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REVIEW

Acidophilic bacteria and archaea: acid stable biocatalysts and their potential applications

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Abstract Acidophiles are ecologically and economically important group of microorganisms, which thrive in acidic natural (solfataric fields, sulfuric pools) as well as artificial man-made (areas associated with human activities such as mining of coal and metal ores) environments. They possess networked cellular adaptations to regulate pH inside the cell. Several extracellular enzymes from acidophiles are known to be functional at much lower pH than the cytoplasmic pH. Enzymes like amylases, proteases, ligases, cellulases, xylanases, α-glucosidases, endoglucanases, and esterases stable at low pH are known from various acidophilic microbes. The possibility of improving them by genetic engineering and directed evolution will further boost their industrial applications. Besides biocatalysts, other biomolecules such as plasmids, rusticynin, and maltose-binding protein have also been reported from acidophiles. Some strategies for circumventing the problems encountered in expressing genes encoding proteins from extreme acidophiles have been suggested. The investigations on the analysis of crystal structures of some acidophilic proteins have thrown light on their acid stability. Attempts are being made to use thermoacidophilic microbes for biofuel production from lignocellulosic biomass. The enzymes from acidophiles are mainly used in polymer degradation.

Keywords Acidophiles · Biocatalysts · Acid stable · Extremophiles

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Introduction

Many microorganisms survive in the physically and geochemically extreme conditions, which challenge the physico-chemical limits of life like extremes of temperature, pH, salinity, pressure, desiccation, radiation, and others. These are termed as extremophiles. Among them, acidophiles are the organisms that thrive in acidic environments with pH less than 4.0. These are diverse group of organisms included in archaea, bacteria, fungi, algae, and protozoa growing in acidic conditions, reported from natural environments like solfataric fields, sulfuric pools and geysers, and artificial environments like areas associated with human activities like mining of coal and metal ores. A few microbes belonging to the genera *Acidithiobacillus* and *Sulfolobus* generate acid through oxidation of sulfur. The species of *Picrophilus*, with optimum pH for growth of 0.7, are obligate acidophiles. Acidic water from mines and geothermal springs are dominated by heterotrophic bacteria belonging to the genera *Acidiphilium* and *Alicyclobacillus* having pH optima for growth between 2.5 and 6.0. While most of the extreme acidophiles belong to archaeal group that includes *Acidianus*, *Desulphurolobus*, *Metallosphaera*, *Stygiolobus*, *Sulfolobus*, *Sulphurisphaera*, *Sufurococcus*, *Thermoplasma* and *Picrophilus* (Bertoldo et al. 2004) (Table 1).

To survive at extremes of pH, organisms require a networked cellular adaptation to surmount different

Table 1 The characteristics of acidophilic bacteria and archaea

Organism	Temp range (°C)	Temp opt. (°C)	pH range	pH opt.	Mole %GC	Locations/ environments	References
Eubacteria							
Acidiphilium angustum	–	–	2.5–6.0	–	67	Coal AMD*	Wichlacz et al. (1986)
Acidiphilium organovorum	20–45	37	2.0–5.5	3	64	Copper mine	Lobos et al. (1986)
Acidiphilium symbioticum	–	37	1.5–6.0	3.0–4.0	59.5		Bhattacharyya et al. (1990)
Acidiphilium aminolyticum	20–37	–	3.0–6.0	–	59		Kishimoto et al. (1995)
Acidiphilium multivorum	17–42	27–35	1.9–5.6	3.2–3.8	66–68	AMD	Wakao et al. (1994)
Acidiphilium cryptum	20–41	35–41	1.9–5.9	3.0	68–70	AMD	Harrison (1981)
Acidiphilium rubrum	Mesophile		2.5–<6				Wichlacz et al. (1986)
Acidiphilium facilis	–	–	2.5–6.0	–	65		Wichlacz et al. (1986); Kishimoto et al. (1995)
Acidimicrobium ferroxidans strain TH3	30–55	48	–	2.0	67–68	Cu leach dump	Clark and Norris (1996); Norris (1990)
Alicyclobacillus acidocaldarius	45–70	–	2.0–6.0	–	61–62	Hot springs, Yellowstone park	Darland and Brock (1971); Wisotzkey et al. (1992)
Alicyclobacillus acidoterrestris	35–55	–	2.2–5.8	–	51–53	Hot springs, Yellowstone park	Wisotzkey et al. (1992)
Alicyclobacillus cycloheptanicus	40–53	–	3.0–5.5	–	54–57	Hot springs, Yellowstone park	Wisotzkey et al. (1992)
Alicyclobacillus acidiphilus	20–55	50	2.5–5.5	3.0	54.1		
Sulfobacillus thermosulfidooxidans	30–60	45–48	1.5–5.5	2.0	48–50	Geothermal volcanic hot springs, coal dumps	Golovacheva and Karavaiko (1978)
Sulfobacillus acidophilus	30–55	45–48	–	2.0	55–57		Norris et al. (1996)
Thiobacillus caldus	30–55	45	1.0–4.0	2.0–2.5	62–64		
Acidithiobacillus thiooxidans	–	–	0.5–5.5	2–3	50–52		
Thiobacillus albertis		28–30	2.0–4.5	3.5–4.0	61.5	Acidic soil of sulfur stock piles	Bryant et al. (1983)
Thiobacillus prosperus	23–41	33–37	1.0–4.5	2.0	63–64	Hydrothermal marine vents	Huber and Stetter (1989)
Thiobacillus acidophilus	<25–37	27–30	1.5–6.5	2.5–3.0	63–64	AMD	Markosyan (1973); Guay and Silver (1975); Harrison (1983)
Leptospirilum ferrooxidans	<10–45	30–37	–	1.5–2.0	51–56	Cu deposits on pyrite, AMD	Markosyan (1972)
Leptospirilum thermoferro-oxidans	<30–55	45–50	–	1.6–1.9	56	–	Golovacheva et al. (1992)
Archaea							
Thermoplasma acidophilum	45–63	59	0.5–4	1.0–2.0	46	Coal refuse piles, solfatara, hot springs	Darland et al. (1970)
Thermoplasma volcanium	33–67	60	1.0–4.0	2.0	38	Acid hot springs, solfatara Yellowstone, soils	Segerer et al. (1988)
Picrophilus oshimae	45–65	60	0–2.2	0.7	36	solfataric hydrothermal areas in Japan	Schleper et al. (1995)

