The common one adaptive characteristic of cold active enzymes is a reaction rate (K_{cat}) that is largely independent of temperature. The majority of psychrophilic enzyme attain temperature insensitive K_{cat} by reducing the activation energy barrier between the substrate and activated state.

For example, reducing the activation energy from 70kJ mol^{-1} for a thermophilic protein alpha-amylase to 35 kJ mol^{-1} for a psychrophilic alpha-amylase enhanced k_{cat} by 21fold at 10^0C (D'Amico *et al*. 2003). At a low energy cost, to aid substrate binding, the active cites of cold shock enzymes tend to be larger and available to substrates. Thus, the binding affinity of substrates for cold shock enzymes is generally lower than that of their thermophilic counterparts (Siddiqui & Caviccchioli 2006).

At low temperature, high rates of catalysis are generally achieved by the flexible structure and concomitant low stability of cold shock enzymes, which is referred to as an activity stability trade off (Siddiqui & Caviccchioli 2006) (Table 2). In an environment characterized by low kinetic energy and retarded molecular motion, cold active enzymes rely on greater disorder as a means of maintaining molecular dynamics and functions (Feller 2007). Many cold active enzymes have a more fluctuating and flexible catalytic region than does the remainder of the protein structure, that is localized flexibility (Siddiqui *et al*. 2005, Feller 2008). The α-amylase from *P. haloplanktis*, AHA has become a model to study the function, structure and stability relationship in cold adapted enzymes (D'Amico *et al*. 2001, 2003; Siddiqui & Cavicchioli 2006; Feller & Gerday 2003; Feller 2008; Siddiqui *et al*. 2005).

The review indicate that the structure of AHA has evolved to have relatively few electrostatic interactions in order to provide enough conformational flexibility to sustain activity at low temperatures, while retaining a sufficient level of overall protein structural integrity. Genomic analyses of psychrophilic archaea have disclosed proteins characterized by a higher content of noncharged polar amino acids (Gln and Thr), a lower content of hydrophobic amino acids (particularly Leu), increased exposure of hydrophobic residues, and a decreased charge that is associated with destabilizing the surface of psychrophilic proteins (Saunders *et al*. 2003). Evolutionary selection of amino acid usage enabled such adaptation (Allen *et al*. 2009). Pro and Arg are associated with an ability to confer increased stability by restricting backbone rotations and by forming multiple hydrogen bonds and salt bridges (Feller & Gerday 2003).

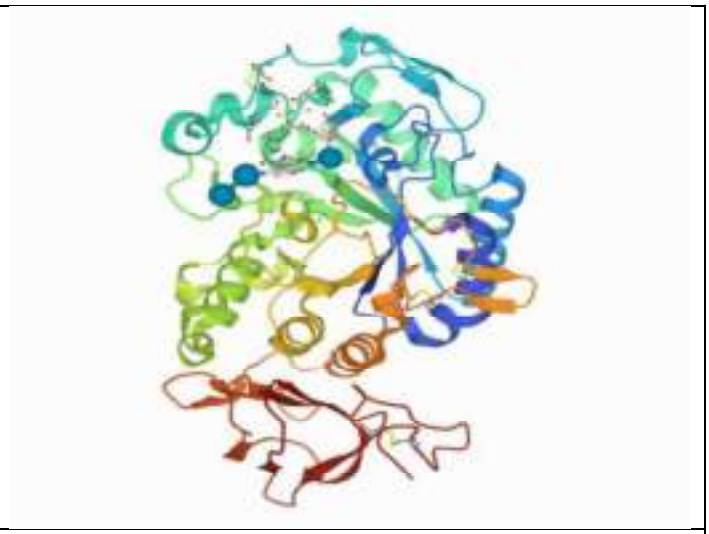
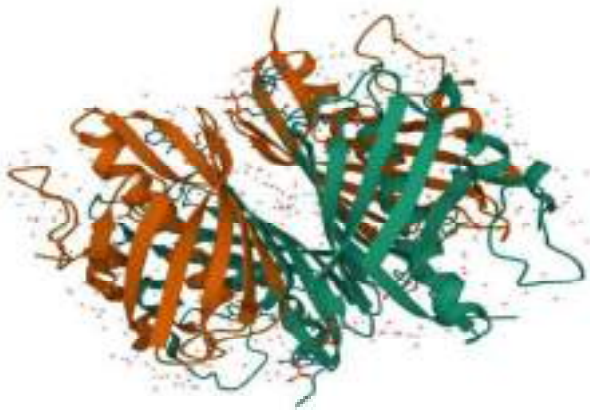
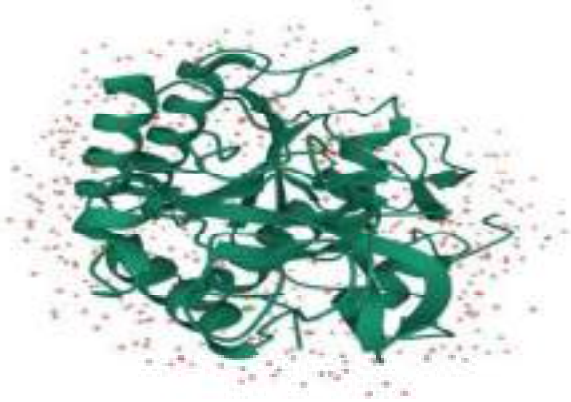
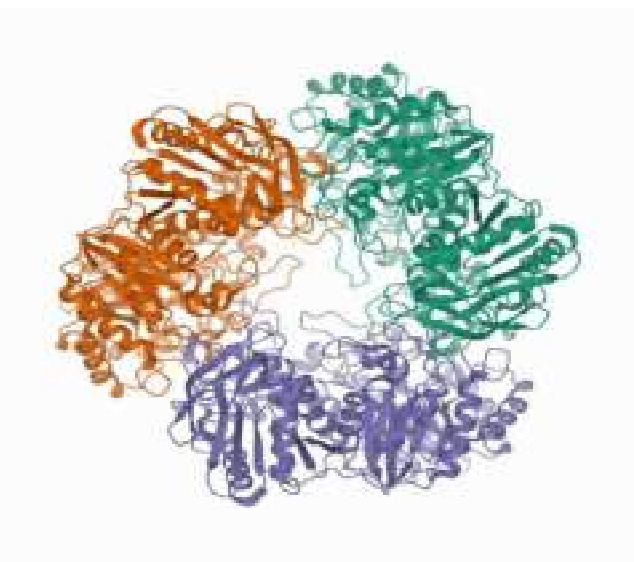
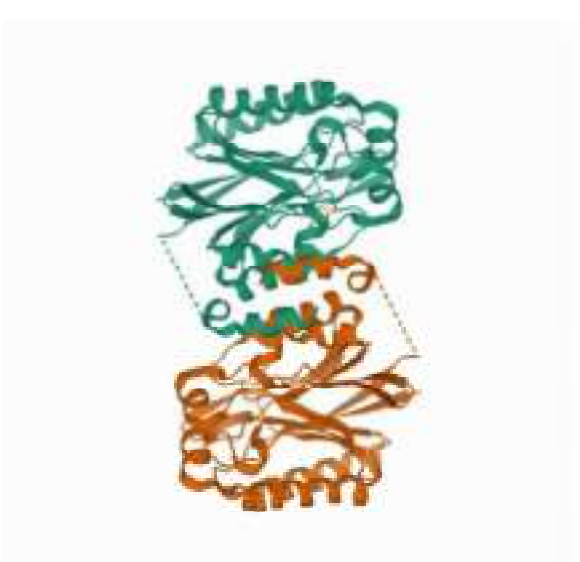
Figure 6: Common structural modifications of psychrophilic enzymes resulting in decreased thermostability, increased activity and increased flexibility (Pieter De Maayer, Dominique Anderson, Craig Cary & Don A Cowan, 2014).

Psychrophilic proteins are characterized by increased surface hydrophobicity, decreased core hydrophobicity, a lower arginine/lysine ratio, intersubunit interactions, weaker interdomain, more glycine residues, more and longer loops, decreased secondary structure content, fewer prolines in loops, more prolines in α-helices, fewer and weaker metal-binding sites, fewer disulfide bridges, fewer electrostatic interactions (aromatic-aromatic interactions, salt bridges, H-bonds, cation-pi interactions), reduced oligomerization, and an increase in the conformational entropy of the unfolded state (Siddiqui & Cavicchioli 2006). Some cold adapted proteins also tend to have flexible 5-turn and strand secondary structures, and they possess large cavities lined predominantly by acidic residues to accommodate water molecules (Paredes *et al*. 2011). Although the above-mentioned structural features can be associated with psychrophilic proteins, any one protein will have a restricted number of, and specific context for, these structural features (Siddiqui & Cavicchioli 2006).

Table 3: Activity-stability relationship of some thermally adapted enzymes

Enzyme	$K_{cat}(min^{-1})$	k_m(Mm)	$T_{opt}(^0C)$	$T_m(^0C)$	$T_{1/2}$(min)	Reference
Aminopeptidase	(10^0C)				(46^0C)	Huston *et al*.
Psychrophile	950	-	39	47	1	2008
Mesophile	114	-	49	58	100,000	
Lactate dehydrogenase						Coquelle *et al*.
Psychrophile						2007
	13,800(0^0C)	0.16(0^0C)	50	50	-	
Thermophile	105,000(44^0C)	0.41(44^0C)			-	
	40,500(90^0C)	0.16(90^0C)	90	90	-	
Cellulase	(4^0C)	(4^0C)			(45^0C)	Garsoux *et al*.
Psychrophile	11	6.0	37	-	40	2004
Mesophile	0.6	1.5	56	-	Unaffected	
Amidase	(25^0C)	(25^0C)			(40^0C)	Huang & Yang
Psychrophile	25,700	1.6	55	-	150	2003
Mesophile	1,500	1.0	>65	-	2,880	
Alpha-Amylase	(10^0C)	(10^0C)				D'Amico *et al*.
Psychrophile	17,640	0.23	28	44	0.23(43^0C)	2003
Mesophile	5,820	0.06	53	52	0.23(60^0C)	
Thermophile	840	-	84	86	0.23(80^0C)	
Alkali phosphatase	(37^0C)	(37^0C)			(50^0C)	Siddiqui *et al*.
Psychrophile	48,740	0.13	40	-	10	2004b
mesophile	6,954	0.11	56	-	38	

Structure of some Psychrophilic Protein with their PDB Entry:

	
Phosphoheptosa isomerase (5BY2)	**Alpha amylase (1G94)**
	
Thioesterase (1Q4S)	**Subtilisin (2GKO)**
	
Beta-galactosidase (6LVW)	**Aliphatic amidase (5JQN)**

Comparative Proteome Analysis of Mesophiles vs Psychrophiles:

In psychrophilic bacteria, amino acid like threonine, alanine, aspartic acid, serine is too much presented in the coli region of secondary structure and amino acid like leucine, glutamic acid, are presented in low rate in the helical regions. Psychrophile contain a higher proportion of amino acids that promote to higher protein flexibility in the coli regions of proteins. In psychrophiles, basic aliphatic, hydrophilic, and aromatic amino acid side chains are present in low rate in the helical region of proteins. The amino acid substitution pattern between the orthologous proteins of mesophiles versus psychrophiles are different for several amino acids when analysed to their substitution in orthologous proteins of psychrophiles and mesophiles.

Thirty proteins obtained from psychrophiles were analysed and compared with mesophiles by bioinformatics tools like BLAST & MSA. Results showed that the some of the amino acids differed in mesophiles proteomes (table 4). The mesophile proteomes showed huge standard deviation for residues indicating that the 6 proteins of mesophile that are used are considerably more divergent than the proteome of psychrophiles.

In 2008, Metpally and Reddy also got similar results where 2816 proteins analysed & 875,219 amino acids per proteome of mesophiles and 3665 proteins with 1169678 amino acids per proteome of psychrophiles. Cold shock proteins (CSP) were identified from psychrophilic bacteria from the well-known Protein Data Bank (PDB) & National Center of Biotechnology Information (NCBI). FASTA sequence of the identified proteins were analysed in the web based ProtParam (https://web.expasy.org/cgi-bin/protparam/protparam) tool of Expasy to extract the amino acid composition of those identified proteins. All the amino acid composition data analysed to identify the ratio of the presence of different amino acids in those identified proteins. In addition to this amino acid composition ratio in the CSP proteins are analysed. Similar proteins were identified from different psychrophilic bacteria & mesophilic bacteria using the web-based algorithm BLASTP (Protein BLAST: search protein databases using a protein query (nih.gov)) of NCBI for each pre-identified proteins described earlier. FASTA sequence of these similar proteins from the different psychrophilic bacteria & mesophilic bacteria used to analyse the amino acid homology in the MUSCLE (MUSCLE < Multiple Sequence Alignment < EMBL-EBI). CLUSTAL multiple sequence alignment & Percent Identity Matrix result was obtained from this MUSCLE analysis.

Amino acid composition preferences:

The analysed result demonstrates an important preference in frequencies of amino acid occurrences and property group in psychrophilic proteomes as compared to mesophilic proteomes (Table 4). The amino acid composition trend is similar in both type of genomes. As compared to mesophiles, in psychrophile, there are few amino acid residues, such as A, S, D are significantly preferred. Amino acid residues E and L are less favoured in psychrophilic proteomes.

During comparison, amino acid group frequencies of occurrences, I observed that neutral and some small amino acid groups are significantly preferred in psychrophile proteomes, where basic, hydrophilic, aromatic and changed group are less favoured (Table 4).

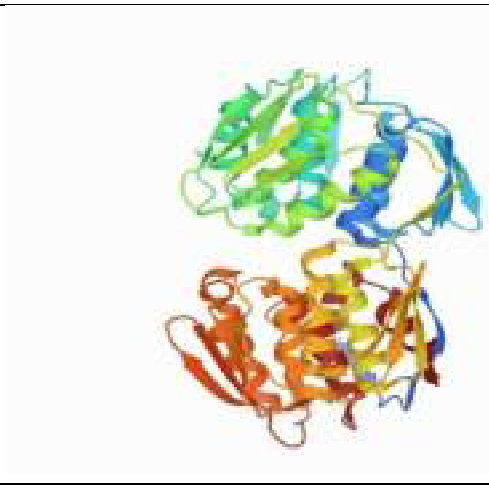
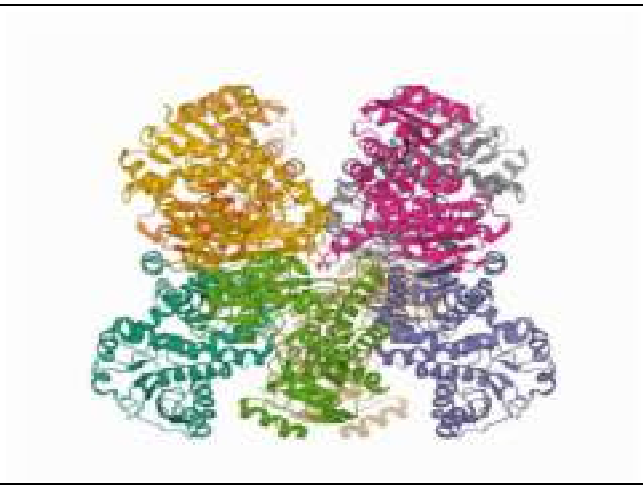
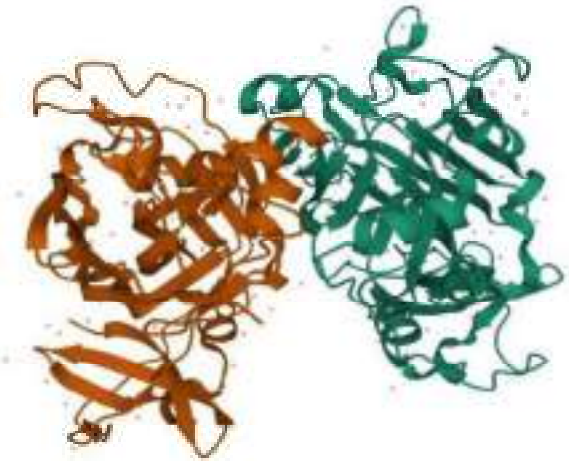
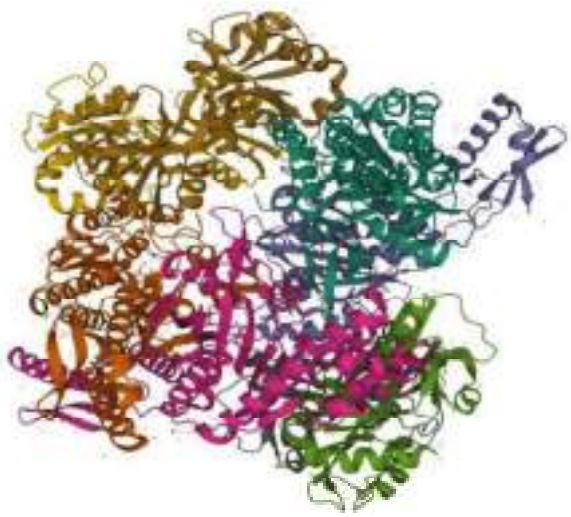
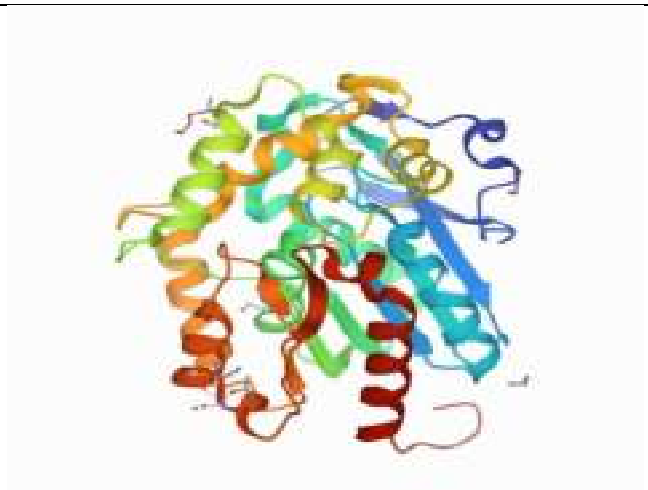
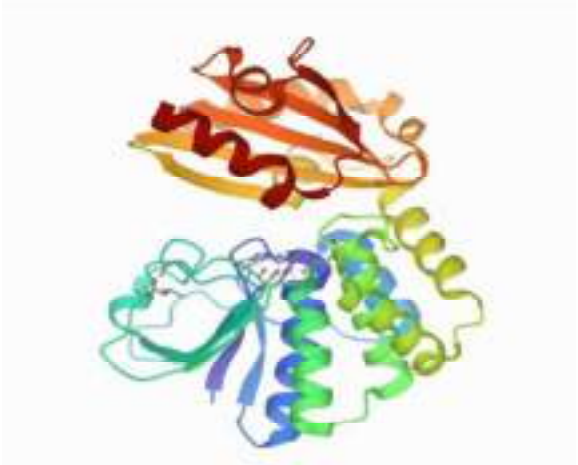
Table 4: The composition of individual amino acids and property groups in protein sequences of psychrophilic and mesophilic proteomes.

