A Comparative Study of Antifreeze Proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis* using Computational Tools and Servers

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Abstract

Antifreeze proteins (AFPs) are structurally diverse polypeptides produced by few vertebrates, plants, fungi and bacteria that permit their survival in subzero environments. AFPs bind to small ice crystals inhibiting growth and re-crystallization of ice. This inhibition causes thermal hysteresis which is the non-colligative depression of the freezing point (T_f) of a solution containing ice blow its melting point (T_m). These, fungal antifreeze protein has been purified and partially characterized only in the species of psychrophillic basidiomycete, *Typhula ishikariensis*. Here, we are reporting a new fungal antifreeze protein from another phychrophile *Antarctomyces psychrotrophicus*, which is a ascomycetes. We examined its computational characteristics and compared them with those of the *T. ishikariensis* antifreeze protein. In the present study an i*n- silico* comparison of the amino acid sequences of these proteins were carried out which shows invigorating similarity between the two proteins. The phylogenetic relationship among the antifreeze proteins and their homologous proteins infer the evolutionary history of these proteins. Their physico-chemical properties, hydropathicity and secondary structure have been identified. The Primary and secondary structure analysis of these fungal AFP's strongly supports its structure-functional relationship to each other. The study will be valuable to understand the structural and functional aspects of these proteins for academic and industrial purposes.

Keywords - Antifreeze proteins (AFPs), Re-crystallization, Thermal hysteresis, *Typhula ishikariensis, Antarctomyces psychrotrophicus*

Introduction

Antifreeze proteins (AFPs) or ice structuring proteins (ISPs) are structurally diverse polypeptides produced by few vertebrates, plants, fungi and bacteria that permit their survival in subzero environments. AFPs bind to small ice crystals inhibiting growth and re-crystallization of ice that would otherwise be fatal. AFPs are involved in cold acclimatization (Fletcher *et al.*, 2001).

This inhibition causes thermal hysteresis which is the non-colligative depression of the freezing point (T_f) of a solution containing ice blow its melting point (T_m) (Knight *et al.,* 1984; Fletcher *et al.,* 1986). Since AFP's work in a non-colligative manner, so their low concentration minimizes their effect on osmotic pressure (Fletcher *et al.*, 2001). The x-ray structure, nuclear magnetic resonance and many spectroscopic studies with AFPs have been instrumental in determining the structure-function relationship. Various studies have pointed out that the mechanism of AFP action is through its adsorption on the ice surface, which creates a curved surface which prevents further growth of ice by the "Kelvin effect" (Venketesh and Dayananda, 2008). The AFPs have different applications like industrial, medical, and agricultural in different fields like food technology, preservation of cell lines, organs, cryosurgery, and cold hardy transgenic plants and animals (Venketesh and Dayananda, 2008).

AFP's have been found in bacteria, plants, invertebrates and fish and were classified according to their structure and TH values (Duman, 2001; Duman and Olsen, 1993; Jia and Davies, 2002). Fish AFP's are grouped into five types and insect AFPs have been grouped into three types (right-handed beta helix and left-handed alpha helix, and a glycine-rich repeat) (Jia and Davies, 2002). Insect AFP's are termed "hyperactive AFP's" because their TH activity is 5-6 degree celcius (Scotter *et al.,* 2006).

Fungal AFP's have been discovered in snow molds that have pathogenic activities against dormant plants under snow cover (Snider *et al.,* 2000; Hoshino *et al.,* 2003; Hoshino *et al.,* 2003; Hoshino, 2005). Snow mold include two major fungal taxa of ascomycetes and basidiomycetes and one psuedofungal toaxon of oomycetes. Only two basidiomycetes are indentified: *Coprinus psychromorbidus* and *Typhula ishikariensis* of these, fungal antifreeze Protein has been purified and partially characterized only in the species of psychrophillic basidiomycete, *Typhula ishikariensis*. *Typhula ishikariensis* is, along with *is* the causal agent of Grey Snow Mould (also called Speckled Snow Mould or Typhula Blight). It's a plant pathogen that can destroy turfgrass when covered for a long period with snow. More importantly, it can damage crops of winter wheat (Schneider and Seaman, 1986). This fungi have the ability to attack plants at low temperature under persistent snow cover. Typhula snow molds caused by *T. phacorrhiza, T. incarnata and T. ishikariensis* are the most important winter diseases of perennial grasses and winter cereals in the United States. During winter dormancy, the carbohydrates reserves are depleted, and the plant become less resistant to disease.