Table 1 continued

Organism	Temp range (°C)	Temp opt. (°C)	pH range	pH opt.	Mole %GC	Locations/ environments	References
Picrophilus torridus	45–65	60	0.2.2	0.7	36	Solfataric hydrothermal areas in Japan	Schleper et al. (1995)
Ferroplasma acidiphilum	15–45	35	1.3–2.2	1.7	36.5		
Acidianus brierley	45–75	70	1.0–6.0	1.5–2.0	31	Solfatara, marine hydrothermal	Brierley and Brierley (1973); Zillig et al. (1980); Segerer et al. (1986)
Acidianus infernus	63–95	90	1.0–5.0	2.0	31	Solfatara	Segerer et al. (1986)
Desulfurolobus ambivalens	55–85	81	0.8–4.0	2.5	32.7	Japan hot springs, solfatara	Brock et al. (1972); Segerer et al. (1985, 1986), Zillig et al. (1986); Zillig and Boeck (1987); Fuchs et al. (1996)
Metalloshaera sedula	50–80	75	1.0–4.5	1.7	45	Solfatara, hot springs	Huber et al. (1989)
Metallsphaera prunae	55–80	75	1.0–4.5	–	46	Uranium mine	Fuchs et al. (1996)
Methylokorus infernorum	–	–	2.0–2.5	–	–	–	
Methylocella	–	–	5.0–5.5	–	–	–	
Methylocapsa	–	–	5.0–5.5	–	–	–	
Stygiolobus azoricus	57–89	80	1.0–5.5	2.5–3.0	38	San Miguel island	Segerer et al. (1991)
Sulfolobus acidocaldarius	55–90	80	1.0–5.5	2.0–3.5	37	Hot springs, solfatara Yellowstone	Brock et al. (1972)
Sulfolobus shibatae	75–85	80	3.0–4.0	3.0	35	Japan	Grogan et al. (1990)
Sulfolobus metallicus	50–75	65–68	1.0–4.5	–	38	–	Huber and stteter (1991)
Sulfolobus yangmingensis	65–95	80	2.0–6.0	4.0	42	Yang-Ming National Park in northern Taiwan	Ren-Long et al. (1999)
Sulfolobus tengchongensis	–	85	–	3.5	34.4	Hot spring in Tengchong	Xiang et al. (2003)
Sulfurisphaera ohwakuensis	63–92	84	1.0–5.5	2.0	33	Hot springs in Japan	Kurosawa et al. (1998)
Sulfurococcus mirabilis	50–86	70–75	1.0–5.5	2.0–2.6	43–46		
Sulfurococcus yellowstonii	40–80	60–65	1.0–5.5	2.0–2.5	44.5	Hot spring (Yellowstone National Park)	Karavaiko et al. (1994)
Thermogymnomonas acidicola		60	1.8–4.0	3.0		Solfataric field, Japan	Itoh et al. (2007)

eco-physiological problems experienced by them. They have several distinctive structural and functional characteristics for the regulation of pH (Golyshina et al. 2000; Crossman et al. 2004). It has been predicted that the organisms develop specific metabolic properties, genetic features, structural and functional characteristics of their macromolecules that are helpful in maintaining the pH and distinguishing them from their neutrophilic counterparts (Baker-Austin and Dopson 2007). Despite being able to survive in extremely acidic conditions, these organisms fail to tolerate such conditions inside the cell because macromolecules such as DNA become unstable so they have evolved mechanisms to pump acid out of the cell in order to maintain neutral to weak acid conditions (pH 5–7) inside the cell (Matin 1999). There are also a few reports of some other organisms having internal pH in the acidic range (Vossenberg et al.1998; Macalady et al. 2004). The genes of several intracellular or cell bound enzymes from *Ferroplama acidiphilum* were cloned and expressed in *Escherichia coli* and the products were purified and characterized (Golyshina

et al. 2006). The characterized enzymes had been found to be stable and function in the acidic pH range (1.7–4.0), suggesting the undetected cellular compartmentalization that provides cytoplasmic pH pachiness and low pH environments for the analyzed enzymes. In this article, an attempt has been to review the progress achieved in the characterization and potential applications of acid stable biocatalysts from acidophilic microbes.

Acid stable enzymes produced by acidophiles and their applications

Many acid stable enzymes are known from acidophiles (Table 2). Some thermoacidophilic proteins have potential application in biofuel production. From the estimated 25,000 enzymes, approximately 3,000 enzymes known to date catalyze different metabolic reactions. Among these, only fewer than hundred enzymes are used industrially because of their high specificity and stability. The world market for industrial enzymes is estimated to be around US $ 3 billion dollars, and even more is estimated from the products obtained from these enzymes. Acid stable cell envelope and acid resistant extracellular enzymes are required as they are having applications in the degradation of polymeric or oligomeric carbon sources (Futterer et al. 2004). The promising characteristics of enzymes from thermoacidophiles such as being active at low pH and high temperature can be exploited in industrial processes like textile industry and fruit juice production processes. The demand for enzymes from extremophiles is more as they can be used under harsh conditions.

Amylolytic enzymes

Amylases are starch-degrading enzymes and are widely distributed in microbes, plants, and animals. The term amylase is conventionally used to designate enzymes capable of hydrolyzing α 1-4 glucosidic bonds in starch, but in the last couple of decades there are reports of various new enzymes involved in the degradation of starch and other related polysaccharides (Buonocore et al. 1976). In order to use natural polymeric substrates like starch, extremophiles belonging to different taxonomic groups produce various amylolytic enzymes. The study of biocatalysis and protein stability of amylolytic enzymes is important because of their biotechnological applications. Amylases are one of the most important groups of enzymes with wider applications in the conversion of starch to sugar syrups, and in the bread and textile industries. For the development of cleaner environmental friendly processes, the demand for enzymes in the industry is increasing. Raw starch from wheat, maize, and tapioca is hydrolyzed to maltodextrins by α-amylase, which are further saccharified and converted into fructose syrups using glucoamylase and glucose isomerase, respectively.

α-Amylases Presently, the α-amylase used in starch industry is active at pH 6.5 and requires Ca^{2+} for stability and/or activity. Since the pH of native raw starches is around 3.2–3.6, the adjustment of pH is made and that necessitates removal of Ca^{2+} in the subsequent stages, which adds to the process operating costs (Crabb and Shetty 1999). Therefore, there is a need for acid stable α-amylases. Acidic α-amylase is an enzyme with pH optimum for activity in the acidic range, and it is more stable at acidic pH. The α-amylase from *Alicyclobacillus acidocaldarius* is the first example of heat and acid stable protein that has been studied in detail (Matzke et al. 1997; Bertoldo et al. 2004). The temperature and pH optima of the enzyme were 75°C and 3, respectively. The enzyme hydrolyzed starch randomly and produced maltotriose and maltose as main end products. Based on the nucleotide sequence of the cloned gene, the enzyme belongs to the family of starch-degrading enzymes with catalytic (beta-alpha) 8-domain. Acidophilic protein contains three exchanges in residues that are uniformly conserved among all members of the enzyme family. The α-amylase gene was expressed in *E. coli* to find whether these exchanges are responsible for the acidic pH optimum. The adaptation of protein to the acidic environment was considered to be due to the reduction of density of the both positive and negative charges on the surface of the protein; this effect avoids the electrostatic repulsion of charged groups at acidic pH and contributes to the acid stability of proteins (Schwermann et al. 1994). The temperature and pH optima of the enzyme produced in *E. coli* were similar to those of the native enzyme (Matzke et al. 1997). The protein engineering of the α-amylase was also attempted to understand the determinants of pH activity profile. Based on the structural studies, it is complex to engineer a protein, as there are many factors that are responsible for the pH activity profile of α-amylases. Site directed mutagenesis of α-amylase produced by *Bacillus* strain was performed in order to understand the pH activity profile of the enzyme. Based on the mutagenic studies, it was concluded that the modification of dynamics of the active site could be an alternative for engineering pH activity profile of the protein. The chance of rational engineering of the enzyme activity is expected to succeed in case the detailed description of enzyme mobility and dynamics of the active site are available while designing the point mutations (Neilsen et al. 2001).