Amino Acids	Psychrophiles[a]								Mesophiles[b]								
	P1	P2	P3	P4	P5	P6	Avg	SD	M1	M2	M3	M4	M5	M6	Avg	SD	t-test
Ala (A)	8.1	8.5	9.2	8.4	8.9	12.3	9.2	1.6	8.3	6.8	9.5	8.4	9.1	6.7	8.1	1.2	1.38
Cys(C)	1.0	1.4	0.9	1.1	1.0	0.7	1.0	0.2	1.0	1.1	0.6	1.0	1.1	0.6	1.0	0.2	0.15

Asp(D)	5.6	5.1	6.0	5.4	5.8	5.3	5.5	0.3	5.0	4.8	5.1	5.0	5.0	5.9	5.1	0.4	1.80
Glu(E)	5.9	6.3	5.5	5.9	5.8	5.6	5.8	0.3	6.5	6.9	5.8	6.2	6.1	6.6	6.3	0.4	-2.55
Phe(F)	4.4	4.3	3.7	4.4	4.3	3.4	4.1	0.4	4.4	5.4	3.9	4.0	4.1	4.3	4.4	0.6	-1.006
Gly(G)	6.4	7.5	6.6	6.5	6.8	8.4	7.1	0.8	6.7	5.9	7.4	7.3	6.7	6.3	6.7	0.6	0.922
His(H)	2.2	2.0	2.3	2.1	2.3	1.9	2.1	0.2	2.1	2.1	2.3	1.9	2.4	1.8	2.1	0.2	0.206
Iie (I)	7.2	7.1	6.9	7.4	6.2	5.0	6.6	0.9	7.1	7.2	6.0	6.3	6.0	7.9	6.8	0.8	-0.298
Lys(K)	6.1	5.7	5.2	6.1	5.1	3.3	5.2	1.0	6.3	8.9	4.4	4.3	4.9	8.1	6.2	2.0	-1.019
Leu(L)	10.3	10.4	10.1	10.7	10.1	10.3	10.3	0.2	10.5	11.2	10.7	11.4	10.8	9.6	10.7	0.6	-1.370
Met(M)	2.4	2.6	2.8	2.4	2.0	2.5	2.5	0.3	2.4	2.3	2.8	2.0	2.7	2.6	2.5	0.3	-0.138
Asn(N)	5.1	3.8	4.6	4.8	4.5	2.9	4.3	0.8	4.9	5.9	3.9	4.1	3.9	5.6	4.7	0.9	-0.883
Pro(P)	3.5	3.9	4.0	3.6	3.9	5.0	4.0	0.5	3.7	3.3	4.4	5.1	4.0	3.2	4.0	0.7	0.140
Gln(Q)	4.6	3.7	4.7	4.3	4.9	3.7	4.3	0.5	4.6	3.7	4.4	5.5	5.2	3.6	4.5	0.8	-0.602
Arg(R)	3.8	5.0	4.4	4.0	4.4	6.1	4.6	0.9	4.5	3.5	5.5	5.1	5.0	3.8	4.6	0.8	0.116
Ser (S)	7.2	6.6	6.7	6.6	6.9	6.8	6.8	0.2	5.8	6.8	5.8	5.9	6.3	6.1	6.1	0.4	3.684
Thr(T)	5.6	5.3	5.8	5.4	5.5	5.6	5.5	0.2	5.2	4.4	5.4	5.5	5.2	5.5	5.2	0.4	1.789
Val(V)	6.6	6.7	6.4	6.6	6.9	8.0	6.8	0.6	6.7	5.6	7.1	6.6	7.0	6.9	6.6	0.5	0.724
Trp(W)	1.0	1.1	1.2	1.1	1.2	1.2	1.5	0.2	1.1	0.7	1.5	1.3	1.6	1.0	1.2	0.3	-0.023
Tyr(Y)	3.1	3.1	3.0	3.1	3.1	2.1	2.9	0.4	3.1	3.7	2.8	2.9	3.0	4.0	3.4	0.5	-1.310

	Amino acid property group																
		Psychrophiles							**Mesophiles**								
	P1	**P2**	**P3**	**P4**	**P5**	**P6**	**Avg**	**SD**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**Avg**	**SD**	**t-test**
Tiny	28.3	29.4	29.1	28.1	29.0	33.8	29.6	2.1	27.0	24.9	29.2	28.0	28.4	25.1	27.1	1.8	2.235
Small	49.1	48.8	50.32	48.4	50.2	55.1	50.3	2.4	47.3	44.4	49.8	48.9	48.4	46.6	47.6	1.9	2.166
Aliphatic	24.1	24.2	23.5	24.5	23.2	23.4	23.8	0.5	24.3	24.0	23.8	24.3	23.9	24.4	24.1	0.3	-1.157
Aromatic	10.8	10.3	10.2	10.7	10.9	8.9	10.3	0.8	10.7	11.9	10.5	10.4	10.8	11.1	10.9	0.6	-1.559
Non polar	54.2	56.5	55.0	55.2	55.0	58.9	55.8	1.7	55.1	53.1	57.3	56.6	55.9	52.9	55.1	1.8	0.615
Polar	45.8	43.5	45.0	44.8	45.0	41.1	44.2	1.7	44.9	46.8	42.7	43.4	44.1	47.1	44.8	1.8	-0.601
Charged	23.4	24.1	23.3	23.4	23.4	22.2	23.3	0.6	24.3	26.1	23.1	22.4	23.5	26.2	24.3	1.6	-1.414
Basic	12.0	12.7	11.8	12.2	11.8	11.3	12.0	0.5	12.8	14.5	12.2	11.3	12.3	13.7	12.8	1.2	-1.663
Acidic	11.5	11.4	11.5	11.2	11.6	10.9	11.3	0.3	11.5	11.6	10.9	11.1	11.2	12.5	11.5	0.5	-0.541
Neutral	25.9	25.2	26.0	25.2	26.3	26.4	25.8	0.5	24.4	22.8	25.3	26.1	25.8	23.3	24.6	1.3	2.057
Hydrophilic	30.8	29.6	30.3	30.6	30.4	26.9	29.8	1.5	31.8	33.6	29.2	30.2	30.2	33.7	31.4	1.9	-1.696
Hydrophobic	44.3	45.0	44.4	45.1	44.2	45.4	44.7	0.5	44.7	44.0	45.5	44.2	45.2	43.5	44.5	0.8	0.527

Structure of some Psychrophilic Protein with their PDB Entry:

	
3-phosphoglycerate 1-carboxyvinyltransferase (5XWB)	**Leucine dehydrogenase (3VPX)**
	
Fumarylase acetate hydrolase (6IYM)	**ATP phosphori bosyltransferase (5M8H)**
	
Haloalkane dehyalogenase (6F9O)	**Inorganic pyrophosphatase (6LL7)**

Secondary structural elements:

In the amino acid composition of mesophilic proteomes and psychrophilic proteomes, there are three major secondary structural elements- alpha-helicases, beta-sheets, and coil. The psychrophilic proteomes contain significantly rich number of residues in the coli region and poor number of residues in alpha-helices regions. In either of two genome sequences, the majority of amino acid exhibit similar compositions. In psychrophilic proteomes, E, F, L, N, Y amino acid show significantly huge frequencies in the coli region, and the E amino acid is significantly poor in the coli region. As compared to the mesophiles, except in an increase in Alanine residues, beta-sheet of psychrophiles did not express any vital changes. In psychrophilic proteome, the small, tiny, hydrophobic, acidic, non-polar, neutral, aliphatic amino acidic groups expressed significantly high frequencies in the coli region.

Conclusion:

All living organisms have developed the mechanisms to respond to environmental stresses, such as temperature fluctuation. In the case of temperature downshift (cold shock response), several factor plays a crucial role in induction of cold shock proteins. Synthesis of cold-shock proteins seems to be regulated mainly at the post-transcriptional level. Thus, the fate of individual mRNA for each cold-shock protein plays a central role in cold shock response. Most of the free living bacteria possess at least one cold-shock-inducible CspA homologue. Thus, it is very important to understand the individual protein's structure, sequence data and function properly.

This study primarily focuses on to determine the similarity/dissimilarity between two major groups of organisms. Simultaneous analysis of structure and sequence data were employed to draw a conclusion over their gene functionalities.

Reference:

1. Casanueva A, Tuffin M, Cary C, Cowan DA (2010) Molecular adaptations to psychrophily: the impact of 'omic' technologies. Trends Microbiol 18:374 – 381

2. D'Amico S, Collins T, Marx J-C, Feller G, Gerday C (2006) Psychrophilic microorganisms: challenges for life. EMBO Rep 7: 385 – 389

3. Rivkina EM, Friedmann EI, McKay CP, Gilichinsky D (2000) Metabolic activity of permafrost bacteria below the freezing point. Appl Environ Microbiol 66: 3230 – 3233

4. Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. Microbiol Mol Biol Rev 70: 222 – 252

5. Margesin R, Feller G (2010) Biotechnological applications of psychrophiles. Env Technol 31: 835 – 844

6. DasSarma S, Capes MD, Karan R, DasSarma P (2013) Amino acid substitutions in cold-adapted proteins from
Halorubrum lacusprofundi, an extremely halophilic microbe from Antarctica. PLoS ONE 8: e58587

7. Williams TJ, Lauro FM, Ertan H, Burg DW, Poljar A, Raferty MJ, Cavicchioli R (2011) Defining the response of a microorganism to temperatures that span its complete growth temperature range (-2°C to 28°C) using multiplex quantitative proteomics. Environ Microbiol 13: 2186 – 2203

8. Rabus R, Ruepp A, Frickey T, Rattei T, Fartmann B, Stark M, Bauer M, Zibat A, Lombardot T, Becker I et al (2004) The genome of *Desulfotalea psychrophila*, a sulfate reducing bacterium from permanently cold Arctic sediments. Environ Microbiol 6: 887 – 902

9. Riley M, Staley JT, Danchin A, Wang TZ, Brettin TS, Hauser LJ, Land ML, Thompson LS (2008) Genomics of an extreme psychrophile, *Psychromonas ingrahamii*. BMC Genomics 9: 210

10. Kuhn E (2012) Toward understanding life under subzero conditions: the significance of exploring psychrophilic
"cold-shock" proteins. Astrobiology 12: 1078 – 1086

11. Cacace G, Mazzeo MF, Sorrentino A, Spada V, Malorni A, Siciliano RA (2010) Proteomics for the elucidation of cold adaptation mechanisms in Listeria monocytogenes. J Proteom 73: 2021 – 2030

12. Kawamoto J, Kurihara T, Kitagawa M, Kato I, Esaki N (2007) Proteomic studies of an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, for global identification of cold-inducible proteins. Extremophiles 11: 819
– 826

13. Deming JW (2002) Psychrophiles and polar regions. Curr Opin Microbiol 5: 301 – 309

14. Durack J, Ross T, Bowman JP (2013) Characterisation of the transcriptomes of genetically diverse Listeria monocytogenes exposed to hyperosmotic and low temperature conditions reveal global stress-adaptation mechanisms. PLoS ONE 8: e73603

15. Bakermans C, Tollaksen SL, Giometti CS, Wilkerson C, Tiedje JM, Thomashow MF (2007) Proteomic analysis of Psychrobacter cryohalolentis K5 during growth at subzero temperatures. Extremophiles 11: 343 – 354

16. Wilson SL, Frazer C, Cumming BF, Nuin PA, Walker VK (2012) Cross-tolerance between osmotic and freeze-thaw stress in microbial assemblages from temperate lakes. FEMS Microbiol Ecol 82: 405 – 415

17. D'Amico S, Claverie P, Collins T, Georlette D, Gratia E, Hoyoux A, Meuwis MA, Feller G, Gerday C (2002) Molecular basis of cold adaptation. Philos Trans R Soc Lond B Biol Sci 357: 917 – 925

18. Wu Z, Kan FW, She YM, Walker VK (2012) Biofilm, ice recrystallization inhibition and freeze-thaw protection in an epiphyte community. Appl Biochem Microbiol 48: 363 – 370

19. Allen M, Lauro FM, Williams TJ, Burg D, Siddiqui KS, et al. 2009. The genome sequence of the psychrophilic archaeon, *Methanococcoides burtonii*: the role of genome evolution in cold adaptation. ISME J. 3:1012–35

20. Richard Morita Y. Psychrophilic Bacteria. Bacteriological Reviews 1975; 39(2):144-167.

21. Stokes JL, Mary L. Redmond. Quantitative Ecology of Psychrophilic Microorganisms. Applied Microbiology 1966; 14(1):74-78.

22. Ramana KV, Lokendra Singh, Dhaked RK. Biotechnological Application of Psychrophiles and their habitat to low temperature. Journal of Scientific and Industrial Research. 2000; 59:87-101.

23. Burhan Hamid, Ravinder Singh Rana, Deepak Chauhan, Poonam Singh, Fayaz Ahmad Mohiddin, Sanjay Sahay et al. Psychrophilic Yeasts and Their Biotechnological Application- A review. Africal Journal of Biotechnology. 2014; 13:2188- 2197.

24. Rosa Margesin, Vanya Mitera. Diversity and Ecology of Psychrophilic Microorganisms. Research in Microbiology 2011; 162:346-361.

25. Vasut RG, Mihaela Dima Robeci. Food Contamination with Psychrophilic Bacteria. Sanitary Veterinary for Food Safety Direction 2009; XLII(2):325-330.

26. Cavicchioli R, Charlton T, Ertan H, Mohd Omar S, Siddiqui KS, Williams TJ. Biotechnological uses of Enzymes from Psychrophiles. Microbial Biotechnology 2011; 4(4):449-460.

27. Salvino D'Amico, Tony Collins, Jean-Claude Marx, Georges Feller, Charles Gerday. Psychrophilic Microorganisms: Challenges for Life. European Molecular Biology Organization 2006; 7(4):385-390.

28. Gounot AM. Microbial life in permanently cold soils. In: Margesin R, Schinner F, editors. Cold-adapted organisms, ecology, physiology, enzymology and molecular biology. New York: Springer; 1999. p. 4–15. https://doi.org/10.1007/978-3-662-06285-2_1

29. Morita R. Psychrophilic bacteria. Bacteriol Rev. 1975;39:144–167.

30. Feller G, Gerday C. Psychrophilic enzymes: Hot topics in cold adaptation. Nat Rev Microbiol. 2003;1:200–208. https://doi.org/10.1038/nrmicro773

31. Georlette D, Blaise V, Collins T, D'Amico S, Gratia E, Hoyoux A, et al. Some like it cold: Biocatalysis at low temperatures. FEMS Microbiol Rev. 2004;28:25–42. https://doi.org/10.1016/j.femsre.2003.07.003

32. Feller G, Gerday C. Psychrophilic enzymes: Molecular basis of cold adaptation. Cell Mol Life Sci. 1997;53:830–841. https://doi.org/10.1007/s000180050103

33. Morita R, Moyer C. Psychrophiles, origin of. Encyclopedia of biodiversity. 2001;4:917–924. https://doi.org/10.1016/B0-12-226865-2/00362-X

34. Witter LD. Psychrophilic bacteria – A review. J Dairy Science. 1961;44:983–1015. https://doi.org/10.3168/jds.S0022-0302(61)89851-2

35. Cavicchioli R. Cold-adapted Archaea. Nat Rev Microbiol.

2006;4:331–343. https://doi.org/10.1038/nrmicro1390

36. Margesin R, Miteva V. Diversity and ecology of psychrophilic microorganisms. Res Microbiol. 2011;162:346–361. https://doi.org/10.1016/j.resmic.2010.12.004

37. D'Amico S, Collins T, Marx JC, Feller G, Gerday C. Psychrophilic microorganisms: Challenges for life. EMBO Rep. 2006;7:385–389. https://doi.org/10.1038/sj.embor.7400662

38. Deming JW. Psychrophiles and polar regions. Curr Opin Microbiol. 2002;5:301–309. https://doi.org/10.1016/S1369-5274(02)00329-6

39. Miteva V. Bacteria in snow and glacier ice. In: Margesin R, Schinner F, Marx J-C, Gerday C, editors. Psychrophiles: From biodiversity to biotechnology. Berlin: Springer; 2008. p. 31–50. https://doi.org/10.1007/978-3-540-74335-4_3

40. Zhang X, Ma X, Wang N, Yao T. New subgroup of Bacteroidetes and diverse microorganisms in Tibetan plateau glacial ice provide a biological record of environmental conditions. FEMS Microbiol Ecol. 2008;67:21–29. https://doi.org/10.1111/j.1574-6941.2008.00604.x

41. Bej AK, Aislabie J, Atlas RM. Polar microbiology: The ecology, biodiversity and bioremediation potential of microorganisms in extremely cold environments. Boca Raton, FL: CRC Press; 2010.

42. Shivaji S, Prakash J. How do bacteria sense and respond to low temperature? Arch Microbiol. 2010;192:85–95. https://doi.org/10.1007/s00203-009-0539-y

43. Weber MH, Marahiel MA. Bacterial cold shock responses. Sci Progr. 2003;86:9–75. https://doi.org/10.3184/003685003783238707

44. Russell NJ. Membrane components and cold sensing. In: Margesin R, Schinner F, Marx J-C, Gerday C, editors. Psychrophiles: From biodiversity to biotechnology. Berlin: Springer; 2008. p. 177–190. https://doi.org/10.1007/978-3- 540-74335-4_11

45. Gilbert JA, Hill PJ, Dodd CE, Laybourn Parry J. Demonstration of antifreeze protein activity in Antarctic lake bacteria. Microbiology. 2004;150:171–180. https://doi.org/10.1099/mic.0.26610-0

46. Sun X, Griffith M, Pasternak JJ, Glick B. Low temperature growth, freezing survival and production of antifreeze protein by the plant growth promoting rhizobacterium Pseudomonas putida GR12–2. Can J Microbiol. 1995;41:776– 784. https://doi.org/10.1139/m95-107

47. Phadtare S. Recent developments in bacterial cold-shock response. Curr Issues Mol Biol. 2004;6:125–136.

48. Hebraud M, Potier P. Cold shock response and low temperature adaptation in psychrotrophic bacteria. J Mol Microbiol Biotechnol. 1999;1:211–219.