Here, we report a new fungal antifreeze protein from another phychrophile *Antarctomyces psychrotrophicus*, which is a ascomycetes, characterized by naked thick-walled, naked asci ellipsoidal to fusiform, echinulate ascospores and blastoconidia isolated from Antarctican soil samples.This fungi generates bipyramidal ice crystals and its thermal activity is maximized under alkaline conditions. We believe that the comparison of the in-silico study between *T. ishikariensis* and *A. Antarctomyces psychrotrophicus* will provide important information on physiochemical characteristics, primary, secondary and tertiary structures.

Materials and Method

The full length amino acid sequences of antifreeze proteins, *Typhula ishikariensis and Antarctomyces psychrotrophicus* were retrieved from NCBI (http://www.ncbi.nlm.nih.gov) protein database, with accession number, Q76CE6 and P86268 respectively. Sequence homology of each protein was obtained by using *BLASTp 2.2.28 (*Stephen *et al.,* 1997; Stephen *et al.,* 2005*)* setting default parameter. Local alignment between the two fungal AFPs was performed by using Lalign server setting default parameters. To map sequence conservation, both the fungal AFPs sequences are aligned with their homologous sequences using multiple sequence alignment tool ClustalW (http://www.ebi.ac.uk/tools/clustalW). Phylogenetic tree was also constructed for both the proteins and its homologous by using ClustalW. The physico-chemical properties such as molecular weight, isoelectric point, total number of negative residues (Asp+Glu) and positive residues (Arg+Lys), extinction coefficient (Gill and Hippel, 1989), instability index (Guruprasad *et al.,* 1990), aliphatic index (Ikai, 1980), and grand average of hydropathiocity (GRAVY) (Kyte and Doolittle, 1982) of the two antifreeze protein *T. ishikariensis and A. psychrotrophicus* were predicted using Expasy's proteomics server PROTPARAM (http://expasy.org/cgi-bin/protparam) (Gasreiger *et al.,* 2005). The secondary structure features of antifreeze protein of *T. ishikariensis, A. psychrotrophicus* predicted using bioinformatics secondary structure prediction tool HNN model.This tool demonstrates that the proteins carries equal percentage of sequence length, alpha helix, beta turn, random coil etc.

Results and Discussion

Homology study

Antifreeze proteins are structurally diverse polypeptides produced by few vertebrates, plants, fungi and bacteria that permit their survival in subzero environments. NCBI protein blast (BLASTp) was carried out intended for scanning Antifreeze proteins of *Typhula ishikariensis and Antarctomyces psychrotrophicus*. A number of homologous proteins of *Typhula ishikariensis* (Table 1) *and Antarctomyces psychrotrophicus* (Table 2) were identified on the basis of score, query coverage, lowest E-value, and higher percentage of sequence similarity-identity. Most significant members are tabulated here.

Phylogenetic study

According to Blast results the members of higher percentage of sequence similarity and identity for both the proteins were allowed for multiple sequence alignment (MSA) and finally a phylogenetic tree was constructed. The phylogenetic relationship among the antifreeze protein Typhula ishikariensis and its 15 homologous proteins (Table 1) infer the evolutionary history of this protein. *The tree divided the sequences into three major clusters in which one cluster is again divided into two sub clusters due to significant variation in their sequences* (Fig. 1).

The phylogenetic relationship among the antifreeze protein *Antarctomyces psychrotrophicus* and its 18 homologous proteins (Table 2) infer the evolutionary history of this protein. *The tree divided the sequences into two major clusters in which one cluster is again divided into two sub clusters due to significant variation in their sequences* (Fig. 2). The result revels that the proteins are conserved and performing the same function.

Table 1: List of Homologous sequences of Antifreeze protein *Typhula ishikariensis.*