Acid stable amylase was also reported from *Bacillus* sp. YX-1. The organism grows at pH 4.5 and produces amylase maximally at pH 5.0. The molecular weight of amylase is 56 kDa and the enzyme is stable in a broad range of pH (4.5–11.0) (Liu and Xu 2008). Sharma and Satyanarayana

Table 2 Acid stable enzymes produced by acidophilic bacteria and archaea

Enzyme	Source	pH opt.	Temp opt. (°C)	Mol. wt (kDa)	PI	Thermostability	Kinetic properties	Subunit type	References
α-Amylase	*A. acidocaldarius*	3.0	75	160	–	–	–	–	Matzke et al. (1997)
	S. solfataricus			120	–	–	–	Homodimer	
	Bacillus sp. YX-1	5.0		59	–	60°C, 1 h (~62%)	–	–	Liu and Xu (2008)
	Bacillus KR-8104			66	–	$T_{1/2}$-~7 min at 70°C	–	–	Sajedi et al. (2005)
	B. acidicola	4.0	60	–	–	$T_{1/2}$ ~27 min at 90°C	–	–	Sharma and Satyanarayana (2010)
	Bacillus sp.	4.5	70	53		75% activity at 70°C upto 45 min	–	–	Asoodeh et al. (2010)
Glucoamylase (extracellular)	*T. acidophilum*	2.0	90	141/95	4.5–4.8	$T_{1/2}$- 24 h at 90°C	K_m soluble starch-1.06 mg/ml	–	Serour and Antranikian (2002)
	P. torridus	2.0	90	133/90	4.5–4.8	$T_{1/2}$-24 h at 90°C	K_m soluble starch-2.5 mg/ml	–	Serour and Antranikian (2002)
	P. oshimae	2.0	90	140/85	4.5–4.8	$T_{1/2}$-20 h at 90°C	K_m soluble starch-4.35 mg/ml	–	Serour and Antranikian (2002)
Glucoamylase (intracellular)	*P. torridus*	4.5–6.5	30–65	290	–	$T_{1/2}$-1 h at 60°C	V_{max} for maltotriose (10 U/mg) Starch (8 U/mg)	Homotetramer	Schepers et al. (2006)
	T. acidophilum				–	$T_{1/2}$-15 min (No Ca^{2+})	V_{max} 3.86 µmol/min	Homodimer	Dock et al. (2008)
						$T_{1/2}$-2 h (with Ca^{2+}) at 80°C	K_m 0.68% (*w/v*) (Amylopectin)		
	S. solfataricus	5.5–6.0	90	260	–	–	–	Tetramer	Kim et al. (2004)
	Clostridium thermosaccharolyticum	5.0	70	75	4.9–5.6	$T_{1/2}$-3 h at 70°C	–	–	Katkocin (1985)
Trehalose dextrin forming enzyme and trehalose forming enzyme	*S. solfataricus* ATCC35092	5	75	86.6	–	85°C-2 h (56%)	–	–	Fang et al. (2004)
	S. solfataricus MT4	5	75		–	80°C-2 h (80%)	–	–	De Pascale et al. (2001)
	S. solfataricus KM1	4.5–5.5	70–80		–	85°C-6 h (91%)	–	–	Kato et al. (1996b, 2000)
	S. acidocaldarius ATCC 33909	5.5–6.0	75	–	–	85°C-1 h (~100%)	–	–	Nakada et al. (1996a)
	S. shibatae DSM 5389	4.5	70	–	–	85°C -2 h (~90%)	–	–	Di Lernia et al. (1998)

Table 2 continued

Enzyme	Source	pH opt.	Temp opt. (°C)	Mol. wt (kDa)	PI	Thermostability	Kinetic properties	Subunit type	References
Cyclomaltodextrinase	*A. acidocaldarius*	5.5		66	–	–	–	–	Matzke et al. (2000)
Proteases	*Xanthomonas*	2.7	NA*	41	–	–	–	–	Oda et al. (1987a)
	Pseudomonas	3.0	NA	43	–	–	–	–	Oda et al. (1987b)
	Bacillus MN-32	3.5	Thermostable	41	–	80°C-10 min		–	Murao et al. (1988)
	Sulfolobus acidocaldarius	2.0	NA	51	–	80°C 48 h	5.3×10^{-5} M (K_m) 14.3 S^{-1} (K_{cat}) (phenyl alanine and *p*-nitrophenylalanyl)	–	Fusek et al. (1990)
	Bacillus sp. strain Wp 22	3.5	60	45	3.8	90°C-3 min 80°C-21 70°C-102 60°C-45 h	K_m 0.036% V_{max} 97 U/mg (heamoglobin)	–	Toogood et al. (1995)
	Thermoplasma volcanium	3.0	60	–	–	–		–	Kocabayak and Ozel (2007)
Endo-glucanase	*Alicyclobacillus acidocaldarius*	4.0	80	100	–	80°C-1 h (60%)	K_m CMC-0.35	–	Eckert and Schneider (2003)
Acid phosphatases	*Sulfolobus acidocaldarius*	5.0–5.5	70	20	–	75°C-60 min (60%)	–	Monomeric	Kurosawa et al. (2000)
β-Glucosidase	*Acidobacterium capsulatum*	3.0	55	90	–	60°C – 10 min (20% activity)	–	–	Kishimoto et al. (1991)
α-Glucosidase	*Ferroplasma acidiphilum*				–				
A-GluFa		2.5–3.0	60	57	6.42	$T_{1/2}$-34 min at 60°C, pH 2.5–3.5	K_{cat}/K_m (S^{-1} mM^{-1}) 293 ± 44.0 (sucrose)	–	Golyshina et al. (2006)
GlyFa1		2.0	60	28.3	–	$T_{1/2}$-23 min at 60°C, pH 1.7–2.5	K_{cat}/K_m (S^{-1} mM^{-1}) 142 ± 27.0 (sucrose)	–	Golyshina et al. (2006)
GlyFa2		3.5–4.0	60	40.5	–	$T_{1/2}$-12 min at 60°C, pH 3.5–4.0	K_{cat}/K_m (S^{-1} mM^{-1}) 142 ± 30.0 (sucrose)	–	Golyshina et al. (2006)
	S. solfataricus	4.5	85	80.5	–	$T_{1/2}$-39 h at 85°C, pH 6.0	K_m 2.16 mM V_{max} 3.08 μmol (*p*-nitrophenyl-D-glucopyranoside)	–	Miura et al. (1999)
Esterases	*Ferroplasma acidiphilum*	2.0–3.5	50	34.7		–	K_{cat}/K_m (S^{-1} mM^{-1}) 64.2 ± 7.1 *p*-Nitrophenyl propionate	–	Golyshina et al. (2006)

Table 2 continued

Enzyme	Source	pH opt.	Temp opt. (°C)	Mol. wt (kDa)	PI	Thermostability	Kinetic properties	Subunit type	References
DNA ligase	*Ferroplasma acidiphilum*	1.5–3.0	–	–	–	–	–	–	Ferrer et al. (2008)
Endonuclease	*Sulfolobus solftaricus*	3 and 7	–	38.2	–	–	–	–	Limauro et al. (2001)

(2010, 2011) have recently reported production of acid stable, high maltose-forming and Ca^{2+}-independent α-amylase from an acidophile *Bacillus acidicola*. Another acid stable α-amylase has been reported from *Bacillus* sp. with pH and temperature optima of 4.5 and 70°C, respectively (Asoodeh et al. 2010). Based on acid induced unfolding of α-amylase of *B. amyloliquefaciens* (BAA), it was concluded that at pH 3.0, BAA acquires partially folded state like molten globules. A reduction of defined tertiary structure without any change in secondary structure was observed. At acidic pH, unfolding of BAA was such that its hydrophobic surfaces are greatly exposed as compared to native conformation at neutral pH. The refolding of molten globule to native form requires Ca^{2+} as a cofactor (Asghari et al. 2004). The structural studies of amylase from other bacteria like *Bacillus* KR-8104 have also been carried out; a shift in *p*Ka value of catalytic residues to acidic pH was recorded. The shifting of pH activity profile towards acidic pH in the acidic amylase from *Bacillus* KR-8104 (KRA) as compared to neutral ones from *B. licheniformis* (BLA) may be because of some amino acid substitutions that affect the putative active site leading to the formation of an extra hydrogen bond between Glu261 and Arg229 (BLA numbering) (Alikhajeh et al. 2007) (Fig. 1). Further studies are in progress to know which residues contribute to the acid pH optimum of the amylase.