50. Chaikam V, Karlson DT. Comparison of structure, function and regulation of plant cold shock domain proteins to bacterial and animal cold shock domain proteins. BMB Rep. 2010;43:1–8. https://doi.org/10.5483/BMBRep.2010.43.1.001

51. Struvay C, Feller G. Optimization to low temperature activity in psychrophilic enzymes. Int J Mol Sci. 2012;13:11643–11665. https://doi.org/10.3390/ijms130911643

52. Gerday C, Aittaleb M, Bentahier M, Chessa JP, Claverie P, Collins T, et al. Cold-adapted enzymes: From fundamentals to biotechnology. Trends Biotechnol. 2000;18:103–107. https://doi.org/10.1016/S0167-7799(99)01413-4

53. Miyazaki K, Wintrode PL, Grayling RA, Rubingh DN, Arnold FH. Directed evolution study of temperature adaptation in a psychrophilic enzyme. J Mol Biol. 2000;297:1015–1026. https://doi.org/10.1006/jmbi.2000.3612

54. Siddiqui KS, Cavicchioli R. Cold-adapted enzymes. Annu Rev Biochem. 2006;75:403–433. https://doi.org/10.1146/annurev.biochem.75.103004.142723

55. Aghajari N, Feller G, Gerday C, Haser R. Structures of the psychrophilic Alteromonas haloplanctis alpha-amylase give insights into cold adaptation at a molecular level. Structure. 1998;6:1503–1516. https://doi.org/10.1016/S0969- 2126(98)00149-X

56. Cavicchioli R, Charlton T, Ertan H, Mohd Omar S, Siddiqui K, Williams T. Biotechnological uses of enzymes from psychrophiles. Microbial Biotechnol. 2011;4:449–460. https://doi.org/10.1111/j.1751-7915.2011.00258.x

57. Banerjee R, Halder A, Natta A. Psychrophilic microorganisms: Habitats and exploitation potentials. Eur J Biotechnol Biosci. 2016;4:16–24.

58. Bisht S. Cold active proteins in food and pharmaceutical industry [article on the Internet]. Biotech Articles. c2011 [cited 2017 Dec. 15]. Available from: http://www.biotecharticles.com/Biotechnology-products-Article/Cold-Active- Proteins-in-Food-and-Pharmaceutical-Industry-719.html

59. D. F. Rodrigues and J. M. Tiedje, “Coping with our cold planet,”Applied and Environmental Microbiology, vol. 74, no. 6, pp.1677–1686, 2008.

60. B. Steven, R. Léveillé,W. H. Pollard, and L. G.Whyte, “Microbial ecology and biodiversity in permafrost,” Extremophiles, vol. 10,no. 4, pp. 259–267, 2006.

61. J. W. Deming, “Psychrophiles and polar regions,” Current Opinion in Microbiology, vol. 5, no. 3, pp. 301–309, 2002.

62. K. Junge, H. Eicken, and J.W. Deming, “Bacterial activity at -2 to-20○C in arctic wintertime sea ice,” Applied and Environmental Microbiology, vol. 70, no. 1, pp. 550–557, 2004.

63. R. Y. Morita, “Psychrophilic bacteria,” Bacteriological reviews, vol. 39, no. 2, pp. 144–167, 1975.

64. N. J. Russell, “Cold adaptation of microorganisms,” Philosophical Transactions of the Royal Society, vol. 326, pp. 595–611, 1990.

65. A. M. Gounot, “Bacterial life at low temperature: physiological aspects and biotechnological implications,” Journal of Applied Bacteriology, vol. 71, no. 5, pp. 386–397, 1991.

66. N. J. Russell, “Molecular adaptations in psychrophilic bacteria: potential for biotechnological applications,” Advances in Bio-chemical Engineering/Biotechnology, vol. 61, pp. 1–21, 1998.

67. R. M. Morgan-Kiss, J. C. Priscu, T. Pocock, L. Gudynaite-Savitch, and N. P. A. Huner, “Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments,” Microbiology and Molecular Biology Reviews, vol. 70, no.1, pp. 222–252, 2006.

68. R. Margesin, G. Neuner, and K. B. Storey, “Cold-loving microbes, plants, and animals—fundamental and applied aspects,” Naturwissenschaen, vol. 94, no. 2, pp. 77–99, 2007.

69. R. Margesin and F. Schinner, Cold-Adapted Organisms: Ecology, Physiology, Enzymology and Molecular Biology, Springer, Hei-delberg, Germany, 1999.

70. C. Gerday and N. Glansdorff, Physiology and Biochemistry of Extremophiles, ASM Press, Washington, DC, USA, 2007.

71. R. Margesin, F. Schinner, J. C. Marx, and C. Gerday, Psychrophiles, from Biodiversity to Biotechnology, Springer, Berlin, Germany, 2008.

72. K. Horikoshi, G. Antranikian, A. T. Bull, F. T. Robb, and K. O. Stetter, Eds., Extremophiles Handbook, Springer, Berlin, Germany, 2011.

73. R. Margesin, G. Feller, C. Gerday, and N. J. Russell, "Cold-adapted microorganisms: adaptation strategies and biotechno-logical potential," in Encyclopedia of Environmental Microbiology, G. Bitton, Ed., pp. 871–885, John Wiley & Sons, New York, NY, USA, 2002.

74. S. D'Amico, T. Collins, J. C. Marx, G. Feller, and C. Gerday, "Psychrophilic microorganisms: challenges for life," EMBO Reports, vol. 7, no. 4, pp. 385–389, 2006.

75. J. B. Bowman, "Genomic analysis of psychrophilic prokaryotes," in Psychrophiles, from Biodiversity to Biotechnology, R. Margesin, F. Schinner, J. C. Marx, and C. Gerday, Eds., pp. 265–284, Springer, Berlin, Germany, 2008.

76. A. Casanueva, M. Tuffin, C. Cary, and D. A. Cowan, "Molecular adaptations to psychrophily: the impact of "omic" technologies," Trends in Microbiology, vol. 18, no. 8, pp. 374–381, 2010.

77. B. A. Methe, K. E. Nelson, J. W. Deming et al., "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 of the United States of America, vol. 102, no. 31, pp. 10913–10918, 2005.

78. G. Feller, "Molecular adaptations to cold in psychrophilic enzymes," Cellular and Molecular Life Sciences, vol. 60, no. 4, pp. 648–662, 2003.

79. G. Feller and C. Gerday, "Psychrophilic enzymes: hot topics in cold adaptation," Nature Reviews Microbiology, vol. 1, no. 3, pp.200–208, 2003.

80. G. Feller, "Protein stability and enzyme activity at extreme biological temperatures," Journal of Physics: Condensed Matter, vol. 22, article 323101, 2010.

81. R. Margesin and G. Feller, "Biotechnological applications of psychrophiles," Environmental Technology, vol. 31, no. 8-9, pp.835–844, 2010.

82. F. Piette, C. Struvay, and G. Feller, "The protein folding challenge in psychrophiles: facts and current issues," Environmental Microbiology, vol. 13, pp. 1924–1933, 2011.

83. F. Roulling, F. Piette, A. Cipolla, C. Struvay, and G. Feller, "Psychrophilic enzymes: cool responses to chilly problems," in Extremophiles Handbook, K. Horikoshi, Ed., pp. 891–916, Springer, Berlin, Germany, 2011.

84. C. Struvay and G. Feller, "Optimization to low temperature activity in psychrophilic enzymes," in International Journal of Molecular Sciences, vol. 13, pp. 11643–11665, 2012.

85. M. Hébraud and P. Potier, "Cold shock response and low temperature adaptation in psychrotrophic bacteria," Journal of Molecular Microbiology and Biotechnology, vol. 1, no. 2, pp.211–219, 1999.

A Bioinformatic analysis of Heat Shock Proteins in Thermophiles

Dissertation Report

in partial fulfilment of the requirement for the degree of

M.sc. in Botany

by

Deblina Saha

(**UID**- 19173013002, **Reg. No**- 00002 of 2019-20)

Under the guidance of

Dr. Sabyasachi Chatterjee
Assistant Professor

PG Department of Botany
Ramananda College
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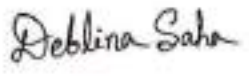
Contents:

DECLARATION

I, Deblina Saha, student of M.Sc. Botany under PG Department of Botany of Ramananda College (Bankura University), Bishnupur, Bankura, hereby declare that all the information furnished in this dissertation project is based on our own intensive research and is genuine. This dissertation does not, to the best knowledge, contain part of our work which has been submitted for the award of our degree either of this college or any other college without proper citation.

Date – 08.08.2021



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Acknowledgement

I would like to acknowledge God for abundant blessings in our life that have allowed us to be where we are today. We express our deep sense of gratitude and profound to our supervisor Dr. Sabyasachi Chantterjee (HOD, UG Department of Botany, Ramananda College) who has helped and encouraged us at all stages of our thesis work with great patience, motivation, enthusiasm and immense care. His suggestions and instructions have served as the major contributor towards the completion of the project.

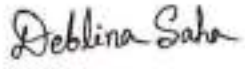
I would like to express our special thanks of gratitude to our teacher Dr. Ajit Kumar Datta (HOD, PG Department of Botany, Ramananda College) as well as our Principal Dr. Swapna Ghorai who gave us the golden opportunity to do the wonderful project, which also helped us in doing a lot of Research and we came to know about so many new things, we are really thankful to them.

I am extremely grateful to our parent for their love, prayers, carrying sacrifices for education and preparing us for our future.

I would like to express our special thanks to Mr. Sourav Singha (SACT, Department of Microbiology, Bankura Sammilani College) for helping us in doing a lot of research and bioinformatics analysis required in this project.

In the accomplishment of this project successfully, many people have best owned upon their blessings and the heart pledged support, this time We are utilizing to thank all the people who have been concerned with this project.

Date: 08.08.2021



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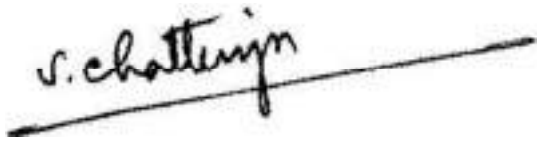
Certificate

This is to certify that the dissertation project entitled "***A Bioinformatic analysis of Heat Shock Proteins in Thermophiles***" has been carried out by Deblina Saha (UID: 19173013002, Regn. No.: BKU/00002 of 2019-20), under my guidance and supervision. To the best of my knowledge, the present work is the result of his original investigation and study done in the PG Department of Botany, Ramananda College, Bishnupur, Bankura. No part of the dissertation has ever been submitted anywhere for any other degree.

The dissertation is fit for submission and the partial fulfilment of the conditions for the award of degree in M.Sc. in Botany.

Date: 10.08.2021



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List of the Figures:

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List of abbreviations:

Sl. No.	Used	Stands For
1	C	celsius
2	HSP	Heat shock protein
3	USA	United States of America
4	UV	Ultraviolet
5	GrpE	Gro-P like protein E
6	ATP	Adenosine Triphosphate
7	kDa	kilodalton
8	NBD	Nucleotide-binding domain
9	SBD	Substrate-binding domain
10	Ala	Alanine
11	Arg	Arginine
12	Asn	Asparagine
13	Asp	Aspartic acid
14	Cys	Cysteine
15	Gln	Glutamine
16	Glu	Glutamic acid
17	Gly	Glycine
18	His	Histidine
19	Ile	Isoleucine
20	Leu	Leucine
21	Lys	Lysine
22	Met	Methionine
23	Phe	Phenylalanine
24	Pro	Proline
25	Ser	Serine
26	Thr	Threonine
27	Trp	Tryptophan
28	Tyr	Tyrosine
29	Val	Valine
30	PDB	Protein Database
31	NCBI	National Canter of Biotechnology Information
32	BLAST	Basic Local Alignment Search Tool
33	MUSCLE	Multiple Sequence Comparison by Log- Expectation
34	DNA	Deoxyribonucleic acid
35	RNA	Ribonucleic acid

1. Introduction

Microbial growth is distinctly dependent on physical factors, especially on temperature. Perhaps due to the major constituent of microbial cells are aqueous chemicals. Therefore, their existence is theoretically confined to a range of temperature (Brock *et al.,* 1970). Consequently, microorganisms can grow at different temperature ranges exhibiting pronounced diversity.

Recent studies have shown that microbial life can exist at temperatures close to or slightly above the boiling point of water. Even in most inhospitable habitats on Earth like thermal vents and hot springs very few of living organisms can flourish (Brock *et al.,* 1970). These microorganisms are referred to as thermophiles. Generally, they can grow at temperatures ranging from 45°C to 75°C, with optimal growth occurring between 50°C and 60°C (Hatman *et al.*, 1989 & Panikov *et al.*, 2003). Thermophilic organisms are categorised into two types: obligatory, which are unable to survive under 40°C–42°C temperature, and facultative, which can exist at low as well as at high temperatures (Farrell and Campbell, 1969). They have also been classified as hyperthermophiles, thermophiles, and moderate thermophiles based on their optimal growth temperature. Hyperthermophiles can be found in all three domains of life: archaea, bacteria, and eukarya, with archaea and bacteria accounting for the vast majority. *Pyrolobus fumari* (Cowan, 2004) has been reported to grow at as high as 110 °C temperature, but *Thermus thermophilus* (Oshima and Imahori, 1974) thrives at temperatures around 70 °C. *Bacillus* species like *B. licheniformis* and *B. brevis* have been shown to grow at 50 (Warth, 1978 & Gupta *et al.*, 2014). Apart from these, several *Bacillus* species thrive in both mesophilic and thermophilic environments, such as *B. methanolicus*, *B. smithii*, and *B. coagulans*, which thrive at temperatures ranging from 37 to 63°C (Bosma *et al.,* 2015; Arfman *et al.*, 1992 & Marshall and Beers, 1967).

Microorganisms have capabilities to thrive as well as adapting to a wide range of environmental stresses due to the activities of several macromolecules, especially a specific group of proteins. The breakdown and denaturation of numerous life-sustaining macromolecules has been identified to occur in cells at high temperature. (Singleton *et al.,* 1973). Proteins are thermolabile in nature, thus, proteins lacking in the essential adaptations undergo irreversible unfolding at such high temperatures, exposing the hydrophobic cores and causing aggregation. Therefore, it is necessary for thermophilic proteins undergo adaptations that allow them to maintain their structure and function at those hostile temperatures (Tomazic *et al.*, 1988). As a result of these environmental changes, the bacteria's genome evolves, express several thermostable proteins, giving them thermal tolerance and the ability to survive at high temperatures (Christopher *et al.*, 2013).

Thermophilic bacteria have originated on Venus and were transported to Earth by solar radiation pressure (Arrhenius, 1927). There have been debates over their origins, perhaps mesophiles evolved from thermophiles or vice - versa. Allen in 1953 has made a compelling argument for thermophiles having a mesophilic origin and further evolved through either adaption or mutation. The presence of thermophilic species in non-thermophilic conditions, as well as the discovery that some mesophilic species may adapt to grow at higher temperatures, are the foundations of this argument (Allen,1953). Mesophiles are thought to have originated in a thermophilic environment, according to current findings. The idea that evolution occurred in a much warmer environment than the current one provides the strongest support for this

concept. The genesis of thermophilic organisms, on the other hand, does not appear to be well established (Tanaka *et al.*, 1971).

Generally, most research has concentrated on the properties of specific molecules, such as protein structural stability or thermophile enzyme activity. Several factors are responsible for thermostability have been explained using many crystalline structures of the observed thermophilic enzymes, like amino acid changes (Arnorsdottir *et al.*, 2009), hydrophobic cores (Bezsudnova *et al.,* 2012; Chen *et al.*, 2004), buried polar contacts and ion pairs (Hakulinen *et al.*, 2003), and interactions between subunits (Nakka *et al.*, 2006; Pang *et al.*, 2007). In the realm of thermophiles, biological analyses based on large-scale data are being used to investigate the major thermophilic factors.

The survival of thermophilic bacteria is mostly owing to the protein's inherent stability. When organisms are exposed to near-lethal temperatures, ubiquitous heat shock reactions are found. A group of proteins with a diverse activity that are induced in response to sudden temperature changes are known as Heat shock proteins (Kagawa *et al.*, 1995). Thermotolerance can result from the production of these proteins, allowing organisms to thrive at even greater temperatures (Hightower, 1991; Lindquist, 1992). Most of HSPs act as molecular chaperones, helping in the refolding of denatured proteins, assisting in the maturation of newly produced proteins, and inhibiting protein aggregation (Hartl, 1996; Hayes, 1996).

The aim of this review is to concentrate on the thermophilic protein stability, the role of Heat Shock Proteins and the proteomic analysis of thermophilic bacteria. Physicochemical data of thermophilic as well as mesophilic bacteria has accumulated, and several proteomic analyses have done on physicochemical data of proteins. Amino acid composition has analysed to identify the specific amino acids, which are responsible to sustain the microorganisms in high temperature. Multiple sequence alignment of similar proteins from different thermophilic & mesophilic bacteria has analysed to identify the conserved sequence of any protein and Percentage identity matrix also obtained to establish the homology of the similar proteins from different thermophilic & mesophilic bacteria.

2. Habitat

Thermophiles have been found in a wide range of thermal habitats, including continental hot springs and geothermal sites. They have been discovered in most soil, mud, and water samples from all around the planet (Brock 1967; Hatman et al., 1989; Panikov et al., 2003). Soil exposed to full sunshine are frequently heated to temperatures above 50°C at midday, with some soils reaching temperatures as high as 70°C, even though the temperature is substantially lower a few millimetres beneath the soil surface. Temperatures of up to 70°C are found in compost piles and silage, where the materials ferment. In fact, bacteria undergo fermentation, or they are carrying out some metabolic activity, as a result temperature rises. However, the most extensive high-temperature environments found in nature, are associated with volcanic activities including hot springs. Hot springs are special places with a wide range of natural conditions and a high temperature. Most hot springs have temperatures that are near or equal to boiling point of water (Yohandini *et al.*, 2015). The Western United States, Central Africa, Central America, New Zealand, Italy, Japan, Indonesia, and Iceland are all home to hot springs (Mohammad *et al*., 2017). Yellowstone National Park in Wyoming has the world's biggest single concentration of hot springs (USA). There are about 70 active volcanoes in

Indonesia, as well as a vast number of geothermal areas and numerous hot springs (Kusumadinata, 1979). A variety of thermophiles can be found in these locations.