Sl. No.	Organism Name	Max. Score	Total Score	Query Coverage	E-value	Identity	Accession no.
1.	*Typhula ishikariensis (243aa)*	461	461	100%	1e-162	100%	BAD02893.1
2.	*Typhula ishikariensis (243aa)*	459	459	100%	6e-162	99%	BAD02894.1
3.	*Typhula ishikariensis (243aa)*	459	459	100%	1e-161	99%	BAD02895.1
4.	*Typhula ishikariensis (243aa)*	457	457	100%	4e-161	99%	BAD02896.1
5.	*Typhula ishikariensis (243aa)*	451	451	100%	1e-158	98%	BAD02892.1
6.	*Typhula ishikariensis (243aa)*	423	423	91%	6e-148	99%	3VN3_A
7.	*Typhula ishikariensis (243aa)*	407	407	100%	4e-141	87%	BAD02892.1
8.	*Typhula ishikariensis (243aa)*	368	368	100%	9e-126	82%	BAD02891.1
9.	*Stereum hirsutum FP-91666 SS1 (248aa)*	256	256	93%	1e-181	58%	EIM91127.1
10.	*Stereum hirsutum FP-91666 SS1 (258aa)*	242	242	93%	4e-76	54%	EIM91118.1
11.	*Stereum hirsutum FP-91666 SS1 (239aa)*	234	234	97%	2e-73	53%	EIM90588.1
12.	*Sphaerocaeta globosa str. Buddy (259aa)*	229	229	97%	3e-71	54%	YP_004248731.1
13.	*Stereum hirsutum FP-91666 SS1 (250aa)*	225	225	93%	9e-70	52%	EIM84895.1
14.	*Cytophaga hutchinsonii ATCC 33406 (368aa)*	219	219	89%	1e-65	55%	YP_676864.1

15.	*Flammulina poluicola (247aa)*	212	212	93%	2e-64	51%	ACL27143.1
16.	*Leucosporidium sp. AY30 (241aa)*	198	198	88%	3e-59	52%	3UYV_A
17.	*Leucosporidium sp. AY30 (241aa)*	198	198	88%	3e-59	52%	3UYV_A
18.	*Lentinula edodes (288aa)*	195	195	90%	2e-57	55%	ACL27145.1

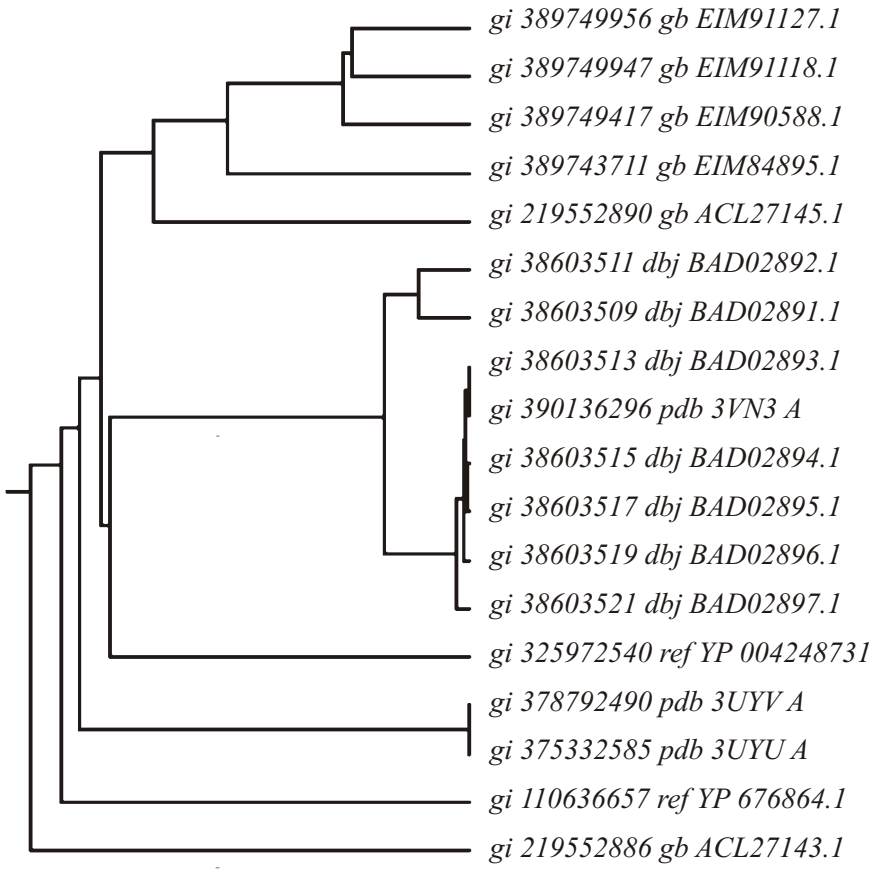

Figure 1: Phylogenetic tree of antifreeze protein *Typhula ishikariensis* through clustalW.

Table 2: List of Homologous sequences of Antifreeze protein *Antarctomyces psychrotrophicus*.