Glucoamylases Glucoamylase (EC 3.2.1.3) is an exo-acting enzyme that hydrolyzes polysaccharides from the non-reducing chain ends by cleaving α-1, 4 and α-1, 6 glycosidic bonds consecutively. Glucoamylases are widely distributed in fungi, yeast and bacteria with temperature and pH optima of 70°C and 3.5–6, respectively. While glucoamylases from archaeal strains have temperature and pH optima of 90°C and 2.0. Archaeal glucoamylases are reported from *Picrophilus torridus*, *P. oshimae*, and *Thermoplasma acidophilum.* All these organisms utilize starch as sole carbon source and produce glucoamylase extracellularly. As compared to glucoamylase obtained from other sources (bacterial and fungal), archaeal glucoamylases are more active and stable at higher temperatures with a half life of 24 h at 90°C for those from *P. torridus* and *T. acidophilum*, and 20 h for *P. oshimae* (Serour and Antranikian 2002). Archaeal extracellular glucoamylases from *P. torridus* and *P. oshimae* and *T. acidophilum* have higher substrate specificity towards larger molecules like starch than small sugars as compared to other recombinant glucoamylases from *S. solfataricus* and *Thermoactinomyces vulgaris* which preferably act on smaller molecules. Recombinant intracellular glucoamylases from extremely thermoacidophilic archaea *T. acidophilum* and *P. torridus* have been reported to possess high activity at 75°C and pH 5, and 30–65°C and pH 4.5–6.5,

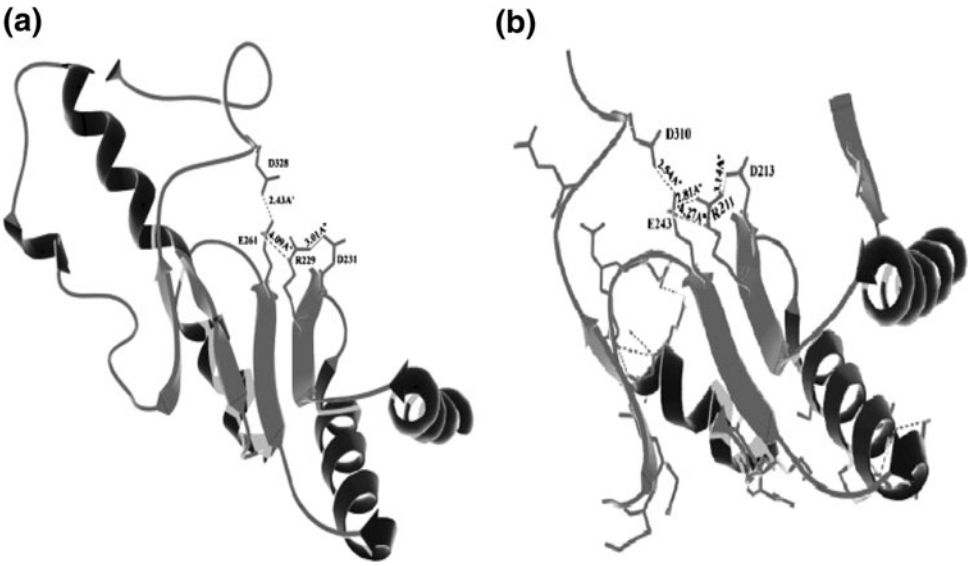

Fig. 1 Depiction and its local hydrogen bonding networks of the real active site of α-amylase of *B. licheniformis* (BLA) (**a**), and putative active site of *Bacillus* KR-8104 (KRA) (**b**). Hydrogen bond distances are shown on the picture in terms of Angstrom. Adapted from Alikhajeh et al. (2007)

respectively (Schepers et al. 2006; Dock et al. 2008). The recombinant intracellular glucoamylase from *T. acidophilum* expressed in *E. coli* was a homodimer (66 kDa/subunit) that requires Ca^{2+} for stability (Dock et al. 2008). Thermoacidophiles are hard to grow, and further produce low titres of native enzymes, and thus, recombinant enzyme production in *E. coli* is attempted. Cloning of glucoamylase from a thermoacidophile *S. solfataricus* in mesophilic host represents the first successful cloning attempt (Kim et al. 2004). The enzyme preferably acts on maltotriose liberating *β*-D-glucose thus distinguishing it from other fungal glucoamylases. Glucoamylase is used in the production of dextrose and fructose syrups, in the baking industry, in the brewing of low calorie beer and in whole grain hydrolysis in the alcohol industry.

α-Glucosidases α-Glucosidases play important roles in carbohydrate metabolism, energy processing and glycosylation of lipids (Batrakov et al. 2002). Rolfsmeier et al (1998) reported α-glucosidase gene from archaeal domain. The structural gene encoding α-glucosidase from *S. solfataricus* was cloned and characterized. Three clones expressing α-glucosidase (α-GluFa, GlyFa1, GlyFa2) have been reported from the genome of *F. acidiphilum*. α-GluFa does not have any sequence similarity to the known glycosyl hydrolases. It exhibited high similarity with an uncharacterized membrane protein belonging to the COG 1287 family found in almost all archaeal genomes and showed highest sequence similarity (96%) with PPE-repeat protein of *Ferroplasma acidarmanus* Gly Fa1 has 40% identity to the dolichol phosphate mannosyltransferase related proteins from *T. acidophilum*, *T. volcanium*, *P. horikoshii* OT3, *Pyrococcus furiosus*, *Aeropyrum pernix* K1 and *Pyrobaculum aerophilum* IM2. While GlyFa2 has been clustered together with archaeal proteins like L-alanine-DL-glutamate epimerase, the related enzymes of *F. acidarmanus*, *O*-succinyl benzoate-CoA synthase of *P. torridus* DSM 9790 (78% sequence similarity), *N*-amino acid racemase of *T. acidophilum* DSM 1728 and *T. volcanium* GSS1 (73% sequence similarity). All of these enzymes expressed in *E. coli* were intracellular and had shown stability and function in the acidic pH range (1.7–4.0), which is less than intracellular pH (5.6). The reasons for this pH optimum anomaly are not yet clear, but some hints regarding cellular compartmentalization providing cytoplasmic pH patchiness and low pH activity of intracellular enzymes have been analyzed (Golyshina et al. 2006). Miura et al. (1999) reported the production of 2,083 bp gene encoding α-glucosidase from *S. solfataricus*.

Other amylases Trehalosyl dextrin-forming enzyme (TDFE) belongs to the class of starch hydrolyzing enzymes. Trehalose (α-D-glucopyranosyl-α-glucopyranoside) is a non-reducing sugar consisting of two glucose molecules linked by an α-1, 1 linkage. It is mainly found in animals, plants, fungi, insects, and bacteria and its intracellular accumulation in some insects, microorganisms and desert plants is helpful in increasing the resistance to various environmental stresses like salts, ethanol, freezing, heat, and desiccation (Page-Sharp et al. 1999; Richards et al. 2002). Trehalose is used as sweetener, preservative, stabilizer for food, cosmetics, and medicines (Richards et al. 2002). The TDFE mainly catalyzes an intracellular transglycosyl reaction to form trehalosyl dextrin from dextrins by converting the α-1, 4-glucosidic linkage at the reducing end to an α-1,1-glucosidic linkage and trehalose-forming enzyme (TFE, also known as maltooligosyltrehalose synthase and maltooligosyltrehalose trehalohydrolase) cleaves the α-1, 4-glucosidic linkage next to the α-1, 1-glucosidic linkage of trehalosyl dextrins forming trehalose from starch. These two enzymes are mainly involved in the formation of trehalose from starch (Kato et al. 1996a,

b; Nakada et al. 1996a, b), and are reported from *S. solfataricus* ATCC 35092, *S. solfataricus* MT4, *S. solfataricus* KM1, *S. acidocaldarius* ATCC33909 and *S. shibatae* DMS 5389 (Fang et al. 2004; de Pascale et al. 2001; Di Lernia et al. 1998; Gueguen et al. 2001; Kato 1999; Kato et al. 1996b; Nakada et al. 1996a). The recombinant enzyme, produced by cloning the gene from *S. solfataricus* ATCC 35092, was optimally active at pH 5 and 75°C (Fang et al. 2004).