Sl. No.	Name of the Organism	Temperature Range	References
1	*Thermus thermophilus HB8*	56-78 °C	Oshima *et al*., 1974
2	*Oceanithermus profundus*	40–68 °C	Miroshnichenko *et al*., 2003
3	*Thermotoga maritima*	55-90°C	Huber *et al*., 1986
4	*Thermus aquaticus*	70-75 C	Brock *et al*., 1969
5	*Bacillus stearothermophilus*	65-69°C	Beffa, 1996
6	*Kosmotoga olearia*	65 °C	Polo M. J. *et al*., 2017
7	*Dictyoglomus thermophilum*	70°C	Patel B. K. *et al*., 1987
8	*Fervidobacterium gondwanense*	65-68°C	Andrews *et al*., 1996
9	*Fervidicola ferrireducens*	55-80 °C	Ogg *et al*., 2009
10	*Meiothermus sp.*	66°C	Ogg *et al*., 2009
11	*Thermus sp.*	75°C	Ogg *et al*., 2009
12	*Flavobacterium thermophilum*	65-72 °C	Oshima *et al*., 1974
13	*Marinithermus hydrothermalis*	50-72 °C	Miroshnichenko *et al*., 2003

Table 1: Temperature range of different thermophilic bacteria

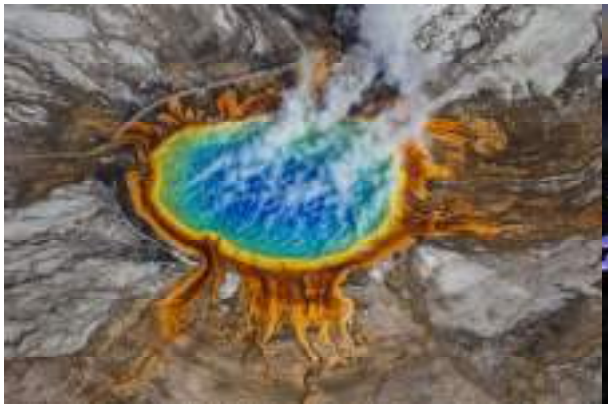


Figure 1: Yellowstone national Park (Hot springs)

Figure 2: Kawah Ijen volcano in Indonesia

3. Thermophilic protein stability:

Proteins, particularly enzymes, are thought to be extremely susceptible structures that are sensitive to changes in environment, such as increased temperatures. Extreme thermophilic microbes, on the other hand, have been found to grow best at temperatures above 70°C. Thermophilic and hyper thermophilic bacteria have generated a variety of enzymes. These thermophilic enzymes are completely active and resistant to high temperatures (Závodszky *et al.,* 1998). They share most physicochemical features with their mesophilic relatives, including the active sites of homologous pairs (Závodszky *et al.,* 1998). The thermophilic enzymes should be as active at room temperature as their mesophilic relatives, based on these similar characteristics and the Arrhenius theory.

There are some suggested mechanisms or indicators of greater thermostability: a more highly hydrophobic core, tighter packing or compactness, deleted or shortened loops, greater rigidity (for example through increased Proline content in loops), higher secondary structure content, greater polar surface area, fewer or smaller voids, smaller surface area to volume ratio,

fewer thermolabile residues, increased hydrogen bonding, higher isoelectric point, and more salt bridges or ion pairs and networks of salt bridges (Taylor *et al.*,2009).

In the research, more ion pairs have been consistently associated to thermostability. At 0°C, water has a dielectric constant of roughly 80, which falls to 55 at 100°C and even lower at high pressures near hydrothermal vents in the deep ocean, where some hyper thermophilic microorganisms exist. A lower dielectric constant makes electrostatic interactions stronger and therefore ion pairs should have a greater stabilizing effect at high temperatures and pressures (Taylor *et al.* 2009).

I. Heat Shock Proteins:

i. The Adverse Effects of Heat

The heat shock response of an organism is triggered when there is an increment few degrees of temperature in the environment (D'Amico *et al*., 2006; Takai *et al.*, 1998). Protein unfolding, unspecific aggregation and entanglement can all be caused by a modest increase in temperature (Courgeon *et al.*, 1984). Protein aggregation and an imbalance in protein homeostasis in general can explain many of the morphological and phenotypic impacts of heat stress. As a result, it is fair to believe that the harmful accumulation of unfolded proteins is a signal to initiate countermeasures. Surprisingly, this situation implies that the cell is unable to recognize temperature by itself. Rather, it indicates that unfolded proteins caused by a range of stimuli, such as oxidative stress, ethanol, heavy metals, or other toxic chemicals, initiate the heat shock response (Courgeon *et al.*, 1984 & Heikkila *et al.*, 1982). Further than the unfolding of individual proteins, heat shock has harmful effects on the cell's internal structure (Szalay *et al*., 2007 & Toivola *et al.,* 2010). These factors combine to cause a cell cycle arrest as well as growth and proliferation stagnation (Lindquist, 1980; Yost and Lindquist, 1986). The accumulation of damage can lead to the cell's death depending on the duration and intensity of the heat stress. Importantly, if heat stress is not fatal, it can lead to a greater tolerance for other, potentially fatal, stresses. This resistance is based on the higher levels of Hsps generated in response to moderate stress situations (Lindquist, 1986). Cross protection is possible: Hsps triggered by one type of stress can protect against other types of stress (Lindquist, 1986).

ii. Role of HSPs

Thermotolerance, a cellular adaptation, allows an organism to tolerate a non-lethal heat stress subsequently survive from lethal heat exposure (Moseley et *al.*, 1997). Localization, regulation, and function of HSPs in the cell has been widely studied to understand their thermotolerance. Initially, stress induced HSP accumulation was related to thermotolerance, or the ability to withstand otherwise fatal heat stress, and later with tolerant to a variety of stresses, such as cytokines (Jäättelä *et al.,* 1993), ischemia (Marber *et al.,* 1995) and UV irradiation (Barbe *et al.,* 1988). The fact that overexpression of multiple HSPs confers tolerance in the lack of conditioning stress and that prevents HSP accumulation using locking antibodies reduces stress tolerance significantly supports the concept that HSPs give stress tolerance directly. The method by which HSPs give stress tolerance is unknown, however it may have something to do with HSPs' key participation in the stress denatured proteins processing (Mizzen *et al.,* 1988). HSPs are also thought to deal with the protein fragments that emerge from stress-induced translational arrest (Chirico *et al.,* 1988). The structural proteins maintenance could possibly be important for HSP-related stress tolerance.

Sl. No.	Name of HSP	organism	PDB/NCBI Id	Referenece
1	grpE	*Thermus thermophilus HB8*	3A6M/ BAA81742	Sunny *et al.* 2020
2	GroEL	*Thermus thermophilus*	4V4O/ BAW02143	Sunny *et al.* 2020
3	HrcA	*Thermotoga maritima*	1STZ/ WP_004080775	Sunny *et al.* 2020
4	radical SAM domain protein	*Thermus thermophilus HB8*	--/ BAD70627	Sunny *et al.* 2020
5	GroES	*Thermus thermophilus*	--/ BAW02144	Sunny *et al.* 2020
6	YidC	*Thermotoga maritima MSB8*	5Y83/5Y83_A	Sunny *et al.* 2020
7	HspA (Hsp20)	*Thermosynechococcus vulcanus*	--/ BAA32501	Sunny *et al.* 2020
8	DnaK	*Thermus thermophilus HB8*	--/ BAA81741	Sunny *et al.* 2020
9	DnaJ 2	*Thermus thermophilus HB8*	4J80/4J80_D	Sunny *et al.* 2020
10	ClpB	*Thermus thermophilus HB8*	1QVR/1QVR_A	Sunny *et al.* 2020

Table 2: List of Heat shock proteins

iii. Chaperonins

Chaperonins are ATP-dependent ring-shaped chaperones that enclose non-native proteins. The GroE machinery in bacteria is the most significant chaperonin (Figure 3). It is made up of 14 GroEL subunits organised in a two-heptameric ring cylinder to which the cochaperone GroES binds (Grallert *et al.*, 2001; Horwich *et al.*,2006). GroEL engulfs one non-native protein in each cavity, and GroES cofactor binding closes each cavity in the presence of ATP (Hartl *et al.*, 2002; Todd *et al.*, 1994). It is simple and easy to understand how GroEL helps to stress resistance. Firstly, it binds a wide variety of nonnative proteins; almost 50% of all E. coli proteins have been found to bind to GroEL (Viitanen *et al.*, 1992). Secondly, during the duration of the ATP hydrolysis cycle, it makes individual polypeptide chains. Depending on the folding characteristics of the protein, they may fold during this period or gain their natural structure after being released from GroE.

As a result, a GroE-bound protein may begin folding in complete isolation, unaffected from nonnative polypeptide chains. The disadvantage of this method is that it requires a large quantity of GroE to capture a significant portion of the proteins that unfold during stress. Due to the restricted amount of upregulation of GroE expression, the protective impact of GroE has a limit (Goloubinoff *et al.*, 1989). This upregulation was far more significant than that observed in stressful conditions. The mechanism that produces such high levels of GroE expression is mysterious. It is simple to see how such a powerful protein-folding machine, which is necessary in bacteria, would also be important in eukaryotic cells' stress management.

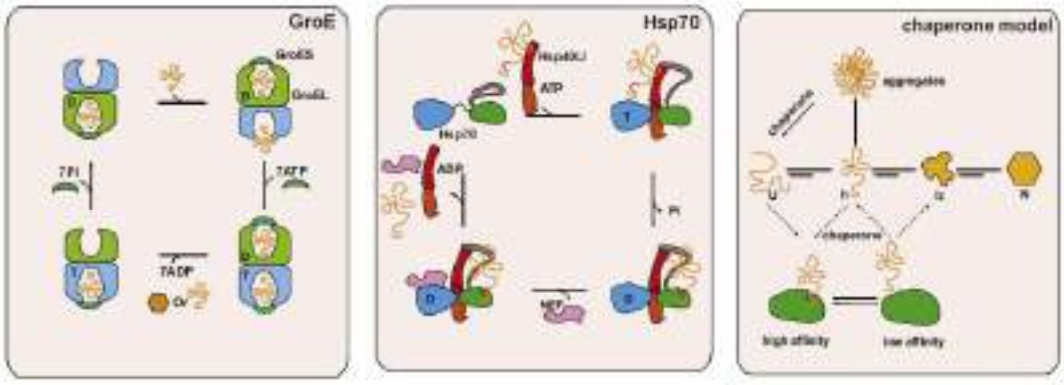
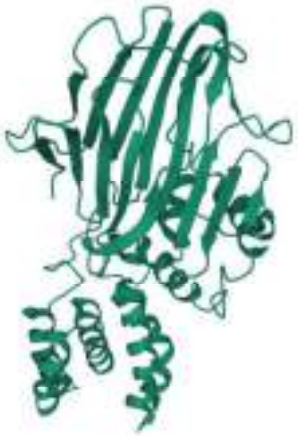


Figure 3: Molecular Chaperone Mechanisms

Sl. No.	Protein name	PDB Entry ID	Organism	Image
1	grpE	3A6M	Thermus thermophilus HB8	
2	groel	4V4O	Thermus Thermophilus	
3	hsp15	3BBV	Thermus thermophilus	

4	pbs lyase	2E9F	Thermus thermophilus HB8	
5	hrcA	1STZ	Thermotoga maritima	
6	Sam family enzyme	3M6V	Thermus thermophilus HB8	

7	groES	4V4O	Thermus thermophilus	
8	yidC	5Y83	Thermotoga maritima MSB8	
9	hsp20 (hspA)	6EWN	Thermosynechococcus vulcanus	
10	dnaK	6PRP	Thermus thermophilus HB8	

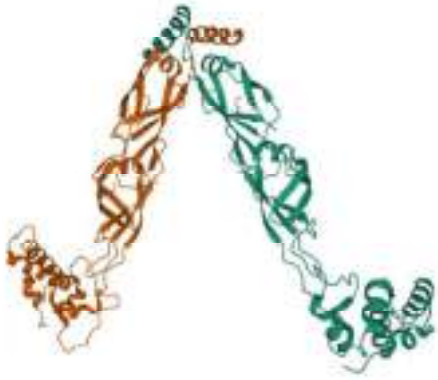
11	dnaJ	4J80	Thermus thermophilus	
12	ClpB	4FD2	Thermus thermophilus HB8	

Table 3: 3D Structure of different Heat shock proteins (HSP)

The 70-kDa heat shock proteins (Hsp70), molecular chaperones, involved in refolding of stress-denatured proteins, protein complex assembly, and transport of newly produced peptides across membranes. HSP70 proteins act by binding and releasing protein substrates in an ATP-dependent manner (F.U. Hartl, 1996; F.U. Hartl *et al.*, 2002). The nucleotide exchange factor of Hsp70 (DnaK, DnaJ, and GrpE) and the J-domain ATPase-activating protein of Hsp40 family are actively involves in the Hsp70 chaperone cycle in Escherichia coli. The nucleotide state of DnaK's N-terminal nucleotide-binding domain (NBD) determines the C-terminal substrate-binding domain's (SBD) affinity for substrates (Raviol *et al.* 2006; Schmid *et al.* 1994). NBD and SBD are linked via a conserved hydrophobic linker. When ADP is connected to NBD, SBD has a high substrate affinity, but when ATP is coupled to NBD, SBD has a reduced substrate affinity (Brehmer *et al.*, 2004; Moro *et al.* 2007). It is still unknown how DnaJ and GrpE, two DnaK domains, interact during the chaperone cycle. GrpE speeds up the conversion of ADP to ATP in DnaK 5000 times. The relevance of full-length DnaK and GrpE for forming a ternary complex and substrate processing has been highlighted in several biochemical and thermodynamic studies. The interdomain linkers SBD and DnaK, which are required for substrate association, are not present in the complex structure (Brehmer *et al.*, 2004).

4. Regulation of HSP Genes:

Heat shock proteins (HSPs) are the most well-known proteins that react to heat stress and protect cells from cellular damage (Mizobata *et al.*, 2000). A particular transcription factor is necessary for the heat shock response (Wu *et al.*, 1986; Wu, 1984). Grossman reported that HSP overexpression in E. coli is caused by the regulatory protein σ32 (Grossman *et al.*, 1984). Under heat stress, the alternative subunit σ32 of the bacterial RNA polymerase replaces the usual regulatory σ70 protein. The activation of σ32 is thought to be induced by a disruption in protein homeostasis. Hsp70 and Hsp40, two chaperones, have the ability to inhibit σ32. σ32 is

present in a cluster with the Hsp70 protein DnaK and its cofactor DnaJ under favourable conditions (Rodriguez *et al*., 2008). According to the generally accepted chaperone titration model (Rhodius *et al*.,2010), heat shock generates σ32 from chaperone complexes. Chaperones are necessary to bind unfolded proteins. The chaperone titration model explains how the heat shock transcription factors are inactivated in the existence of unemployed chaperones, but dramatically activated when chaperones are busy in the presence of unfolded proteins (Rhodius *et al*.,2010). The unfolding of outer membrane porins appears to be the activation signal (Walsh et al., 2003; Kim et al., 2010; Hasenbein et al., 2010). When the cell recovers normal function, the surplus of free chaperones causes the transcriptional regulator to be downregulated again (Rhodius *et al*., 2010).

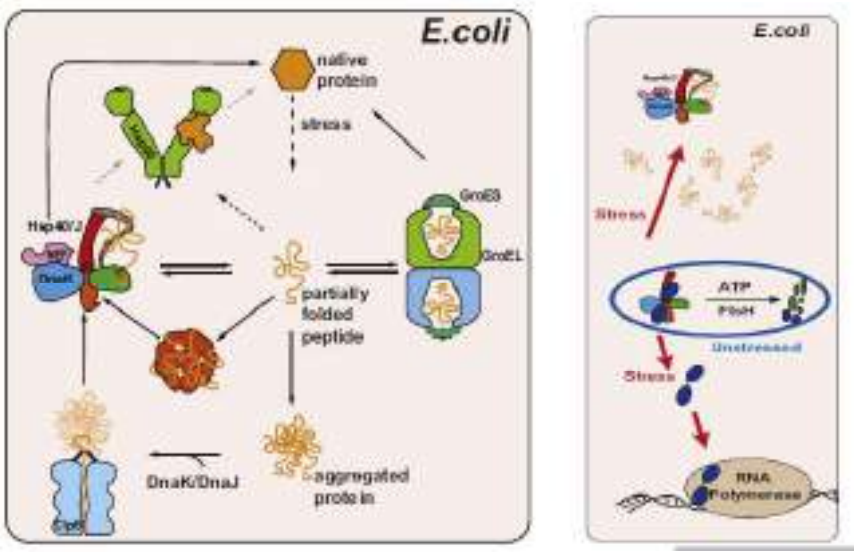


Figure 4: Regulation of the Heat Shock Response

5. Proteome Analysis

I. Amino Acid Composition

As per the study conducted by Jaenicke and Bohm in 1998, Val and Leu are the most thermostable amino acids when thermophiles are retained at a temperature of 100°C or above, followed by His, Tyr, Lys, Ile and evidently Arg, Glu, Asp, and Cys are the least thermostable of all the typical amino acids (Jaenicke *et al.* 1998). There have been statistically significant differences in sequence composition between thermophilic and mesophilic proteins. The amino acids Gln, Asn, Cys and Met are thermolabile, which means they deamidate (Asn and Gln) or oxidise (Met and Cys) at high temperatures (Kumar *et al.* 2000). In thermophilic proteins, these amino acids are less abundant. Despite the high sequence similarity between the protein structural pairs, the overall amino acid composition in thermophilic proteins and mesophilic proteins is distinguishable. When thermophilic proteins are compared to their mesophilic homologs, the proportions of thermolabile residues Ser and Cys decrease significantly, whereas those of Tyr and Arg increase significantly (Kumar *et al.* 2000). Jaenicke and Bohm analysed the genomes of thermophiles and mesophiles and discovered that the genome of thermophiles encodes for more charged amino acids and fewer polar/uncharged residues than the mesophilic genome. They also discovered that as the temperature rose, glutamine deamidation increased (Jaenicke *et al.* 1998).