Sl. No.	Organism Name	Max. Score	Total Score	Query Coverage	E-value	Identity	Accession no.
1.	*Typhula ishikariensis (243aa)*	57.1	57.1	100%	1e-09	100%	P86268.1
2.	*Typhula ishikariensis (243aa)*	31.6	31.6	75%	8.0	73%	YP_001379664.1
3.	*Typhula ishikariensis (243aa)*	29.9	29.9	80%	29	71%	BAN32830.1
4.	*Typhula ishikariensis (243aa)*	29.9	29.9	80%	29	71%	ACJ35559.1
5.	*Typhula ishikariensis (243aa)*	29.1	29.1	65%	55	77%	YP_495511.1
6.	*Typhula ishikariensis (243aa)*	29.1	29.1	60%	53	83%	WP_018774336.1
7.	*Typhula ishikariensis (243aa)*	29.1	29.1	70%	57	71%	CBJ48445.1
8.	*Typhula ishikariensis (243aa)*	28.6	28.6	65%	78	77%	YP_004171135.1
9.	*Stereum hirsutum FP-91666 SS1 (248aa)*	28.6	28.6	75%	78	73%	WP_016463472.1
10.	*Stereum hirsutum FP-91666 SS1 (258aa)*	28.6	28.6	75%	78	73%	WP_005047967.1
11.	*Stereum hirsutum FP-91666 SS1 (239aa)*	28.2	28.2	90%	107	72%	WP_007943713.1
12.	*Sphaerocaeta globosa str. Buddy (259aa)*	27.8	27.8	90%	151	72%	WP_007883880.1
13.	*Stereum hirsutum FP-91666 SS1 (250aa)*	27.8	27.8	65%	151	73%	WP_004620553.1
14.	*Cytophaga hutchinsonii ATCC 33406 (368aa)*	27.8	27.8	67%	151	73%	WP_020813961.1
15.	*Flammulina poluicola (247aa)*	27.8	27.8	70%	152	79%	YP_001158336.1
16.	*Leucosporidium sp. AY30 (241aa)*	27.8	27.8	75%	152	75%	WP_007560414.1
17.	*Leucosporidium sp. AY30 (241aa)*	27.4	27.4	65%	210	73%	YP_006397228.1
18.	*Lentinula edodes (288aa)*	27.4	27.4	50%	211	90%	YP_004415435.1

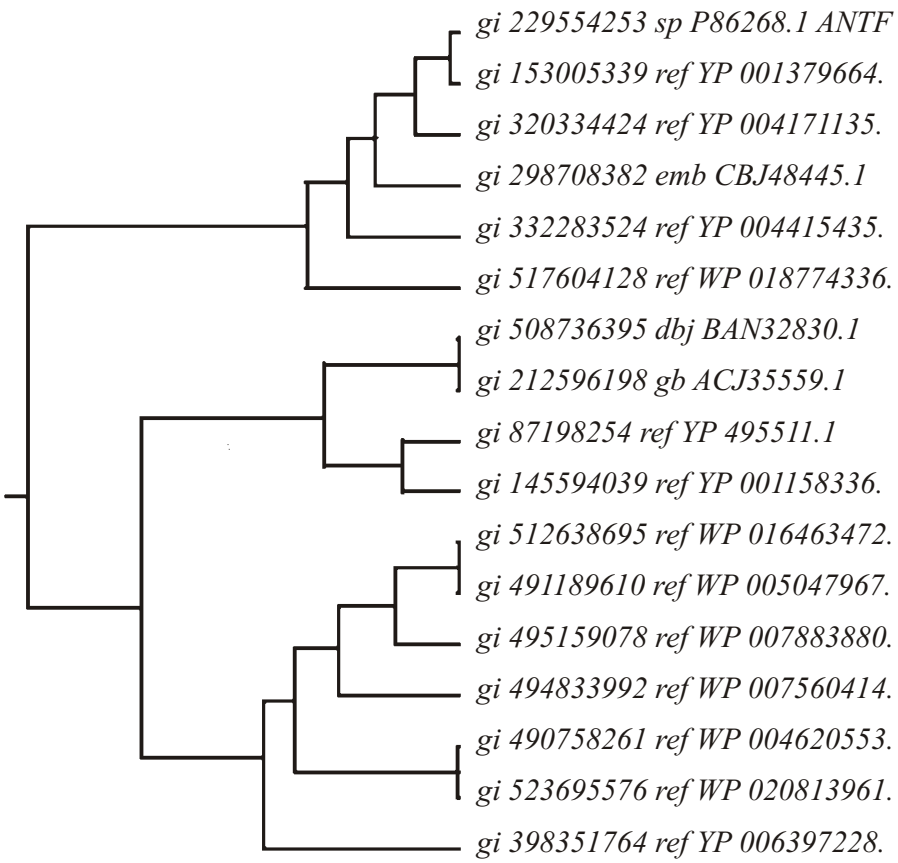

Figure 2: Phylogenetic tree of antifreeze protein *Antarctomyces psychrotrophicus* through clustalW.