A gene encoding a cyclomaltodextrinase (neopullulanase) was cloned in *E. coli* from the thermoacidophilic bacterium *A. acidocaldarius* ATCC27009 with its pH optimum of 5.5. Due to the absence of N-terminal signal sequence in encoded CdaA protein and its similarity with a family of bacterial proteins, it was described as maltogenic α-amylase, neopullulanase or cyclomaltodextrinase. It was a 66-kDa protein that degraded pullulan in sodium dodecylsulfate polyacrylamide gel. The activity of neopullulanase was detected in cytoplasm suggesting it to be cytoplasmic cyclomaltodextrinase. CdaA degraded cyclomaltodextrins more rapidly than pullulan to panose (Matzke et al. 2000).

Proteolytic enzymes

Proteases are enzymes with wider applications in commercial and physiological fields. Proteases catalyze the hydrolysis of proteins by cleaving the peptide bonds. Proteases are also known to modify the proteins by limited proteolysis like the activation of zymogenic forms of enzyme, blood clotting and lysis of fibrin clots, and the processing and transport of secretory proteins across the membrane. Since these are having physiological importance, they are ubiquitously in plants, animals and microorganisms. Proteases from microbial sources are preferred over plant and animal sources as they posses all the properties preferred for biotechnological applications. Depending on the mode of action, proteases are categorized as exopeptidases and endopeptidases. Exopeptidases cleave the peptide bond proximal to the amino and carboxy termini of the substrate, while endopeptidases cleave the peptide bonds in the inner regions of the polypeptide chain. On the basis of the functional group present at the active site, proteases are classified as serine proteases, aspartic proteases, cysteine proteases, and metalloproteases.

Asparatate proteins correspond to the acidic proteinase group. It is also referred as acid or carboxyl group proteinase, and this is a well-established group of proteolytic enzymes that digests proteins and peptides in acidic solutions. They have been reported in a variety of organisms such as mammals, fungi, plants, and retroviruses, and recently in archaea and bacteria. The presence of two aspartate residues at the active site (Asp32 and Asp215, according to pepsin numbering) results in the shift of optimum pH of the enzyme to the acidic range (Davies 1990). Thermopsin, however, is a dissimilar class of acid protease produced by *S. acidocaldarius* and does not have aspartyl residues in its active site and does not show any sequence homology to other known proteases, with its maximum activity at pH 2.0 and 90°C (Fusek et al. 1990). Acid proteases are generally inhibited by their transition-state inhibitor pepstatin (Marciniszyn et al. 1976) and the active-site-modifying reagents diazoacetyl-DL-norleucine methyl ester (DAN) (Rajagopalan et al. 1966) and 1, 2-epoxy-3- (*p*-nitrophenoxy) propane (EPNP) (Tang 1971). However, certain pepstatin-insensitive proteases are reported from *Xanthomonas* sp., *Pseudomonas* sp. and *Bacillus* spp. (Oda et al. 1987a, b; Prescott et al. 1995). A novel type of protease named kumamolysin produced by *Bacillus* novo sp. MN-32 (Murao et al. 1988) and protease produced by *T. volcanium* with temperature and pH optima at 55°C and 3.0 belong to pepstatin-insensitive protease group. Serine- (PMSF), cysteine- (DTT), metallo- (EDTA) and aspartate- (pepstatin) protease inhibitors did not inhibit the caseinolytic activity of the protease enzyme from *T. volcanium* (Kocabayak and Ozel 2007). Cell associated aspartic protease is also produced by thermoacidophilic *Bacillus* sp. strain Wp 22.A1 with growth optimum at pH 3.0 and 60°C, and it exhibited maximum proteolytic activity at pH 3.5. (Toogood et al. 1995). Collagen is a structural protein of animals and it is a by-product of animal livestock. Proteolytic products of collagen are of medical and industrial application like non-allergic preservatives for medicines and cosmetics (Gaffney et al. 1996; Honda 1998). A thermostable collagenase is reported from *Bacillus* strain NTAP-1, an acidophilic bacterium, with optimum pH for Azocoll hydrolysis at 3.9. It is also not completely inhibited by EDTA (Nakayama et al. 2000). Another monomeric pepstatin-insensitive carboxyl proteinase of molecular mass 37 kDa and active towards collagen was reported from *A. sendaiensis* NTAP-1 (Tsuruoka et al. 2003). Acidic proteases (rennin) are generally used in cheese industry.

Cellulases

Cellulose is the most abundant biopolymer comprising D-glucose units linked in a linear chain by β-1, 4 glucosidic bonds. Cellulose and related compounds are heterogenous in nature containing amorphous and crystalline regions resistant to enzyme hydrolysis. Therefore, the assembly of enzymes is required for the microbial conversion of cellulose to soluble compounds. There are various types of glucanases known like endo-β-glucanases, exoglucanases, glucan glucohydrolases, and β-glucosidases. Endo-β-glucanases randomly hydrolyze the internal glycosidic bonds

to decrease the length of the cellulose chains. Exoglucanase removes cellobiose from either reducing or non-reducing ends. Glucan glucohydrolases act on cello-oligomers while ß-glucosidases act on cellobiose to form glucose. There are reports of cellulases from acidophilic bacteria and archaea. *A. acidocaldarius* produces protein with its activity towards carboxy methylcellulose and oat spelt and active at pH 4.0 and 80°C (Eckert and Schneider 2003). Gene encoding endoglucanase was also reported from *S. solfataricus*, which was found to be functional gene and has shown significant similarity to endoglucanase gene from *Thermotoga maritima*, *T. neopolitana,* and *P. furiosus*. The gene was cloned and sequenced from the library of *S. solfataricus* MT4 (Limauro et al. 2001). Further, three hypothetic endo-β-glucanases SSO1354, SSO1949 and SSO2534 were encoded by thermoacidophilic archeaon *S. solfataricus* P2. Purified recombinant enzyme SSO1949 was a unique extremophilic enzyme with a pH and temperature optima at 1.8 and 80°C and half-life of 8 h at 80°C, pH 1.8 and hydrolyses carboxymethylcellulose as well as cello-oligomers, with cellobiose and cellotriose as main reaction products. The Ser/Thr rich N-terminus explains that these amino acids stabilize the enzyme in acidic conditions. The distinctive combination of temperature and pH stability can be used in the production of bioethanol from cellulose (Huang et al. 2005). Structural analysis and homology modeling has revealed that the catalytic domain of SSO1949 has similarity with other mesophilic, acidophilic, and neutral cellulases.

Blanco et al. (1998) reported cloning of endoglucanase encoding gene Cel A from *Bacillus* sp. BP-23 in *E. coli* with a molecular mass of 44.8 kDa. The recombinant enzyme showed activity on CMC and lichenin but no activity was found on avicel.