There are 15 different proteins (non-HSP) as well as 10 Heat shock proteins (HSP) were identified from thermophilic bacteria from the well-known Protein Data Bank (PDB) & National Center of Biotechnology Information (NCBI). FASTA sequence of the identified proteins were analysed in the web based ProtParam (https://web.expasy.org/cgi-bin/protparam/protparam) tool of Expasy to extract the amino acid composition of those identified proteins. All the amino acid composition data (Table 4 & 5) analysed to identify the ratio of the presence of different amino acids in those identified proteins. In addition to this amino acid composition ratio in the HSP proteins (Table 4) & non-HSP proteins are analysed separately (Table 5).

Heat Shock Protein	**grpE**	**chaperonin GroEL**	**HrcA**	**MqnE**	**chaperone GroES**	**YidC**	**HspA**	**Chaperone protein DnaK**	**Chaperone protein DnaJ 2**	**Chaperone protein ClpB**	**Average**
ORGANISM	*Thermus thermophilus HB8*	*Thermus thermophil...*	*Thermotoga maritima*	*Thermus thermophilus HB8*	*Thermus thermophil...*	*Thermotoga maritima MSB8*	*Thermosynechococcus vulcanus*	*Thermus thermophilus HB8*	*Thermus thermophilus HB8*	*Thermus thermophilus HB8*	
PDB Id/ NCBI Id	3A6M/ --	4V4O /--	1STZ / WP_ 004080775	--/ BAD70627	--/ BAW02144	5Y83/5Y83_A	--/ BAA32501	4J80/4J80_D	4J80/4J80_D	1QVR/ 1QVR_A	
Ala (A)	11.90%	13.60%	3.00%	8.90%	5.90%	4.40%	6.90%	11.20%	9.90%	10.50%	8.62%
Arg (R)	10.70%	5.00%	8.60%	9.40%	5.00%	3.50%	8.30%	6.70%	8.50%	9.80%	7.55%
Asn (N)	1.70%	3.30%	4.40%	1.90%	1.00%	3.80%	2.10%	2.80%	1.40%	1.40%	2.38%
Asp (D)	5.60%	4.20%	3.60%	5.40%	5.00%	4.20%	4.80%	4.90%	4.20%	4.80%	4.67%
Cys (C)	0.00%	0.00%	0.30%	0.80%	0.00%	0.20%	0.00%	0.30%	0.00%	0.00%	0.16%
Gln (Q)	2.30%	1.80%	1.80%	2.70%	3.00%	2.20%	3.40%	3.40%	2.10%	3.70%	2.64%
Glu (E)	17.50%	12.00%	10.90 %	8.30%	12.90%	5.10%	13.10%	11.20%	9.90%	12.60%	11.35%
Gly (G)	8.50%	8.50%	5.90%	7.50%	10.90%	5.80%	3.40%	8.00%	10.60%	6.80%	7.59%
His (H)	1.10%	0.40%	0.60%	3.80%	0.00%	2.20%	0.70%	1.50%	3.20%	1.50%	1.50%
Ile (I)	1.70%	6.60%	6.80%	5.40%	7.90%	7.10%	5.50%	6.20%	2.80%	6.30%	5.63%
Leu (L)	13.00%	9.00%	13.30 %	10.80%	7.90%	12.40%	11.00%	9.10%	8.10%	13.10%	10.77%
Lys (K)	6.20%	8.70%	8.30%	4.80%	10.90%	7.80%	7.60%	6.70%	4.90%	5.70%	7.16%
Met (M)	1.70%	1.30%	1.80%	2.40%	1.00%	1.60%	2.80%	1.50%	0.00%	1.10%	1.52%
Phe (F)	4.00%	2.20%	4.40%	3.80%	1.00%	7.10%	3.40%	2.30%	3.90%	2.00%	3.41%
Pro (P)	4.00%	2.80%	2.70%	4.60%	5.00%	4.00%	6.20%	5.00%	9.90%	4.00%	4.82%
Ser (S)	1.10%	3.10%	6.80%	2.20%	1.00%	4.70%	4.80%	2.90%	2.10%	2.80%	3.15%
Thr (T)	0.60%	6.60%	5.60%	5.60%	5.00%	6.70%	6.20%	6.20%	3.50%	4.00%	5.00%
Trp (W)	0.00%	0.00%	0.30%	2.20%	0.00%	2.00%	0.70%	0.20%	0.40%	0.70%	0.65%
Tyr (Y)	1.70%	1.10%	4.40%	3.00%	2.00%	6.00%	1.40%	1.00%	4.20%	2.10%	2.69%
Val (V)	6.80%	9.80%	6.50%	6.70%	14.90%	9.30%	7.60%	9.10%	8.10%	6.90%	8.57%
Pyl (O)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Sec (U)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	

Table 4: Amino acid composition table of HSP Proteins of Thermophiles

Non-HSP Protein	**Cytochrome ba3**	**Magnesium transport**	**LeuT Leucine**	**polysulfide reductase**	**Mg2+ transporter**	**V-type ATPases**	**Probable SecDF**	**Photosystem II**	**Cytochrome ba3 with**	**SecYEG translocon in**	**Sulfide:quinone**	**V-type ATP synthase**	**apo-FtsH ATP-**	**complex I (NADH-**	**TAQ DNA POLYMER**	**Average**
ORGANISM	*Thermus thermophilu*	*Thermotoga maritima*	*Aquifex aeolicus*	*Thermus thermophilu*	*Thermus thermophilu*	*Thermus thermophilu*	*Thermus thermophilu*	*Thermosynechococcus*	*Thermus thermophilu*	*Thermotoga maritima*	*Aquifex aeolicus*	*Thermus thermophilu*	*Thermotoga maritima*	*Thermus thermophilu*	*Thermus aquaticus*	
PDB Id	1EHK	2HN2	2QJU	2VPZ	2ZY9	3A5C	3AQP	3WU2	3BVD	3DIN	3HYW	3K5B	3KDS	3M9S	1TAQ	
Ala (A)	11.00%	2.30%	10.60%	8.60%	8.90%	9.50%	11.50%	9.90%	10.90%	5.50%	9.10%	23.10%	9.70%	7.50%	10.80%	9.93%
Arg (R)	4.10%	6.20%	3.90%	6.90%	6.60%	6.60%	6.70%	3.80%	4.20%	6.40%	3.30%	10.60%	7.10%	6.80%	9.10%	6.15%
Asn (N)	2.50%	3.10%	2.70%	2.40%	1.10%	1.70%	2.70%	6.70%	2.50%	3.80%	5.10%	0.00%	3.40%	2.10%	1.40%	2.75%
Asp (D)	2.10%	5.90%	2.30%	4.20%	6.60%	4.50%	3.40%	2.30%	2.10%	6.00%	4.00%	1.00%	5.60%	3.90%	5.00%	3.93%
Cys (C)	0.00%	0.30%	0.00%	1.00%	0.00%	0.50%	0.00%	1.50%	0.00%	0.30%	1.60%	0.00%	0.60%	1.40%	0.40%	0.51%
Gln (Q)	2.00%	2.00%	1.20%	2.90%	1.90%	2.90%	3.00%	2.30%	1.90%	2.90%	1.60%	1.90%	1.30%	2.30%	1.90%	2.13%
Glu (E)	2.30%	9.60%	4.50%	8.00%	8.70%	9.20%	6.30%	4.40%	2.30%	10.30%	7.00%	22.10%	11.20%	8.20%	10.50%	8.31%
Gly (G)	7.80%	4.00%	8.80%	8.80%	6.60%	9.50%	8.00%	9.00%	7.70%	6.50%	7.90%	3.80%	8.40%	11.20%	7.10%	7.67%
His (H)	2.10%	2.00%	1.20%	3.10%	2.50%	1.00%	1.60%	3.20%	3.20%	2.10%	2.10%	0.00%	1.90%	2.10%	2.20%	2.02%
Ile (I)	4.30%	7.90%	10.60%	4.30%	3.20%	6.10%	6.50%	7.80%	4.20%	7.00%	8.10%	1.00%	7.50%	4.80%	3.00%	5.75%
Leu (L)	16.90%	10.20%	11.70%	9.50%	17.80%	8.10%	15.40%	9.00%	16.70%	9.20%	5.80%	14.40%	9.50%	9.80%	14.90%	11.93%
Lys (K)	2.00%	6.80%	3.50%	6.10%	2.70%	4.20%	3.50%	0.60%	1.80%	9.60%	7.20%	11.50%	6.90%	5.30%	5.00%	5.11%
Met (M)	3.00%	2.80%	2.30%	1.80%	1.90%	3.30%	0.90%	3.20%	3.00%	3.00%	3.00%	1.00%	1.90%	3.00%	1.90%	2.40%
Phe (F)	6.60%	5.40%	9.80%	3.80%	2.10%	3.30%	4.30%	7.80%	6.50%	4.10%	6.00%	0.00%	3.40%	3.70%	3.20%	4.67%
Pro (P)	6.90%	4.20%	4.70%	8.00%	4.70%	6.10%	4.50%	4.10%	6.90%	2.40%	7.90%	1.00%	4.50%	7.10%	5.80%	5.25%
Ser (S)	3.90%	4.20%	3.50%	2.70%	4.00%	4.00%	4.70%	6.70%	3.90%	4.70%	3.30%	1.00%	3.20%	4.80%	3.40%	3.87%
Thr (T)	3.90%	5.90%	5.30%	4.30%	4.90%	4.80%	5.40%	4.70%	3.90%	3.70%	4.90%	1.00%	3.70%	5.00%	3.60%	4.33%
Trp (W)	4.30%	1.40%	3.10%	2.50%	1.30%	1.40%	0.70%	2.90%	4.20%	0.80%	0.90%	0.00%	0.20%	1.80%	1.70%	1.81%
Tyr (Y)	3.90%	4.80%	3.30%	4.40%	3.00%	3.60%	2.20%	4.10%	3.90%	3.80%	3.30%	1.90%	1.70%	4.10%	2.90%	3.39%
Val (V)	10.30%	11.00%	6.80%	6.50%	11.80%	9.70%	8.80%	6.10%	10.20%	7.80%	7.90%	4.80%	8.20%	5.30%	6.10%	8.09%
Pyl (O)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Sec (U)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 5: Amino acid composition table of Non-HSP Proteins of Thermophiles

It is observed that Ala, Glu, Gly, Leu & Val amino acids are found plenty in the non-HSP proteins, whereas only Glu & Leu amino acids are found in higher proportion in the HSP proteins. In addition to this amino acid composition analysis, we identified the similar proteins in the mesophilic bacteria (Escherichia coli name of mesophilic) to find the homology between proteins of thermophiles and mesophiles. The amino acid composition of the similar proteins found in the thermophiles as well as mesophiles are compared in tabular form to analyse the composition of the amino acids. Few of HSP/non-HSP protein comparison are shown below:

PROTEIN:	grpE	GRPE	heat-inducible transcription	heat-inducible transcription	HspA	sHSP20-GI	DnaK	DnaK	Chaperone protein ClpB	chaperone ClpB
ORGANISM	*Thermus thermophilus HB8*	*Escherichia coli*	*Thermotoga maritima*	*Mesotoga infera*	*Thermosynechococcus vulcanus*	*Escherichia coli*	*Thermus thermophilus HB8*	*Escherichia coli*	*Thermus thermophilus HB8*	*Escherichia coli*
PDB Id/NCBI Id	3A6M/--	1DKG/--	1STZ/WP_004080775	--/WP_169699550	--/BAA32501	--/WP_074468313	4J80/4J80_D	--/WP_023278178	1QVR/1QVR_A	--/WP_042107122
Ala (A)	11.90%	12.20%	3.00%	4.90%	6.90%	8.60%	11.20%	11.00%	10.50%	9.20%
Arg (R)	10.70%	5.60%	8.60%	7.50%	8.30%	7.90%	6.70%	3.90%	9.80%	7.70%
Asn (N)	1.70%	4.10%	4.40%	4.90%	2.10%	3.90%	2.80%	4.10%	1.40%	3.50%
Asp (D)	5.60%	6.60%	3.60%	6.10%	4.80%	7.20%	4.90%	8.60%	4.80%	6.00%
Cys (C)	0.00%	0.00%	0.30%	0.30%	0.00%	0.00%	0.30%	0.30%	0.00%	0.40%
Gln (Q)	2.30%	4.10%	1.80%	3.70%	3.40%	3.90%	3.40%	5.80%	3.70%	5.70%
Glu (E)	17.50%	13.20%	10.90%	7.20%	13.10%	9.90%	11.20%	7.80%	12.60%	9.70%
Gly (G)	8.50%	4.10%	5.90%	5.80%	3.40%	5.30%	8.00%	7.20%	6.80%	7.50%
His (H)	1.10%	1.50%	0.60%	0.90%	0.70%	0.70%	1.50%	1.30%	1.50%	2.00%
Ile (I)	1.70%	6.60%	6.80%	8.90%	5.50%	3.90%	6.20%	6.90%	6.30%	6.40%
Leu (L)	13.00%	8.10%	13.30%	8.60%	11.00%	8.60%	9.10%	7.50%	13.10%	11.90%
Lys (K)	6.20%	6.60%	8.30%	6.30%	7.60%	7.20%	6.70%	7.80%	5.70%	5.10%
Met (M)	1.70%	4.60%	1.80%	2.00%	2.80%	3.30%	1.50%	2.40%	1.10%	2.50%
Phe (F)	4.00%	1.50%	4.40%	5.20%	3.40%	3.90%	2.30%	2.40%	2.00%	2.20%
Pro (P)	4.00%	4.60%	2.70%	2.30%	6.20%	5.90%	5.00%	3.60%	4.00%	3.30%
Ser (S)	1.10%	3.60%	6.80%	9.20%	4.80%	4.60%	2.90%	3.90%	2.80%	4.60%
Thr (T)	0.60%	4.10%	5.60%	3.70%	6.20%	5.30%	6.20%	6.90%	4.00%	3.70%
Trp (W)	0.00%	0.00%	0.30%	0.30%	0.70%	1.30%	0.20%	0.20%	0.70%	0.20%
Tyr (Y)	1.70%	0.50%	4.40%	4.00%	1.40%	0.70%	1.00%	1.10%	2.10%	2.00%
Val (V)	6.80%	8.60%	6.50%	8.10%	7.60%	7.90%	9.10%	7.40%	6.90%	6.50%
Pyl (O)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Sec (U)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 6: Composition comparison between similar HSP found in Thermophilic & Mesophilic bacteria

It is observed that proportion of Glu & Leu amino acids are higher in the thermophiles as compared to the mesophilic counterpart. The same comparison study also done for the non-HSP protein (Table 7), where it is also found that most of the non-HSP proteins of thermophiles has higher composition of Glu & Leu amino acids compared to the non-HSP mesophilic proteins.

PROTEIN:	Mg2+ transporter MgtE	RNase HI in complex with Mg2+	Probable SecDF protein-export	Protein translocase subunit	V-type ATP synthase	ATP synthase subunit	apo-FtsH ATP-dependent metalloprot	FtsH	TAQ DNA POLYMERASE	DNA POLYMERASE I
ORGANISM	*Thermus thermophilus HB8*	*Escherichia coli*	*Thermus thermophilus HB8*	*Escherichia coli*	*Thermus thermophilus HB8*	*Escherichia coli*	*Thermotoga maritima*	*Escherichia coli*	*Thermus aquaticus*	*Escherichia coli*
PDB Id/NCBI Id	2ZY9/--	1RDD/--	3AQP/--	5MG3/--	3K5B/--	6OQW/--	3KDS/--	1LV7/--	1TAQ/--	1QSL/--
Ala (A)	8.90%	9.00%	11.50%	9.00%	23.10%	16.40%	9.70%	10.90%	10.80%	10.20%
Arg (R)	6.60%	6.50%	6.70%	5.00%	10.60%	6.20%	7.10%	7.00%	9.10%	6.00%
Asn (N)	1.10%	4.50%	2.70%	2.40%	0.00%	3.40%	3.40%	2.30%	1.40%	3.80%
Asp (D)	6.60%	4.50%	3.40%	1.50%	1.00%	5.10%	5.60%	6.60%	5.00%	5.80%
Cys (C)	0.00%	1.90%	0.00%	0.70%	0.00%	0.00%	0.60%	0.80%	0.40%	0.20%
Gln (Q)	1.90%	5.20%	3.00%	4.80%	1.90%	5.10%	1.30%	3.10%	1.90%	4.10%
Glu (E)	8.70%	7.70%	6.30%	3.30%	22.10%	9.60%	11.20%	8.20%	10.50%	8.90%
Gly (G)	6.60%	9.00%	8.00%	9.40%	3.80%	4.50%	8.40%	10.90%	7.10%	5.50%
His (H)	2.50%	3.20%	1.60%	1.10%	0.00%	1.10%	1.90%	0.80%	2.20%	2.60%
Ile (I)	3.20%	4.50%	6.50%	10.30%	1.00%	5.60%	7.50%	6.20%	3.00%	6.40%
Leu (L)	17.80%	7.70%	15.40%	10.50%	14.40%	10.20%	9.50%	7.80%	14.90%	11.40%
Lys (K)	2.70%	7.10%	3.50%	3.90%	11.50%	4.50%	6.90%	5.80%	5.00%	6.30%
Met (M)	1.90%	2.60%	0.90%	3.50%	1.00%	4.00%	1.90%	3.90%	1.90%	2.50%
Phe (F)	2.10%	1.30%	4.30%	7.00%	0.00%	3.40%	3.40%	5.10%	3.20%	2.60%
Pro (P)	4.70%	3.20%	4.50%	4.40%	1.00%	1.70%	4.50%	5.10%	5.80%	4.50%
Ser (S)	4.00%	2.60%	4.70%	5.20%	1.00%	6.80%	3.20%	2.30%	3.40%	4.50%
Thr (T)	4.90%	6.50%	5.40%	5.90%	1.00%	2.30%	3.70%	3.50%	3.60%	4.80%
Trp (W)	1.30%	3.90%	0.70%	1.10%	0.00%	0.60%	0.20%	0.00%	1.70%	0.80%
Tyr (Y)	3.00%	3.20%	2.20%	3.30%	1.90%	0.60%	1.70%	0.40%	2.90%	3.50%
Val (V)	11.80%	5.80%	8.80%	7.90%	4.80%	9.00%	8.20%	9.30%	6.10%	5.60%
Pyl (O)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Sec (U)	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%

Table 7: Composition comparison between similar non-HSP found in Thermophilic & Mesophilic bacteria

II. Blast Analysis

Similar proteins were identified from different thermophilic bacteria & mesophilic bacteria using the web-based algorithm BLASTP (Protein BLAST: search protein databases using a protein query (nih.gov)) of NCBI for each pre-identified 10 HSP & 15 non-HSP proteins described earlier. Around 5 thermophilic bacteria & 2 mesophilic bacteria were found from the BLASTP search for each protein. FASTA sequence of these similar proteins from the different thermophilic bacteria & 2 mesophilic bacteria used to analyse the amino acid homology in the MUSCLE (MUSCLE < Multiple Sequence Alignment < EMBL-EBI). CLUSTAL multiple sequence alignment & Percent Identity Matrix result was obtained from this MUSCLE analysis. Percentage identity matrix (Table: 8) of grpE protein shows that homology of proteins across the same genus bacteria (>99%) is higher compared to the bacteria belongs to another genus (around 27-60%), whereas GroEL proteins from different thermophilic & mesophilic bacteria have homology between 62 to 92 % (Table: 9). The CLUSTAL multiple sequence

alignment (Figure 6 & 7) for bacteria belongs to same genus & bacteria belongs to different genus confirms the same result of Percentage identity matrix of grpE protein (Table 8). Due to the higher homology, more numbers of conserved domain are observed in the CLUSTAL multiple sequence alignment result of GroEL protein from MUSCLE software (Figure 8).