Primary structure study

Amino Acid composition and physiochemical properties determines the fundamental properties of the protein. The Physiochemical properties of *Typhula ishikariensis and Antarctomyces psychrotrophicus* were computed using ProtParam (Table 3). The computed isoelectric point for *Typhula ishikariensis* is 4.89 and for *Antarctomyces psychrotrophicus* is 3.67 indicating that both the proteins are highly acidic in nature. Isoelectric point is a pH at which a protein carries no net charge. It is of significance in protein purification as it is the pH at which solubility is often minimal and mobility in an electrofousing system is zero. The extinction coefficient for *Typhula ishikariensis* is calculate as 23950and for *Antarctomyces psychrotrophicus* it is 0. This measure indicates how much light is absorbed by a protein at a particular wavelength. Computed value of instability index of for *Typhula ishikariensis* is 22.92 and for *Antarctomyces psychrotrophicus* is 0.01. Instability index relies upon the occurrence of certain dipeptides along the length of the protein. Aliphatic index for *Typhula ishikariensis* is computed as 100.82 and for *Antarctomyces psychrotrophicus* is computed as 103.0. Higher aliphatic index of proteins indicated their structural stability. The aliphatic index refers to the relative volume of a protein that is occupied by aliphatic side chains. An increase in the aliphatic index increases the thermo stability of enzyme. Grand average of hydropathicity (GRAVY) values of the protein *Typhula ishikariensis* was found to be 0.537 and for *Antarctomyces psychrotrophicus* was found to be 1.130. A positive GRAVY value for proteins designate it to be hydrophobic in nature.

Table 3: Primary structure analysis of antifreeze proteins *Typhula ishikariensis* and *Antarctomyces psychrotrophicus*.

Organism Name	***Typhula ishikariensis***	***Antarctomyces psychrotrophicus***
Molecular weight	24093.3	1848.2
Theoritical pI	4.89	3.67
Total no. of negatively charged residues	11	2
Total no. of positively charged residues	9	0
Extinction coefficient ($M^{-1}Cm^{-1}$) At 280 nm	23950	-
Instability index	22.92	0.01
Aliphatic index	100.82	103.0
Gravity	0.537	1.130

Secondary structure study

The secondary structure indicated whether a given amino acid lies in a helix, strand or coil. The secondary structure features of antifreeze protein of *T. ishikariensis, A. psychrotrophicus* predicted using bioinformatics secondary structure prediction tool HNN model and successive comparative studies were carried on. The result showing significant percentage of alpha helices, extended strands, beta turns and random coils is 25.00%, 5.00%, 0.00% and 70.00% in *Antarctomyces psychrotrophicus* and 20.58%, 27.98%, 0.00% and 51.44% in *Typhula ishikariensis* respectively (Table 4). The results revealed that in both the proteins random coils dominated among secondary structure elements followed by alpha helices, which shows that amino acid sequences of these proteins are hydrophobic in nature and that's why these are highly conserved.

Table 4: Secondary structure analysis of antifreeze proteins *Typhula ishikariensis* and *Antarctomyces psychrotrophicus*.

Features	***Antarctomyces psychrotrophicus***	***Typhula ishikariensis***
Alpha helix (Hh)	5 is 25.00%	50 is 20.58%
3_{10} helix(Gg)	0 is 0.00%	0 is 0.00%
Pi helix(Ii)	0 is 0.00%	0 is 0.00%
Beta bridge(Bb)	0 is 0.00%	0 is 0.00%
Extended strand(Ee)	1 is 5.00%	68 is 27.98%
Beta turn(Tt)	0 is 0.00%	0 is 0.00%
Bend region(Ss)	0 is 0.00%	0 is 0.00%
Random coil(Cc)	14 is 70.00%	125 is 51.44%
Ambiguous states(?)	0 is 0.00%	0 is 0.00%
Other states	0 is 0.00%	0 is 0.00%

Conclusion

In the present study, an attempt has been made to perform a comparative computational analysis between two fungal antifreeze proteins from *Antarctomyces psychrotrophicus* and *Typhula ishikariensis* including its homology study, physicochemical characterization, secondary structure prediction and phylogenetic analysis. Based on all the findings it can be concluded that both the antifreeze proteins may comprise same structural and functional properties. We wish that this study will inspire new experim entalefforts in this area; specially, the assessment of computational approach can be readily tested for their biochemical relevance. The above study will be useful to understand the structural and functional aspects of these proteins and will be of importance for commercial and academic understanding.

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