Acidic β-glucosidase is produced by *Acidobacterium capsulatum*, an acidophilic, mesophilic and chemoorganotrophic bacterium. The temperature and pH optima of the enzyme are 55°C and 3.0, respectively. The enzyme hydrolyzes aryl-β-glycosides and β-linked disaccharides (Kishimoto et al. 1991).

Xylanolytic enzymes

Xylan is a major component of hemicellulose in the plant cell wall. Xylan is a heteropolysaccharide consisting of a backbone of β-1, 4-linked xylopyranosyl units, half of which are linked to acetyl, methylglucuronyl, or L-arabinofuranosyl residues (Biely 1985). The complete breakdown of xylan requires the combined action of many enzymes (endo-β-1, 4-xylanase, β-D-xylopyranosidase, α-L-arabinofuranosidase, acetyl xylan esterase, and α-D-glucuronidase); the key enzyme is the endo-β-1, 4-xylanase as it cleaves the internal glycosidic bond of the polysaccharide. A majority of microbial xylanases is active at neutral to slightly acidic pH (Collins et al. 2005). Acid stable xylanases are reported from few acidophilic archaea and fungi. Xylanase from *A. capsulatum* was cloned and purified in *E. coli* with its maximum activity and stability in the acidic pH range with MW. 41–40 kDa. Xyn A gene of *A. capsulatum* has two conserved glutamates Glu 167 and Glu 282 with hydrolytic activities towards carboxymethylcellulose and *p*-nitrophenyl-β-D-cellobioside in addition to xylan (Inagaki et al. 1998). *S. solfataricus* strain Oα was also reported to produce xylanase. The enzyme produced by the strain displayed activity on carboxymethylcellulose with optimum temperature and pH of 95°C and 3.5 and half-life of 53 min at 95°C. *S. solfataricus* strain in combination with SSO1354 yields glucose and xylose when acted on cellulose and xylan suggesting its role in saccharification (Maurelli et al. 2008).

Increasing energy demand is the cause of high carbon emissions, which has led to the global climate change. Thus efforts are being made to reduce these carbon emissions, and a significant decrease in carbon emissions could be achieved by using biofuels from lignocellulosic biomass. One of the major drawbacks of biofuel production from lignocellulosics is the unavailability of acidic and thermostable biocatalysts like esterases, cellulases and alcohol dehydrogenases at an affordable cost. The acidic and thermostable enzymes will be good candidates for efficient biofuel production as the present industrial processes for biomass degradation involves low pH and high temperature (Galbe and Zacchi 2007). An appropriate expression system is, therefore, a challenge for the production of thermostable and acid stable proteins, since the co-expression of stress–response machinery from thermoacidophilic archaea can help in the development of novel expression of strains, which are well adapted to the production of such proteins.

DNA ligases and others

DNA ligase form two covalent phosphodiester bonds between 3′ hydroxyl ends of one nucleotide with the 5′ phosphate end of another. ATP is required for the ligase reaction. DNA ligase has applications in both DNA repair and DNA replication. In addition, DNA ligase has broad use in molecular biology laboratories for genetic recombination experiments. A highly acid stable purple colored DNA ligase is known from acidophilic iron oxidizing archaeon *F. acidiphilum* with pH optimum of 1.5–3.0. DNA ligases have been isolated and characterized from five acidophiles, including *F. acidiphilum*, *T. acidophilum*, *P. torridus*, *S. acidocaldarius*, and *Acidithiobacillus ferrooxidans*. Unlike DNA ligase from *F. acidiphilum*, the latter four are metalloenzymes with pH optima in neutral

range and are not active in acidic pH range, and require Mg^{2+} and K^{+} ions for catalysis. LigFa contains two Fe^{3+} tyrosinate centers, which organize the 3D structure of the protein and play significant role in substrate binding (Ferrer et al. 2008).

Intracellular esterase enzyme was detected in the membrane fraction of *F. acidiphilum*, a 308 amino acid long protein of molecular mass 34.7 kDa with temperature and pH optima of 50°C and 2.0–3.5, respectively. It belongs to ester hydrolase family IV of the Arpigny and Jaeger (1999) classification. It is very similar to homologous proteins predicted from the genomes of *F. acidarmanus* (94%), *P. torridus* DSM 9790 (65%), and *T. volcanium* GSS1 (61%) (Golyshina et al. 2006).

Acid phosphatase (APase) was also reported from thermoacidophile *S. acidocaldarius.* The enzyme was partially purified and characterized with optimum pH 5 and 70°C. Apase was a 25 kDa monomeric protein belonging to low molecular mass APase family (Kurosawa et al. 2000). A membrane bound acid pyrophosphatase (PPase) has been reported from a thermoacidophilic archaeon *S. tokodaii* with optimum pH for activity of 2–3. This acid PPase is a dolicholpyrophosphatase that plays a role in glycoprotein biosynthesis (Manabe et al. 2009). This belongs to the phosphatidic acid phosphatase class 2 superfamily that includes both membrane-intrinsic and soluble enzymes with diverse functions ranging from dephosphorylation of undecaprenyl-pyrophosphate and phospho-monoesters such as glucose-6-phosphate to vanadium-containing chloroperoxidation (Manabe et al. 2011).

Maltose-binding proteins (MBP) are primary receptors in bacterial transport and chemotaxis systems. The first crystal structure of the protein was determined from *A. acidocaldarius* (Fig. 2). The thermo- and acid-stability of this family of proteins was determined by comparing the protein structures of the related proteins from *E. coli* (a mesophiles), and two hyperthermophiles (*P. furiosus* and *Thermococcus litoralis*). As compared to the structures of MBP of *E. coli, P. furiosus*, and *T. litoralis*, the thermoacidophilic protein of *A. acidocaldarius* has fewer charged residues (Schafer et al. 2004). The content of acidic and basic residues was equal but more basic residues are exposed on the surface, while acidic residues are buried inside resulting in highly positive surface area. Fewer salt bridges are buried than in other MBP structures, but their exposure on the surface does not seem to be unusual. All these characteristics can be correlated to the acid stability of MBP of *A. acidocaldarius* (Schafer et al. 2004).

The clustered regularly interspaced short palindromic repeats (CRISPR) and their associated protein genes (cas genes) are used as a defense system in archaea and bacteria. The CRISPR has been identified to provide acquired immunity against viruses and plasmids by targeting nucleic acid in a sequence specific manner (Hovarth and Barrangou 2010). The protein SSO2001 is a part of cas gene cluster reported from *S. solftaricus* and it is an endonuclease that specifically digests double stranded oligonucleotides. The enzyme exhibits optimum catalytic activity at pH neutral and 3.0 (Han and Krauss 2009).