	T1701	**T1702**	**T1703**	**T1704**	**T1705**	**T1706**	**T1707**	**M1701**	**M1702**	**M1703**
T1701	100%	100%	99%	30%	27%	26%	31%	27%	29%	28%
T1702	100%	100%	99%	30%	26%	25%	31%	26%	28%	27%
T1703	99%	99%	100%	30%	27%	26%	31%	27%	29%	28%
T1704	30%	30%	30%	100%	61%	63%	54%	54%	50%	53%
T1705	27%	26%	27%	61%	100%	68%	51%	49%	50%	50%
T1706	26%	25%	26%	63%	68%	100%	47%	48%	47%	49%
T1707	31%	31%	31%	54%	51%	47%	100%	54%	55%	54%
M1701	27%	26%	27%	54%	49%	48%	54%	100%	85%	84%
M1702	29%	28%	29%	50%	50%	47%	55%	85%	100%	86%
M1703	28%	27%	28%	53%	50%	49%	54%	84%	86%	100%

Table 8: Percentage identity matrix of similar proteins (grpE) from different thermophilic & mesophilic bacteria

Protein Name: grpE
T1701: Thermus thermophilus HB8, **T1702:** Thermus aquaticus, **T1703:** Thermus islandicus **T1704:** Oceanithermus profundus, **1705:** Meiothermus silvanus, **T1706:** Calidithermus terrae, **T1707:** Meiothermus ruber, **M1701:** Escherichia coli, **M1702:** Shigella flexneri, **M1703:** Enterobacteriaceae

NCBI Accession No	CAD60062 51.1	WP_128647 057.1	WP_050900 260.1	WP_119313 550.1	WP_013156 973.1	HE126203.1	WP_013458 511.1	WP_033399 210.1	BAA81742. 1	WP_003045 305.1
Name of Organism	*Thermus thermophilus HB8*	*Thermus aquaticus*	*Thermus islandicus*	*Oceanithermus profundus*	*Meiothermus silvanus*	*Calidithermus terrae*	*Meiothermus ruber*	*Escherichia coli*	*Shigella flexneri*	*Enterobacteriaceae*
No of Amino Acid	177	179	183	191	191	188	184	197	202	197

Figure 6: CLUSTAL multiple sequence alignment of grpE protein from Thermus genus

* Mark indicates the absolute similarities of amino acid across all proteins.
: Mark indicates partial similarities of amino acid across all proteins.
. mark indicates random similarities of amino acid across all proteins.

Marked area shows the highly conserved sequences.

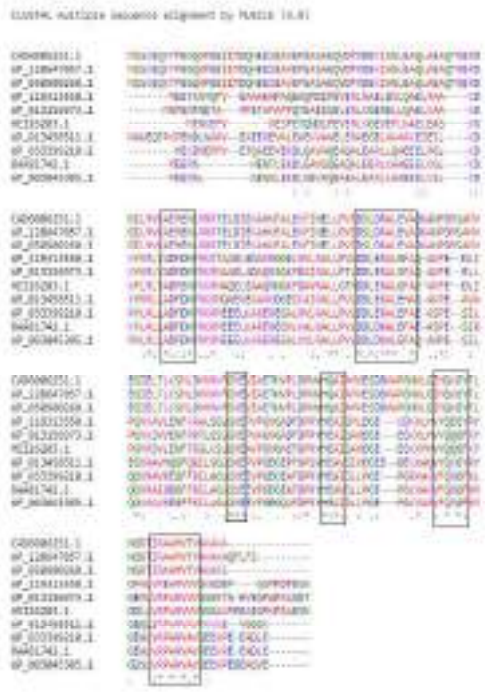


Figure 5: CLUSTAL multiple sequence alignment of grpE protein from bacteria belongs to different genus

	T1800	T1801	T1802	T1803	T1804	T1805	M1800	M1801	M1802	M1803
T1800	100	91.54	91.73	91.73	62.29	63.96	63.22	64.94	64.99	65.36
T1801	91.54	100	99.82	99.63	61.81	62.43	62.73	62.96	62.71	62.45
T1802	91.73	99.82	100	99.82	61.99	62.62	62.92	63.15	62.89	62.64
T1803	91.73	99.63	99.82	100	61.81	62.43	62.73	62.96	62.71	62.64
T1804	62.29	61.81	61.99	61.81	100	92.48	91.74	80.11	83.18	82.99
T1805	63.96	62.43	62.62	62.43	92.48	100	92.66	82.5	84.13	84.29
M1800	63.22	62.73	62.92	62.73	91.74	92.66	100	79.74	83.18	83.73
M1801	64.94	62.96	63.15	62.96	80.11	82.5	79.74	100	86.69	86.32
M1802	64.99	62.71	62.89	62.71	83.18	84.13	83.18	86.69	100	96.68
M1803	65.36	62.45	62.64	62.64	82.99	84.29	83.73	86.32	96.68	100

Table 9: Percentage identity matrix of similar proteins (GroEL) from different thermophilic & mesophilic bacteria

Protein Name: GroEL
T1800: Thermus thermophilus, **T1801**: Oceanithermus profundus, **T1802**: Meiothermus ruber, **T1803**: Meiothermus silvanus, **T1804**: Thermus scotoductus, **T1805**: Calidithermus chliarophilus, **M1800**: Escherichia coli, **M1801**: Klebsiella pneumoniae, **M1802**: Acinetobacter baumannii, **M1803**: Salmonella enterica

NCBI Accession No	EBF75015 40.1	WP_11103 4267.1	AOX4813 0.1	WP_20531 8135.1	WP_02789 2654.1	WP_01315 7123.1	WP_01301 3372.1	WP_01345 8370.1	BAW0214 3.1	WP_01955 0604.1

Name of Organism	Thermus thermophilus	Oceanithermus profundus	Meiothermus ruber	Meiothermus silvanus	Thermus scotoductus	Calidithermus chliarophilus	Escherichia coli	Klebsiella pneumoniae	Acinetobacter baumannii	Salmonella enterica
No of Amino Acid	543	543	545	546	542	545	546	546	546	544

Many studies have compared amino acid compositions of the proteome or a specific set of proteins in mesophiles and thermophiles. A trend has been observed in all sets of results that the thermophilic proteins favour large, charged, hydrophobic as well as aromatic residues (Tamakoshi *et al.,* 1995). Whereas they disfavour uncharged polar residues. It has been reported by several researchers that a set of amino acid comprises of Ile, Val, Tyr, Trp, Arg, Glu, and Leu typically present in all thermotolerant proteins, especially in HSPs. The altered amino acid composition of thermophiles appears to be related to the altered overall nucleotide composition of the genomic DNA, which co-evolved with the translational machinery to prevent melting of the double helix at the higher temperature (Petukhov *et al*., 1996 & Bryan *et al*., 2010).

Identical or comparable DNA, RNA, or amino acid (protein) sequences that exist in different or the same species throughout generations are referred to as conserved sequences. Over generations, these sequences show extremely little changes in composition, or no changes at all. Coding and non-coding sequences are both examples of conserved sequences present in various genomes. Amino acids and nucleic acids are frequently preserved as coding sequences to maintain a protein's structure and function. These scenes are just slightly altered. When modifications occur, an amino acid or nucleic acid is generally replaced by one that is biochemically identical.

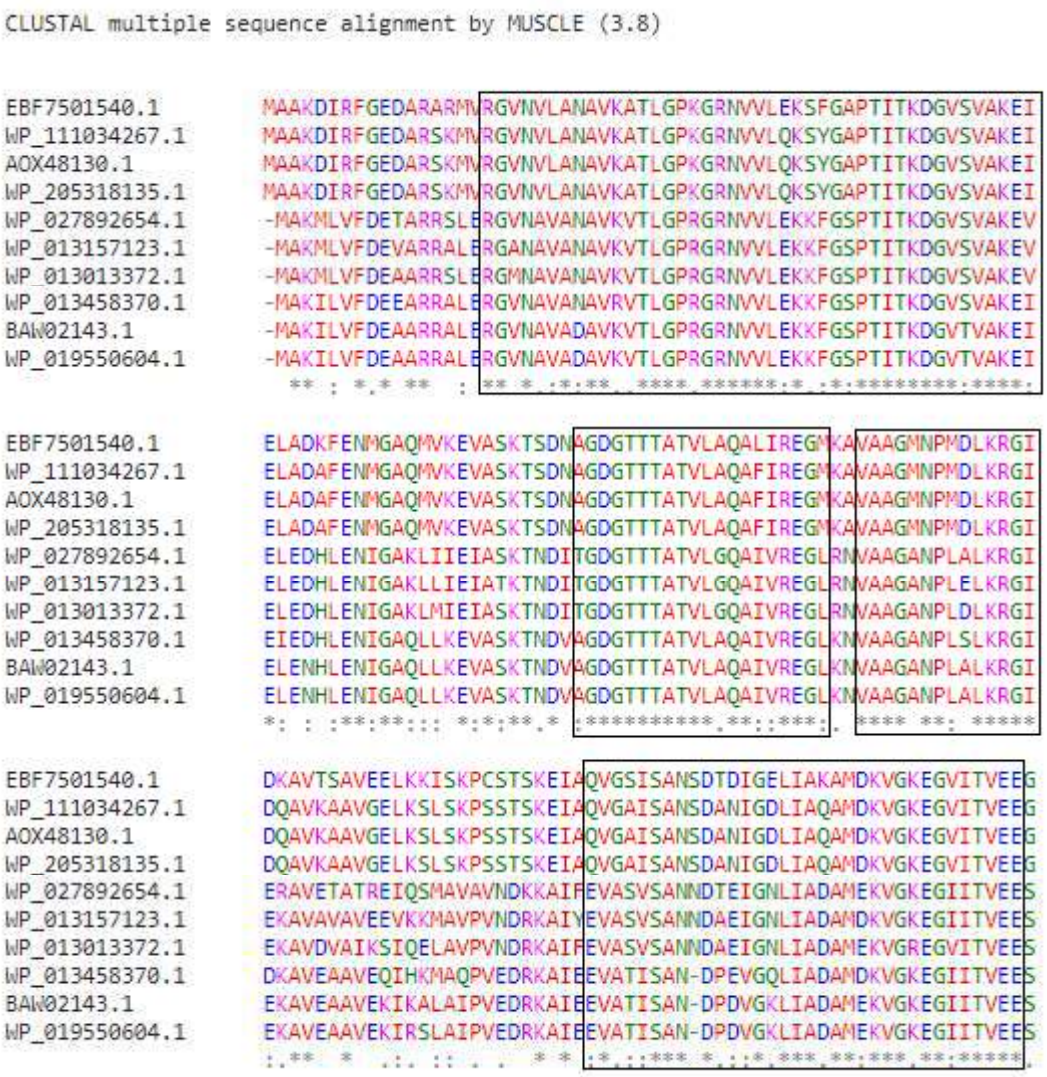


Figure 7: CLUSTAL multiple sequence alignment of GroEL protein from bacteria belongs to different genus

Conclusion:

Thermophilic bacteria have great importance in the field of research and academics. The fundamental mechanisms behind the survival of microorganisms needs to be explored to have better understanding of life processes. Therefore, this study unveils the molecular aspect of the thermophilic proteins (i.e., HSPs), their probable nature, structure and function by the computational approach. Comparing thermophilic HSPs with mesophilic ones reveals that, there are continuous evolution resulting into genetic change which aid to adopt in such intense conditions. Proteome analysis of macromolecular structures also provide the evidence which suggests that change in secondary structure could be a strategy for stabilising more thermolabile molecules. Still there are so many unknown factors which facilitate survival at extreme temperature. A detailed study would be required in this regard which demand considerable experimental innovations and a better knowledge of intracellular conditions than we currently possess.

References:

1. Wang Q., Cen Z., and Zhao J. (2015). The Survival Mechanisms of Thermophiles at High Temperatures: An Angle of Omics. Physiology. 30: 97–106.
2. Richard W. C. (1969). Thermophilic blue green Algae & the thermal environment. Bacteriological Reviews. 33: 476-504.
3. Christopher J. R., Hunter L., Eric T., Vern W., and Caryn E. (2013). Protein Adaptations in Archaeal Extremophiles. Archaea. doi:10.1155/2013/373275.
4. Santos H. and Costa M. S. (2002). Compatible solutes of organisms that live in hot saline environments. Environmental Microbiology. 4(9): 501–509.
5. Costa M. S., Santos H., and Galinski E. A. (1998). An overview of the role and diversity of compatible solutes in Bacteria and Archaea. Biotechnology of Extremophiles. doi:10.1007/bfb0102291.
6. Brock T. D., Brock K. M., Belly R. T., and Weiss R. L. (1972). Sulfolobus: a new genus of sulfur-oxidizing bacteria living in low pH and high temperature. Archiv für Mikrobiologie. 84: 54–68.
7. Stetter K. O. (2006). History of discovery of the first hyperthermophiles. Extremophiles. 10: 357–362.
8. . Kashefi K. and Lovley D. R. (2003). Extending the upper temperature limit for life. Science. 301: 934.
9. Zavodszky P., Kardos J., Svingor A., and Petsko G. A. (1998). Adjustment of conformational flexibility is a key event in the thermal adaptation of proteins. Proceedings of the National Academy of Sciences USA. 95: 7406–7411.
10. Taylor T. J. and Vaisman I. I. (2009). Discrimination of thermophilic and mesophilic proteins. BMC Structural Biology. 10(1): S5.
11. Kumar S., Tsai C. J., and Nussinov R. (2000). Factors enhancing protein thermostability. Protein Engineering. Design and Selection. 13(3): 179–191.
12. Balsam T. M. and Atef J. *et al*. (2017). Isolation and Characterization of Thermophilic Bacteria from Jordanian Hot Springs: Bacillus licheniformis and Thermomonas hydrothermalis Isolates as Potential Producers of Thermostable Enzymes. International Journal of Microbiology. doi:10.1155/2017/6943952.
13. Yohandini H., Julinar, and Muharn (2015). Isolation and Phylogenetic Analysis of Thermophile Community within Tanjung Sakti Hot Spring, South Sumatera, Indonesia. HAYATI Journal of Biosciences. 22(3): 143-148.
14. Jaenicke R. and Bohm G. (1998). The stability of proteins in extreme environments. Current Opinion in Structural Biology. 8(6): 738-748.
15. Averhoff B. and Muller V. (2010). Exploring research frontiers in microbiology: recent advances in halophilic and thermophilic extremophiles. Research in Microbiology. 161(6): 506-514.
16. Boto L. (2014). Horizontal gene transfer in the acquisition of novel traits by metazoans. Proceedings of the Royal Society B: Biological Sciences. doi:10.1098/rspb.2013.2450
17. Feng S., Powell S. M., Wilson R., and Bowman J. P. (2014). Extensive gene acquisition in the extremely psychrophilic bacterial species Psychroflexus torquis and the link to sea-ice ecosystem specialism. Genome Biology and Evolution. 6(1): 133–148.
18. Courgeon A.M., Maisonhaute C., and Best-Belpomme M. (1984). Heat shock proteins are induced by cadmium in Drosophila cells. Experimental Cell Research. 153(2): 515–521.
19. Heikkila J.J., Schultz G.A., Iatrou K., and Gedamu L. (1982). Expression of a set of fish genes following heat or metal ion exposure. The Journal of Biological Chem. 257(20): 12000-12005.