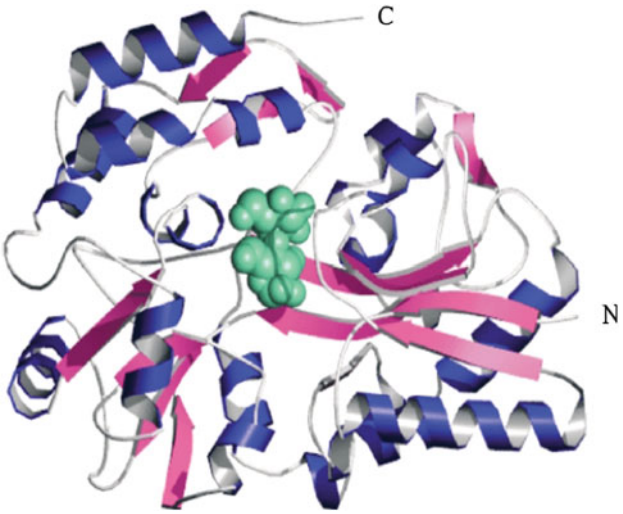

Fig. 2 The overall structure of *Alicyclobacillus* maltose binding protein (AcyMBP) with β-strands (*magenta color*), and α-helices (*blue*). The marked N-terminal domain consists of residues 30–140 and 287–340, while the C-terminal domain consists of residues 141–286 and 341–399. Bound maltose is shown in *green*. Adapted from Schafer et al. (2004)

Iron-oxidizing bacterium *T. ferrooxidans* produces type I copper protein rusticyanin. It is an acid stable protein, and is therefore, different from other type I copper proteins, which are unstable at low pH. This protein has been found to be active in electron transfer reactions with inorganic reagents in the pH range between 1 and 3 (Blake et al. 1991). Various mechanisms have been developed by thermoacidophiles to tolerate heavy metals that are physiologically toxic to microorganisms (Fig. 3). These mechanisms can be enzyme dependent as in case of *S. solfataricus* mercuric reductase that reduces soluble intracellular Hg^{2+} to volatile elemental Hg^{0} although the level of resistance towards mercury was less as compared to bacteria (Schelert et al. 2006). Polyphosphate based mechanism was observed in *S. metallicus* to tolerate high levels of copper. The survival of these microorganisms in sulfidic mineral environments with high toxic metal concentrations may account for their ability to accumulate and hydrolyze polyp. It has been shown that multiple mechanisms operate synchronously to provide cumulative tolerance. For e.g. in *Sulfolobus* spp., efflux of ATPases in addition to poly P pathway is involved in contributing to copper and cadmium tolerance (Romonsellez et al. 2006; Ettema et al. 2006).

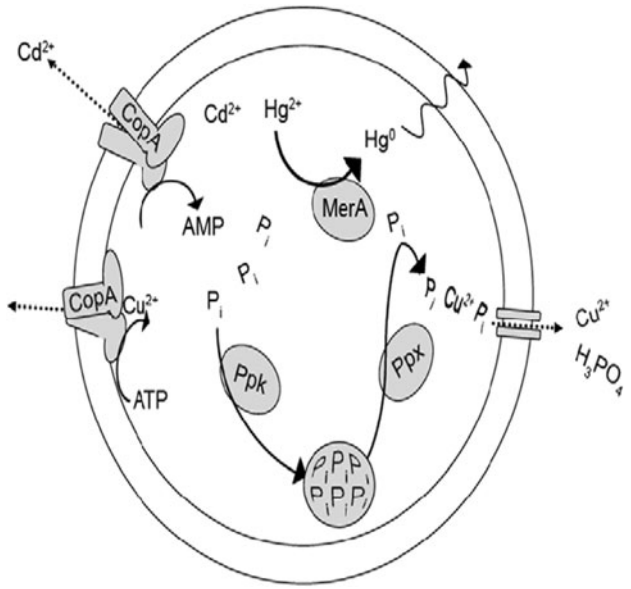

Fig. 3 Metal resistance mechanisms in extreme thermoacidophiles. CopA is the P-type ATPase shown to be involved in copper and cadmium cation efflux in *S. solfataricus* (Ettema et al. 2006). MerA is the mercuric reductase that reduces soluble Hg^{2+} to volatile elemental Hg and is constitutively expressed in *S. solfataricus* (Schelert et al. 2006). Ppk (polyphosphate kinase) and Ppx (exopolyphosphatase) consist of the polyP system explained in *S. metallicus* (Romonsellez et al. 2006). Adapted from Auernik et al. (2008a)

Several plasmids are reported from acidophilic microorganisms. Plasmid pTA1 reported from *T. acidophilum* strains (Yasuda et al. 1995) consist of 18 open reading frames and products of these open reading frame has suggested that this plasmid might have originated from chromosomal DNA. This plasmid can be used for the construction of vectors (Yamashiro et al. 2006). Another plasmid pDL10 reported from *Acidianus ambivalens* is 7,598 bp plasmid with 10 open reading frames organized in five putative operons (Kletzin et al. 1999). *S. islandicus* also has 5,350 bp plasmid pRN1, and it is first plasmid to be sequenced in its group with a high copy number and wide host range. Some part of the plasmids has sequence similarity with helicase domain of viral primase proteins and Cop small protein from promiscuous eubacterial plasmids (Keeling et al. 1996). Another distantly related homologue 6,959 bp pRN2 has been reported from *S. islandicus* with 3 open reading frames (Keeling et al. 1998).

A high potential iron sulphur protein (HiPIP) has been reported from *A. ferrooxidans*. The structural analysis of this protein has shown the presence of two additional cysteines and a high content of proline residues. High proline residues are essential to stabilize the protein folding at low pH. An unusual disulphide bridge is also present that anchors at the N terminal extreme of the protein. It is a redox protein and has high redox potential that is necessary to achieve electron transfer reactions at extreme pH (Nouailler et al. 2006).

Problems in expressing genes encoding proteins of extreme thermoacidophiles

Although the overexpression of thermoacidophilic proteins in mesophilic hosts (*E. coli*) is desirable and simplified, the codon usage and inclusion bodies are the problems encountered in this approach. Some strategies to overcome these problems have been proposed recently. The genes from *S. solfataricus* have rare codons clustering at the 5′ transcript end as compared to *E. coli* that inhibits the target translation (Kim and Lee 2006). The genes from *S. solfataricus* and *T. acidophilum* were expressed in *E. coli*. The level of expression in *E. coli* BL21 (DE3) was much less as compared to those expressed in *E. coli* BL21 (DE3)-codon plus-RIL strain, which contains extra copies of rare codons for arginine, isoleucine, and leucine. The addition of rare codons to tRNAs of *E. coli* strains such as BL21 (DE3)-Codon plus-RIL or Rosetta (DE3) or by changing rare codons by primer design to those more frequently translated by the host has helped in solving over expression problems (Kim and Lee 2006). In another approach, the yields of proteins of *P. torridus* and *S. tokodaii* produced in *E. coli* were increased by growth and expression at elevated temperatures up to 46 °C, suggesting that at higher temperatures protein synthesis is slow, and thus, increasing the rate of proper protein folding (Koma et al. 2006).

Genomics of acidophiles

In order to construe the attractive and unique features of acidophiles, the genomes have been sequenced and analyzed. Only genomes of a few archaea such as *P. torridus* (Futterer et al. 2004), *T. acidophilum* (Ruepp et al. 2000), *T. volcanium* (Kawashima et al. 2000), *S. acidocaldarius* (Chen et al. 2005), *S. solfataricus* (She et al. 2001), *S. tokodaii* (Kawarabayasi et al. 2001), *Methylacidiphilum infernorum* (Hou et al. 2008), and *Metallosphaera sedula* (Auernik et al. 2008a) have been sequenced completely. The characteristics of genomes of acidophilic archaea are presented in Table 3. The analysis of genomes suggested major differences in the DNA replication mechanism, transcriptional apparatus and cell cycle processes in Crenarchaeotes and Euryarchaeotes (She et al. 2001). A number of genes are shared among the species of *Sulfolobus*. A few genes have been found to be exclusively present in *S. acidocaldarius*: the genes for characteristic restriction modification system, UV damage excision repair system, thermopsin and aromatic ring dioxygenase. It lacks

Table 3 Characteristics of genome sequences of the thermoacidophilic bacteria and archaea

Organism	Size (Mbp)	Coding region (%)	G + C content (%)	Total no. of ORFs	ORFs with function	ORF's without function	rRNAs	tRNAs	Other characteristics	References
P. torridus	1.55	91.7	36	1,535	983	553	5 s (1) 16 s (1) 23 s (1)	47	Genes for proton pumping NADH dehydrogenase, A1–A0 type ATPase, genes for acid stable enzymes like proteases and glucoamylases, secondary transporters more as compared to primary, genes for protein like superoxide dimutase, peroxiredoxin like proteins, alkyl hydroperoxide (for protection from oxygen stress)	Futterer et al. (2004)
T. acidophilum	1.56	87	46	1,509	685	446	5 s (1) 16 s (1) 23 s (1)	45	252 ORF's for protein degradation pathway and transport proteins	Ruepp et al. (2000)
T. volcanium	1.58	–	–	–	–	–	–	–	–	Kawashima et al. (2000)
S. solfataricus	2.99			3,032					40% archaeal specific genes, 12% bacterial, 2.3% eukaryotic. Major metabolic carrier is ferrodoxin, large number of extracellular and intracellular proteases, sugar and sulfur metabolizing enzymes	She et al. (2001)
S. tokodaii	2.69	83.9	32.8	2,826	911	145	5 s (1) 16 s (1) 23 s (1)	46	Genes involved in sulfide metabolism, TCA cycle, respiratory chain, and eukaryotic type genes present in genome, which are not identified in other archaea, lack CCA sequence in tRNA genes	Kawarabayasi et al. (2001)
S. acidocaldarius	2.22	–	37	–	–	–	–	–	305 coded genes specific for *S. acidocaldarius*, and 866 specific to *sulfolobus* genus Presence of integrated, and encaptured, pARN type conjugative plasmid, genes for restriction modification system, UV damage excision repair system, thermopsin and an aromatic ring dehydrogenase	Chen et al. (2005)

Table 3 continued

Organism	Size (Mbp)	Coding region (%)	G + C content (%)	Total no. of ORFs	ORFs with function	ORF's without function	rRNAs	tRNAs	Other characteristics	References
Methylacidiphilum infernorum	2.3	91.2	45.5		1,522	–	1 operon for rRNA (23S, 16S and 5S)	46	731 hypothetical proteins, 3 small RNA, 2 riboswitches, 25 CRISPR repeats, ~9 transposons, Possible intergrated plasmid Minf_1152–Minf_1200	Hou et al. (2008)
Metallosphaera sedula	2.2	–	46	–	–	–	–	–	Genes related to autotrophic carbon fixation, metal tolerance and adhesion, one putative rusticyanin and a putative tetrathionate hydrolase for iron and sulfur oxidation, respectively, and presence of fox gene cluster	Auernik et al. (2008a, b)

sugar transporter genes, and therefore, its growth on various carbon sources is limited. Based on the genomic studies of various metal leaching thermoacidophiles, it has been predicted that the presence of fox gene cluster, Rus and TetH genes in the genome appears to distinguish thermoacidophilic bioleachers from non-bioleachers (Auernik et al. 2008b).

Directed evolution

Naturally occurring enzymes are amazing biocatalysts with abundant potential applications in industries and medicine. To be compatible with the specific requirements of the given application, the catalytic properties of the enzyme need to be tailored. Directed evolution mimics Darwinian evolution and has emerged as a powerful tool for engineering enzymes with new or improved functions. Directed evolution can be used to alter various enzyme properties like activity, selectivity, substrate specificity, stability, and solubility (Rubin-Pitel and Zhao 2006). A number of strategies are used for directed evolution like error prone PCR, DNA shuffling, staggered extension process, random priming recombination, heteroduplex recombination, random chimera genesis on transient templates, recombinant extension on truncated templates, incremental truncation for the creation of hybrid enzymes, degenerate oligonucleotide gene shuffling, random drift mutagenesis, sequence saturation mutagenesis and nucleotide excision, and exchange technology (Sen et al. 2007). The gene-targeted mutants of extremely thermoacidophilic archaea are a significant challenge, but some progress has already been made in this area. The mutant of *S. solfataricus* 98/2 termed *S. solfataricus* PBL 2025 lacks about 50 genes including *lacS* (Schelert et al. 2004). The inability of the mutant to grow on lactose based minimal media provides a selectable marker (Albers and Driessen 2007). A deficient mutant of *S. solfataricus* was used to study the function and regulation of α-amylase (Worthington et al. 2003). The gene targeted strategy has been used for developing mercury reductase deficient *S. solfataricus* (Schelert et al. 2004).

In the recent years, starch industry has grown to be the largest market for enzymes after detergent industry. The properties of enzymes acting on starch and α-glucan have been altered by directed evolution as the naturally occurring enzymes from hyperthermophilic bacteria and archaea are not appropriate for the harsh industrial process conditions or have limited shelf lives. Richardson et al. (2002) generated two chimeric α-amylases by DNA shuffling and high throughput screening for improved properties suited for industrial applications. Error prone PCR (epPCR) was used to improve the performance of a maltogenic amylase (Novamyl) in baking (Jones et al. 2008). Site directed mutagenesis and saturated mutagenesis have been employed for altering the pH optimum of enzymes like α-amylase from *B. licheniformis* (Verhaert et al. 2002) and soyabean *β*-amylase (Hirata et al. 2004), but the catalytic rate of the enzymes was affected. In contrast, the directed evolution approach ensures the selection of variants with sufficient activity at the desired pH. The pH of phytase has also been altered so that it could be used as the feed

additive in feed industry (Zhao et al. 2010). High throughput techniques like FACS, chemical complementation, and robot-assisted ELISA assays have been used as novel screening methods for introducing the glycosidic linkages. The enzymes are being evolved for their application in oligosaccharide, glycolipid, and glycoprotein synthesis.

Concerted efforts are being made to reduce carbon emissions in order to mitigate global warming. A substantial decrease in greenhouse gas emissions could be attained by using biofuels from lignocellulosic biomass instead of fossil fuels. Second-generation bioethanol produced from a variety of lignocellulosic materials, such as wood, agricultural or forest residues, has the potential to be an important substitute for gasoline. But the major obstacle in bioethanol production from lignocellulosics is the non-availability of efficient and inexpensive biocatalysts like alcohol dehydrogenases, cellulases, and esterases that are active and stable at high temperatures and low pH values, as the recalcitrant material like soft wood can be hydrolyzed using acid in order to attain high sugars and ethanol yields (Galbe and Zacchi 2007). Considering the dependence on acid and heat pretreatment in breakdown of lignocellulosics, the importance of thermoacidophilic enzymes for efficient biofuel production has been emphasized.

Huang et al. (2005) reported the acid- and heat-tolerant cellulase such as SSO1949 from *S. solfataricus* that could simplify the reaction process and reduce production costs. At present, several glycoside hydrolases with industrial significance have been identified from thermoacidophilic organisms (Serour and Antranikian 2002; Dock et al. 2008), and the speedy improvement of sequencing technologies will further aid the identification of genes encoding novel biocatalysts within newly sequenced genomes.

The European Commission (http://ec.europa.eu/index_en.htm) and the U.S. Department of Energy (http://www.doe.gov) have emphasized on substituting 25–30% of the fuel used in the transportation sector with clean and CO_2 efficient biofuel by 2030 (Hess 2008). The application of metatranscriptomics and metaproteomics to the samples obtained from hot and acidic environments will focus on the metabolic requirements for correct gene expression and protein folding under desirable conditions that resemble the industrial bioconversion of biomass to alcohol.

Conclusions

A number of acid stable enzymes involved in polymer degradation are known from acidophilic bacteria and archaea that are active at acidic pH. The enzymes capable of acting at low pH and high temperature are rare, and therefore, further research is called for finding novel sources for heat- and acid-stable enzymes and understanding the structural properties of acid stable enzymes produced by acidophiles. The understanding of the structural properties of heat and acid stability of enzymes will enable the development of enzymes/proteins with the desired properties in the nearest future.

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