20. Szalaya M. S. and Kovacsa I. A. *et al.* (2007). Stress-induced rearrangements of cellular networks: Consequences for protection and drug design. FEBS Letters. 581(19): 3675–3680.
21. Toivola D. M., Strnad P., Habtezion A., and Omary M.B. (2010). Intermediate filaments take the heat as stress proteins. Trends Cell Biol. 20(2): 79–91.
22. Lindquist S. (1980). Varying patterns of protein synthesis in Drosophila during heat shock: implications for regulation. Developmental Biology. 77(2): 463–479.
23. Lindquist S. (1986). The heat-shock response. Annu. Rev. Biochem. 55: 1151– 1191.
24. Barbe M. F., Tytell M., Gower D. J., and Welch W. J. (1988). Hyperthermia protects against light damage in the rat retina. Science. 24: 1817–1820.
25. Marber M. S., Mestril R., Chi S. H., Sayen R., Yellon Y. M., and Dillman W. H. (1995). Overexpression of the rat inducible 70-kDa heat stress protein in a transgenic mouse increases the resistance of the heart to ischemic injury. Journal of Clinical Investigation. 95: 1446– 1456.
26. Jaattela M, and Wissing D. (1993). Heat shock proteins protect cells from monocyte cytotoxicity: possible mechanism of self-protection. Journal of Experimental Medicine. 177: 231–236.
27. Mizzen L., and Welch W. J. (1988). Effects on protein synthesis activity and the regulation of heat shock protein 70 expression. Journal of Cell Biology. 106: 1105–1116.
28. Chirico W. J., Waters M. G., and Blobel G. (1988). 70K heat shock related proteins stimulate protein translocation into microsomes. Nature. 332: 805–810.
29. Grallert H. and Buchner J. (2001). Review: a structural view of the GroE chaperone cycle. Journal of Structural Biology. 135: 95–103.
30. Horwich A. L., Farr G. W. and Fenton W. A. (2006). GroEL-GroES-mediated protein folding. Chemical Reviews. 106: 1917–1930.
31. Hartl F. U. and Hayer-Hartl M. (2002). Molecular chaperones in the cytosol: from nascent chain to folded protein. Science. 295: 1852–1858.
32. Todd M. J., Viitanen P. V., and Lorimer G. H. (1994). Dynamics of the chaperonin ATPase cycle: implications for facilitated protein folding. Science. 265: 659–666.
33. Viitanen P. V., Gatenby A. A., and Lorimer G. H. (1992). Purified chaperonin 60 (groEL) interacts with the nonnative states of a multitude of Escherichia coli proteins. Protein Science. doi:10.1002/pro.5560010308.
34. Goloubinoff P., Gatenby A. A., and Lorimer G. H. (1989). GroE heat-shock proteins promote assembly of foreign prokaryotic ribulose bisphosphate carboxylase oligomers in Escherichia coli. Nature. 337: 44–47.
35. Wu B. J., Kingston R. E. and Morimoto R. I. (1986). Human HSP70 promoter contains at least two distinct regulatory domains. Proceedings of the National Academy of Sciences USA. 83: 629–633.
36. Rodriguez F., Arsene-Ploetze F., Rist W., Rudiger S., Schneider-Mergener J., Mayer M. P., and Bukau B. (2008). Molecular basis for regulation of the heat shock transcription actor sigma32 by the DnaK and DnaJ chaperones. Molecular Cell. 32(3): 347–358.
37. Rhodius V. A., and Mutalik V. K. (2010). Predicting strength and function for promoters of the Escherichia coli alternate sigma factor, s. E. Proceedings of the National Academy of Sciences USA. 107: 2854–2859.
38. Kim D. Y., Kwon E., Choi J., Hwang H. Y., and Kim K. K. (2010). Structural basis for the negative regulation of bacterial stress response by RseB. Protein Science. 19: 1258–1263.
39. Walsh N. P., Alba B. M., Bose B., Gross C. A., and Sauer R. T. (2003). OMP peptide signals initiate the envelope-stress response by activating DegS protease via relief of inhibition mediated by its PDZ domain. Cell. 113: 61–71.

40. Hasenbein S., Meltzer M., Hauske P., Kaiser M., Huber R., Clausen T., and Ehrmann M. (2010). Conversion of a regulatory into a degradative protease. Journal of Molecular Biology. 397: 957–966.
41. Hartl F. U. (1996). Molecular chaperones in cellular protein folding. Nature. 381: 571-579.
42. Hartl F. U. and Hayer-Hartl M. (2002). Molecular chaperones in the cytosol: from nascent chain to folded protein. Science. 295: 1852-1858.
43. Gestwicki J. E., Crabtree G. R., Graef I. A. (2004). Harnessing chaperones to generate small-molecule inhibitors of amyloid β aggregation. Science. 306: 865-869.
44. Jinwal U. K., Miyata Y., Koren J. 3rd, Jones J. R., Trotter J. H., Chang L., O'Leary J., Morgan D., Lee D. C., Shults C. L., Rousaki A., Weeber E. J., Zuiderweg E. R., Gestwicki J. E., Dickey C. A. (2009). Chemical manipulation of Hsp70 ATPase activity regulates tau stability. The Journal of Neuroscience. 29: 12079-12088.
45. Schmid D., Baici A., Gehring H., Christen P. (1994). Kinetics of molecular chaperone action. Science. 263: 971-973.
46. Raviol H., B. and Mayer M. P. (2006). Human and yeast Hsp110 chaperones exhibit functional differences. FEBS Letters. 580: 168-174.
47. Brehmer D., Gässler C., Rist W., Mayer M. P. and Bukau B. (2004). Influence of GrpE on DnaK-substrate interactions. Journal of Biological Chemistry. 279: 27957-27964.
48. Moro F., Taneva S. G., Velazquez-Campoy A. and Muga A. (2007). GrpE N-terminal domain contributes to the interaction with DnaK and modulates the dynamics of the chaperone substrate binding domain. Journal of Molecular Biology. 374: 1054-1064.
49. Brock, T. D. (1967). Life at high temperatures. Science. 158: 1012-1019.
50. Brock, T. D. and G. K. Darland. (1970). Limits of microbial existence: temperature and pH. Science. 169: 1316-1318.
51. Singleton R. Jr. And Amelunxen R. E. (1973). Proteins from Thermophilic Microorganisms, Bacteriological Reviews. American Society for Microbiology. 37(3): 320-342.
52. Tomazic S. J. and Klibanov A. M. (1988). Mechanisms of irreversible thermal inactivation of Bacillus α-amylases. The Journal of Biological Chemistry. 263(7): 3086–3091.
53. Arnorsdottir J., Sigtryggsdottir A. R., Thorbjarnardottir S. H., Kristjansson M. M. (2009). Effect of proline substitutions on stability and kinetic properties of a cold adapted subtilase. Journal of Biochemistry. 145: 325–329.
54. Bezsudnova E. Y., Boyko K. M., Polyakov K. M., Dorovatovskiy P. V., Stekhanova T. N., Gumerov V. M., Ravin N. V., Skryabin K. G., Kovalchuk M. V. and Popov V. O. (2012). Structural insight into the molecular basis of polyextremophilicity of short-chain alcohol dehydrogenase from the hyperthermophilic archaeon Thermococcus sibiricus. Biochimie. 94: 2628–2638.
55. Chen J. and Stites W. E. (2004). Replacement of staphylococcal nuclease hydrophobic core residues with those from thermophilic homologues indicates packing is improved in some thermostable proteins. Journal of Molecular Biology. 344: 271–280.
56. Hakulinen N., Turunen O., Janis J., Leisola M., and Rouvinen J. (2003). Three-dimensional structures of thermophilic beta-1,4-xylanases from Chaetomium thermophilum and Nonomuraea flexuosa. European Journal of Biochemistry. 270(7): 1399–1412.
57. Nakka M., Iyer R. B. and Bachas L. G. (2006). Intersubunit disulfide interactions play a critical role in maintaining the thermostability of glucose-6-phosphate dehydrogenase from the hyperthermophilic bacterium Aquifex aeolicus. The Protein Journal. 25: 17–21.
58. Pang J. and Allemann R. K. (2007). Molecular dynamics simulation of thermal unfolding of Thermatoga maritima DHFR. Physical Chemistry Chemical Physics. 9: 711–718.
59. Pollo S. M. J., Adebusuyi A. A., Straub T. J., Foght J. M., Zhaxybayeva O. & Nesbo C. L. (2017). Genomic insights into temperature-dependent transcriptional responses of

Kosmotoga olearia, a deep-biosphere bacterium that can grow from 20 to 79 °C. Extremophiles. 21: 963–979.
60. Patel B. K., Morgan H. W., Wiegel J. & Daniel R. M. (1987). Isolation of an extremely thermophilic chemoorganotrophic anaerobe similar to Dictyoglomus thermophilum from new zealand hot springs. Archives of Microbiology. 147: 21–24.
61. Kagawa H. K., Osipiuk J., Maltsev N., Overbeek R., Quaite-Randall E., Joachimiak A., and Trent J. D. (1995). The 60 kDa heat shock proteins in the hyperthermophilic archaeon Sulfolobus shibatae. Journal of Molecular Biology. 253: 712–725.
62. Hightower L. E. (1991). Heat shock, stress proteins, chaperones, and roteotoxicity. Cell. 66(2): 191–197.
63. Lindquist S. (1992). Heat shock proteins and stress tolerance in microorganisms. Current Opinion in Genetics & Development. 2(5): 748–755.
64. Hartl F. U. (1996). Molecular chaperones in cellular protein folding. Nature. 381:571–580.
65. Hayes S. A., Dice J. F. (1996). Roles of molecular chaperones in protein degradation. Journal of Cell Biology. 132:255–258.
66. Miroshnichenko M. L., L'Haridon S., Jeanthon C., Antipov A. N., Kostrikina N. A., Tindall B. J., Schumann P., Spring S., Stackebrandt E. and Bonch-Osmolovskaya A. (2003). Oceanithermus profundus gen. nov., sp. nov., a thermophilic, microaerophilic, facultatively chemolithoheterotrophic bacterium from a deep-sea hydrothermal vent. International Journal of Systematic and Evolutionary Microbiology. 53(3): 747–752.
67. Huber R., Langworthy T. A., König H., Michael T., Woese C. R., Sleytr U. B. & Stetter K. O. (1968). Thermotoga maritima sp. nov. represents a new genus of unique extremely thermophilic eubacteria growing up to 90°C. Archives of Microbiology. 144: 324–333.
68. Brock T. D. and Freeze H. (1969). Thermus aquaticus gen. n. and sp. n., a Nonsporulating Extreme Thermophile. Journal of bacteriology. 98(1): 289-297.
69. Kambourova M. (2018). Thermostable enzymes and polysaccharides produced by thermophilic bacteria isolated from Bulgarian hot springs. Engineering in Life Sciences. 18: 758–767.
70. Andrews K. T. and Patel B. K. (1996). Fervidobacterium gondwanense sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. International Journal of Systematic and Evolutionary Microbiology. 46(1):265-269.
71. Ogg C. D. and Patel B. K. C. (2009). Fervidicola ferrireducens gen. nov., sp. nov., a thermophilic anaerobic bacterium from geothermal waters of the Great Artesian Basin, Australia. International Journal of Systematic and Evolutionary Microbiology. 59: 1100–1107.
72. Ogg C. D. and Patel B. K. C. (2009). Thermotalea metallivorans gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer. International Journal of Systematic and Evolutionary. Microbiology. 59: 964–971.
73. Oshima T. and Imahori K. (1974). Description of Thermus thermophilus (Yoshida and Oshima) comb. nov., a Nonsporulating Thermophilic Bacterium from a Japanese Thermal Spa. International Journal of Systematic Bacteriology. 24: 102-112.
74. Beffa T., Blanc M., Pierre-Franc¸ Ois L., Vogt G., Marchiani M., Fischer J. L., and Aragno M. (1996). Isolation of Thermus Strains from Hot Composts (60 to 80°C). Applied And Environmental Microbiology. 62(5): 1723–1727.
75. Mizobata T., Kagawa M., Murakoshi N., Kusaka E., Kameo K., Kawata Y., Nagai J. (2000). Overproduction of Thermus sp. YS 8–13 manganese catalase in Escherichia coli production of soluble apoenzyme and in vitro formation of active holoenzyme. European Journal of Biochemistry. 267: 4264–4271.
76. Hiroki O., Kwang K., Ryoji M., and Seiki K. (2014). Lysine Propionylation is a Prevalent Post-translational Modification in Thermus thermophilus. Research. 13(9): 2382-2398.

77. Sunny J. S., Mukund N., Natarajan A., and Saleena L. M. (2020). Identifying heat shock response systems from the genomic assembly of Ureibacillus thermophilus LM102 using protein-protein interaction networks. Gene. Doi:10.1016/j.gene.2020.144449
78. Bryan, P. N. and Orban J. (2010). Proteins that switch folds. Current Opinion in Structural Biology. 20(4): 482–488.
79. Tamakoshi, M., Yamagishi A., and Oshima T. (1995). Screening of stable proteins in an extreme thermophile, *Thermus thermophilus*. Molecular Microbiology. 16(5): 1031–1036.

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Date- 26/07/2021

This is to certify that the dissertation thesis titled "***A Comprehensive Review of In Silico Studies on Mycobacterium abscessus***" submitted by Tanushree Patra for partial fulfillment of MSc degree from the Department of Botany, Ramananda College, Bankura University represents the record of original study carried out by her under my supervision. The dissertation thesis is worthy for partial fulfillment of MSc degree in Botany. The work has not been submitted for any degree of Bankura University or any other University.

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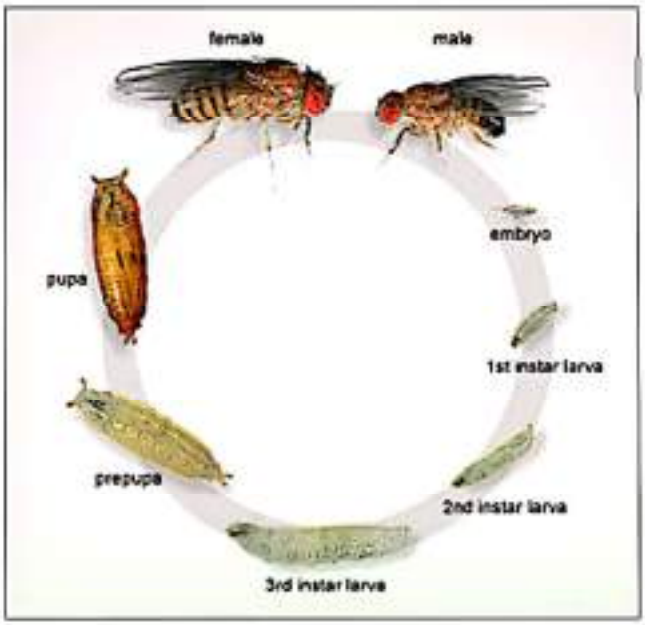
Zoology

BANKURA UNIVERSITY



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A PROJECT REPORT ON DROSOPHILA CULTURE

UID NO :- 18173126008

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COURSE TITLE :- DEVELOPMENTAL BIOLOGY
COURSE CODE :- SH/ ZOO/601/C-13
COURSE ID :- 62601

CONTENTS

ACKNOWLEGDEMENT

→ First and foremost, I would like to express my utmost gratitude and appreciation to my project supervisor, Dr. Nilanjana Chatterjee, for her guidance, supervision and assistance throughout my project Research and thesis writing. Her expertise and ever ready guidance contributed a major part in making this project a success.

Secondly, I would to like the principal of our college Dr. Swapna Ghorai and our HOD of our Zoology Department Dr. Nilanjana Chatterjee for providing me the opportunity and Lab equipments to perform this final Semester project.

I would like to express my gratitude to my family especially my parents for their on-going supports, encouragement and Motivation.

Last but not the least, I would like to show my appreciation to my friends and those who lent me a hand, supported and guided me in the process of completing this final year project.

DECLARATION

→ I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously or concurrently submitted for any other degree at RAMANANDA COLLEGE or the other institutions.

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Introduction:- *Drosophila melanogaster* is a small

Common fly found near unripe and rotted fruit. It has been in use for over a century to study genetics and behavior. "Thomas hunt Morgan" was the preeminent biologist studying Drosophila early in the 1900's. He was the first to discover sex-linkage and genetic recombination, which placed the small fly in the forefront of genetic research. Due to its small size, ease of culture and short generation time, geneticists have been using Drosophila ever since.

Fruit flies are easily obtained from the wild and many biological science companies carry a variety of different mutations. In Addition these companies sell any equipment needed to culture the flies. Cost are relatively low and most equipment can be used year after years. there are a variety of laboratory exercises one could purches, Although the necessity to do so is questionable.

Why use *Drosophila*?

i. They are small and easily handled.
ii. They can be easily anesthetized and Manipulated individually with unsophisticated equipment.
iii. They are sexually dimorphic (males and females are different), making it is quite easy to differentiate the sexes.
iv. Virgins fruit flies are physically distinctive from mature adults, making it easy to obtained Virgin males and female for genetic crosses.

(V) Flies have short generation time (10-12) days and do well at room temperature.

(VI) The care and culture of fruit flies required little equipment, is low in cost and uses little space even for large cultures.

By using Drosophila, we will:—

i. Understand Mendelian genetics and inheritance of traits.

ii. Draw conclusions of heredity patters from data obtained.

iii. Construct traps to catch wild population of D. melanogaster.

iv. Gain and understanding of the life cycle of D. melanogaster, an insect which exhibits complete Metamorphosis.

(V) Construct crosses of caught and known wild type and mutated flies.

(VI) Learn techniques to manipulate flies, sex them, and keep concise journal notes.

(VII) Learn culturing techniques to keep the flies healthy.

(VIII) Realise many science experiments cannot be conducted with in one or two lab sessions.

Our Goals:-

i. Identify questions and concepts that guide scientific investigations.

ii. Design and conducted scientific investigation.

iii. Formulate and revise scientific explanations and models using logic and evidence.

iv. Communicate and defence a scientific argument.

Classification:-

Domain:- Eukarya
Kingdom:- Animelia
Phylum:- Arthropoda
Class:- Insecta
order:- Diptera
family:- Drosophilidae
Genus:- Drosophila
species:- melanogaster

Life cycle of Drosophila melanogaster:-

→ D. melanogaster exhibits complete metamorphism, meaning the life cycle includes an egg, larval form, pupa and finally emergence (enclosure) as a flying adult. This is the same as the well known metamorphosis of butterflies. The larval stage have three instars, or molts.

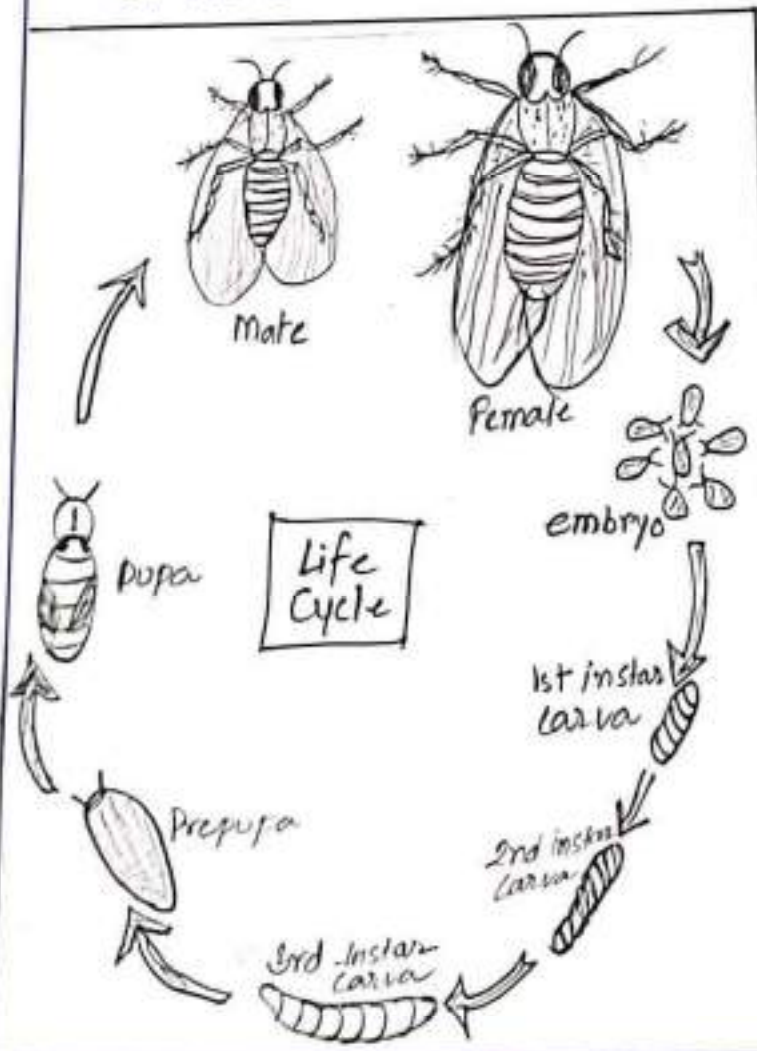


FIG: LIFE CYCLE OF D. MELANOGASTER

Day 00:- Female lays eggs.
Day 01:- Eggs hatch.
Day 02:- First Instar (1 day in length)
Day 03:- Second instar (1 day in length)
Day 05:- Third and Final instar (2 days in length)
Day 07:- Larvae begin roaming stage. pupariation occurs 120 hours after egg laying.
Day 11→12:- Eclosin (Adults emerge from the pupa case) females become sexually mature 8-10 hours after enclosion.

- The generation time of D. melanogaster varies with temperature. The above cycle is for a temparature of about 22°C (71°F). Flies raised at lower-temparature (to 18°C or 64°F) will take about twice as long to develop.
- Females can lay up to 100 eggs/day.
- Virgin females are able to lay eggs; however they will be sterile and few in number.
- After the eggs hatch, small larva are visible in the growing medium. Bcz of our white media, a small black area are found at the head of the larvae. Some dried - premixed media is blue to help identify larvae however this is not a necessity and with a little patence and practice, larva are easy seen by HP. In Addition as the larvae are feeded they disrupt from smooth Surface of the media and so by looking only at the surface one can tell if larvae are present. However it is always a good idea to double check using a stereo microscope. After the third instar, larvae will begin to migrate up the culture vial in order to pupate.

Sexing Drosophila :→

→ The Abdomen of the female has seven segments, several dark transverse stripes and is pointed at the HP. The Abdomen of the male has only five segments, two dark stripes, and a more rounded, heavily pigmented HP. In immature males the pigmentation may not be developed.

Male →

Female

Materials:-

- Drosophila melanogaster (Male- Female)
- Drosophila Medium (Each Vial contain 10 mL Medium and 10 mL distilled water)
- Anesthesfly Solution.
- Vial tube with Sponge Cover.
- Soft paint brush.
- Marker (pen)
- Magnifying glass.
- q-tips.

METHODS:-

- For Anesthetizing System:-

— At the beginning, anesthesfly solution was dropped on the cotton which placed under the etherize cap and closed the bottle for a few seconds under the ether glass fullfill the entire bottle. Then the base of the bottle was stroke lightly on the palm of the hand so that the flies will drop to the bottom. Next the bottle cap was removed, quickly replaced it and the mouth of etherizer the bottle was inverted over the etherizer and shaked the flies into the etherizer. Didn't invert the bottle over the etherizer bcz the ether is heavier than air and it could flow to the culture tube and kill the larvae and pupa. Both culture tube and etherizer were inverted and strock slowly until the Adult drosophila dropdown . The flies were then subjected to the ether for a minute or until they ceased moving. After that the etherized flies were transfered on the A4 paper. The etherized flies were examined with a magnifying glass. A soft brush was used for moving the flies about on the stage of the Magnifying glass.

Finally, after finishing our experiment, the drosophilas were discarded in a morque. After this step, a check was made in 5 hours. Drosophila was ~~separated~~ seperated according to sex. Then separated flies were put in different mediums. Mean while. medium culture prepare was mixed 10 mL of media and 10 mL of distilled water.

Drosophila's Medium Culture.

#Produces for monohybrid crosses :- Until the cross G'nK (wild type X sepia) step, we were obtained 3 female and 11 male (wild type) For monohybrid crosses were used 6 wild type (Read eyes normal drosophila) and 6 sepia (sepia eyes normal wings). 6 male (wild type) and 6 female (sepia) drosophila were shifted into the vial which contains new medium and the vial was closed with the cotton.

Cross of sepia with type.

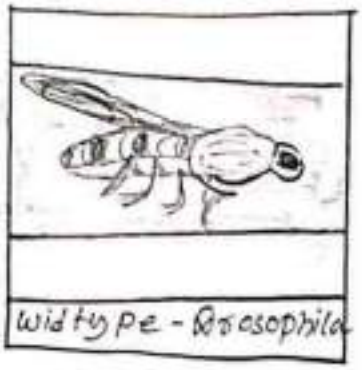
— The rest was killed and the traits were observed. the vial was held horizontally until the drosophila wake up.

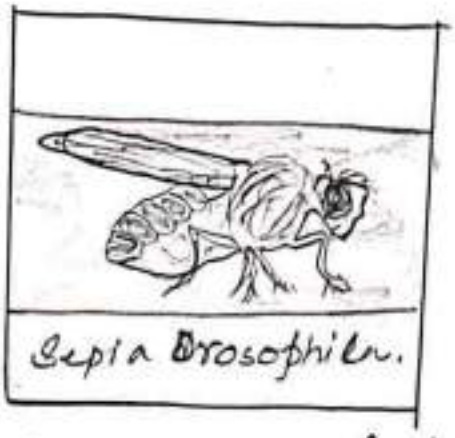
— Drosophila was so held for 2 weeks, then they were kept at 18°c, so that their development will slow down.

Temperatur cycling :- It is posible to maximize the number of virgins by using temperature cycling. when cultures are maintained at temperature of 18°c. development is slowed so female will not mow enclosure totally. we were obtained 19 female drosophila and 20 male drosophila. drosophila to until this stage constitutes the F1 phenotype.

RESULT:→

Monohybrid Crosses





the Cross between wild type (female) X Sepia (male)

Se+ dominant Allele for wild type.
Se is recessive for Sepia.

Male Normal Eye (wild type) female Sepia Eye

Parent — Se+Se+ Se Se

Gamete → Se → se

F1 Se+Se

All Wild type.

During the Mendel's law of segregation we were examined the inheritance of eye colour by crossing two pure breeding strains of drosophila melanogaster that is wild type & Sepia. We determine which allele is dominant by Setting up the cross se+ se+ males x sese female are described above.
until crosslinker for F1 were were obtained 21 male (wild type) and 16 female (wild type). F1 crossed result 3 females and 11 males were obtained.

Table:- Result's of drosophila's Number:-

DROSOPHILA	TOTAL OBTAINED	Total Virgin
Parent (male)	24	11 (virgin)
Parent (female)	16	3 (Virgin)
F1 (male)	20	
F1 (Female)	19	
Using for Cross F1 (Wild type) Male	6	
Using for cross F1 (Sepia) Female	6	

DISCUSSION :→

→ In this experiment, Parental generation 24, male and 16, female drosophila were obtained. However in the F1 Cross over phase there are only 8 females and 11 male drosophila. The reason for the decrease of flies :-

(1)→ the flies need to be inspected every 5 hours, against a possible Mating. But, me and my group of friends have not done the check regularly. At the Control over 5 hours. we put female drosophila in morgh and put their male drosophila in medium culture. If the controls are not exceeded in 5 hours. we puts the female in the female medium.

(2)→ Drosophila should be careful when taking them to the Medium when they are unconcious, because the drosophila stick to the Medium culture and died there. That's why when the flies are unconcious and the fake should be. on the horizontal side. However we were experimenting. we did not pay Attention to this Step.

⑤ Flies of the Medium Culture may be died from the oxygen deficiency.

④ During the Anesthesia procedure, when the Vial hit the bench. Medium culture was poured. puppies of the Media died. Living drosophila may have been died the Same reason.

— The reson I have mentioned above and there may have been a number of decrease in flies due to many reasons I can't count.

CONCLUSION:-

In this experiment I learn on how to conduct a genetic experiment which spans of generation and learn how to design genetic crosses to illustrate segregation, independent assortment and sex linkage. There are four stages of Drosophila melanogaster life cycles that's are egg, larva, pupa and Adult. From study of its life cycle. I'm able to perform this experiment. I can differentiate the Male and female of Drosophila melanogaster based on several characteristic such as Site of Adult Shape of Abdomen, Markings on the Abdomen etc. This making easier for me to differentiate them especially in the experiment about Sex Unkage.

REFERENCE :->

1. Paul Arnold (2009), Humangenetics and fruitfly. Drosophila melanogaster. Retrieved March 29, 2010. from · http:// www. bIOL.org / Drosfics. html.

2. Celesta A. Berg. ph. D. University of Washington, from http:// depts. washington. edu/ cberglab/ wordpress/ outreach/ an-introduction-to-fruit-flies/ 2010. http/ www. google. com. my) Search ? hlen & qdrosophila + melanogaster + phenotype & revld.

3. Retrieve on 8 April 2010 at. http:// www. mun. ca/ biology/ dinner/ B2250/ Drosophila Genetics. PDF.

4. Picture found in http:// gfe. uni-muenster. de/ Media / find Media output. php? thema = Genetics.

5. Quoting from this site http :/ www. biologyjunction. com/ lab_7_ Sample-3-fruitflies. htm.

BANKURA UNIVERSITY

ESTD 2014

RAMANANDA COLLEGE

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CLASS - B.Sc SEM-VI (HONS)

PREPARED AND SUBMITED BY

REG. NO- 16960 OF 2018 - 2019

UID- 18173126007

ROLL NO- 246

CONTENT

ACKNOWLEDGEMENT

This Project is not only the outcome of the hard work Put by me also the one who helped me through out this Process.

I warmly acknowledge the continious encouragement invaluable Supervision timely Suggestions and inspired guidance offered by our guided Professors, our honourable Dr. Nilanjana Chatterjee Department of zoology in bringing the Project to a Successful Completation. I also thanks other faculty members of zoology Dept.

I am also greatful to our honourable Principle Dr. Swapna Ghorai and at last but not the least I express my sincere thanks to all my friends and my Parents for extending their helping hand towards me to acomplish this understanding.

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INTRODUCTION

Drosophila is genus of small flies belonging to the family Drosophilidae, whose members are often called fruit flies. One species of Drosophila in particular D. melanogaster, has been heavily used in research in genetics, and is a common model organism in developmental biology.

This species of fruit flies not only possesses of well-defined genetics information, they also have short generation time which one generation only required about two weeks. In addition, one pair of parents flies is able to produce several hundreds offspring which ease the process of genetics.

It is an ideal organism in genetic flied or biological research for several reason:-

- Fruit flies are handy with simple food requirement and occupy little space.
- The reproductive cycle is complete in about 12 days at room temperature, allowing quick analysis of various experiment.
- Fruit flies produce large number of offspring to allow sufficient data to be collected.
- They are small and easily handled.
- They can be easily anesthelized and manipulated individually with unsophisticated equipment.

Systematic Position of *Drosophila melanogaster*

Phylum - Anthropoda

Class - Insecta

Order - Diptera

Family - Drosophilidae

Genus - *Drosophila*

Species - *melanogaster*

All the Drosophila belong to the Phylum - Anthropoda, class - Insecta, order - Diptera.

The life cycle of
Drosophila melanogaster

LIFE CYCLE

There are four stages to the life cycle of fruit flies, these are- egg, larvae (maggots), Pupa and adult.

1. **Eggs:→** The female adult fly lays eggs (1-20) into the maturing and ripening fruit of host Plant. The eggs hatch into larvae inside the fruit after a few days (2-4 days). Fruit fly eggs are very small. During its lifetime, a female fruit fly may lay 400 eggs or more.

2. **Larva:→** The larva is a white, segmented, worm-shaped burrower with black mouth Parts in the head region. For tracheal breathing it has a Pair of Spiracles at both the anterior and Posterior ends. Since insect skin will not stretch, the young small larvae must Periodically shed their skins in order to reach adult size. There are two molts in Drosophila larval development - ① The 1st molt ⑪ 2nd molt.

3. **Pupa:→** Soon after everting its anterior Spiracles, the larval body shortens and the cuticle becomes hardened and Pigmented. A headless and wingless PrePupa forms. This stage is followed by the formation of the Pupa with everted head, wing, Peds and legs. The Puparium thus utilizes the cuticle of the 3rd larval instar. The adult structure that Seem to appear 1st during the Pupal Period have actually been Present as small areas of dormant tissues as far back as the embryonic stage. These localized Preadult tissue are called anlagen.

The main function of the Pupa is to Permit development of the anlagen to adult Proportions.

4. Adult :→

Adult exhibit a typical insect anatomy, including Compound eyes, 3-Parts bodies (head, thorax, abdomen) wings and six Jointed legs. The various types of bristles and hairs found on the body.

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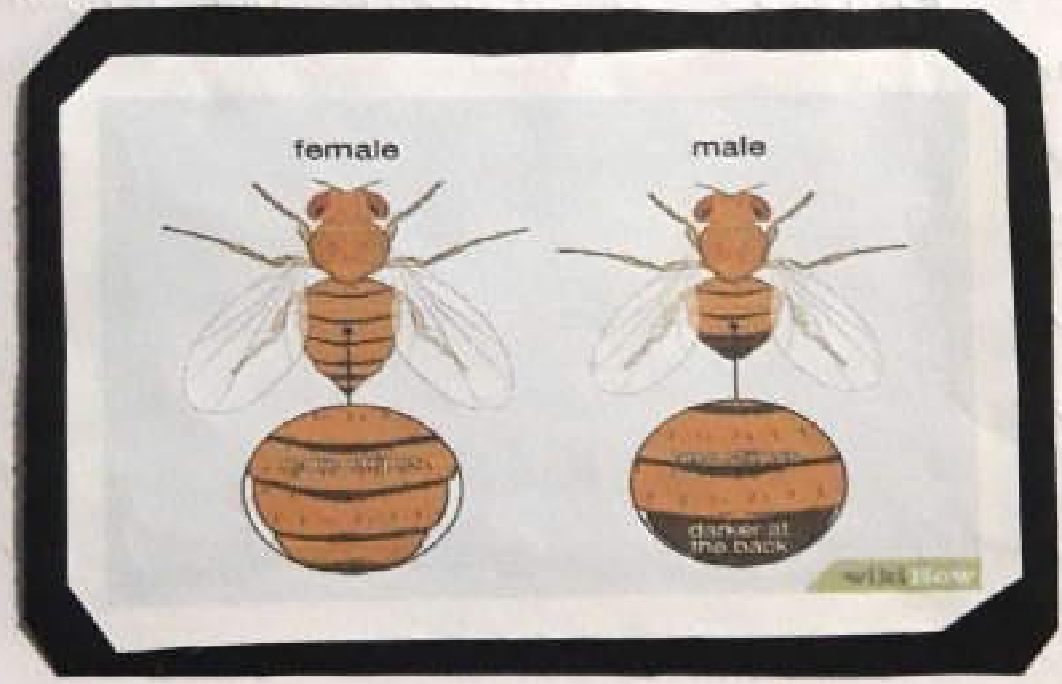
female
male
darker at the back

Sexing Drosophila

The abdomen of the female has seven segments, several dark transverse stripes and is pointed at the tip. The abdomen of the male has only five segments, two dark stripes, and a more rounded, heavily pigmented tip. In immature males the pigmentation may not be developed.

• Equipment →

1. A shelf for storing the bottles of flies.
2. Clean transparent vials / jars / bottles. Large test tubes, falcon tubes or a clean clear container with a suitable narrow neck.
3. A cotton wool plug, foam chunks cut to size, clean guaze or material to cover tied down with a rubber band.
4. Magnifying glasses and / or microscopes for observation and sorting.
5. Petri dishes.
6. A variety of small paints brushes.

• Ingredients →

(i) 27 g agar
(ii) 200 g Cornmeal (organic, fine ground)
(iii) 140 g Sugar.
(iv) 50 g Yeast
(v) 20 ml Propionic acid.
(vi) A dash of Nipagin.

Instruction

① Dissolved agar in 2L tap water by boiling.

② Dissolved Cornmeal, Sugar and Yeast in 1L cold tap water so that it is free of lumps.

③ Once agar is dissolved, the Cornmeal mixture should be added and boiled.

④ Stir it Continuously, boil for 15 minutes.

⑤ Open the stove and let it Cool. Propionic acid and nipagen should be added.

⑥ The fly food should be distributed in vials/ bottles as required. Only fill up to 1.5 cm in each Container.

⑦ Cover with Paper towels and allow to cool and dry (Over night) at room temperature.

⑧ Vials / bottles should be Plugged before storing in a fridge.

Handing Flies

Once the flies have been knocked, the flies need to be moved around using a clean Paint brush Using the Paint brush carefully means, that flies should not be harmed during selection.

Transferring flies from one container to another involves tapping the bottle gently, the flies dislodge from the walls and fall to bottom. Removing the lid from the bottle the flies need to be transferred to the top of the bottle and flies need to be kept. Then tap and the flies will fall from one bottle to another, quickly Put the lids on the bottles.

Anaesthetising Flies

There are 2 main ways - ① Freezer method ② Carbon dioxide. We choose Carbon dioxide.

Carbon dioxide

A tube attached to a Soda stream bottle and directed into a vial of flies with make them fairly sluggish and easy to handle for a short time. It is a good idea to have a Petri dish lid nearby to trap the active fish. To avoid blasting and damaging the fish, to Pump a few shots of CO_2 into the vital through a gauze or cotton flug. CO_2 is heavy and should not be forgotten to tap to the flies to bottom for optimum result so they are sitting in the CO_2 gas.

REFERENCE

① Robert. E.K, Lords of the fly "Drosophila Genetics and the experimental life". University of chicago Press; 1999.

② Milislav D, Biology of Drosophila. 10th ed. Cold spring Harbor Laboratory; 1950.

③ Harry D, Mendelian genetics of Drosophila. Cambridge University Press; 2001; 7-10.