Analyses of *Thermotogales* secretomes

by

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ABBREVATIONS

APS	ammonium persulfate
BSA	bovine serum albumin
CMC	carboxy methylcellulose
Ethanoic acid	acetic acid
Ethanthiol	2-mercaptoethanol
2-hydroxypropanate	lactate
LC-MS	liquid chromatography mass spectrometer
kDa	kiloDalton
Resazurin	7-hydroxy-3H-one-10-oxide
TEMED	N’N’N’N’ tetramethylethen diamine
TGS-buffer	TRIS-Glycine-SDS
TRIS	tris hydroxy aminoethane
PIPES	piperazine-N,N’bis-2-ethane sulfonic acid
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

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1 ABSTRACT

The aim of this study was to analyse the secretomes of the deeply branching order, *Thermotogales* using the species of *Thermosipho africanus*, *Petrotoga mobilis* and *Kosmotoga olearia*, which have all been isolated from hot and deep north-Sea oil reservoirs, as experimental systems. These strains are thermophilic and anaerobic heterotrophs, and were grown in mineral media supplemented with yeast extract and carbohydrate. This is the first study of the exoproteomes in this bacterial order.

Proteins from culture supernatants, which were concentrated by ultrafiltration, were analysed by both SDS-PAGE and proteomics-based methods. A large number (60-90) of extracellular proteins with signal peptides, including solute-binding proteins, various classes of hydrolytic enzymes, as well as previously uncharacterised (hypothetical) proteins, were identified in spent media of *P. mobilis* and *K. olearia*. *T. africanus* culture supernatants contained almost only typical intracellular proteins and almost no proteins with signal peptide, possibly due to extensive leakage of cytoplasmic proteins from the cells or a high degree of cellular lysis during growth. *P. mobilis* grown on maltodextrin as a carbon source, a specific alpha amylase accumulated in the medium, demonstrating a substrate-controlled regulation.

For *K. olearia*, a particular large number (33) of uncharacterised proteins with signal peptides, were identified, a few of which were among the most abundant extracellular proteins. Some of these proteins are shared with other *Thermotogales* representatives, while others are unique to *K. olearia*. This organism is thus a rich source of new extracellular functions. *K. olearia* also secreted 19 different extracellular solute-binding proteins, belonging to carbohydrate- ,oligo/dipeptide-, amino acid-binding families, and 12 different hydrolases, including amylases, peptidases and nucleotidases. This species appears to be an efficient scavenger with a large arsenal of enzymes and proteins for digestion and uptake of various types of nutrients. A smaller number of the same types of the extracellular proteins were identified in the *P. mobilis* secretome.

A large number of proteins without signal peptides were also detected in the exoproteomes. For *T. africanus* and *P. mobilis*, these are possibly due to unspecific leakage or partial cell lysis, but for *K. olearia*, the fraction with extracellular proteins without signal peptides (160) appeared to be more limited and specific. These proteins may be secreted by a hitherto unknown mechanism often referred to as non-classically protein secretion. They are also sometimes termed “moon-lighting” proteins, indicating that they may have dual functions, one known intracellular function and one unknown extracellular function.

Keywords: *Thermotogales*, secretome, signal peptides.

2 INTRODUCTION

The *Thermotogales* order represents a diverse and deep-branching group of mostly thermophilic, Gram-negative and non-sporulating bacteria with capability of growth of a large group of substrates, including carbohydrates and proteinaceous polymers. These organisms thrive in a number of thermal environments like hot springs, hot thermal, hot marine vents and in subsurface environments like oil reservoirs. It is expected that these organisms secrete a number of exoenzymes, which degrade polymeric substances, but so far, a thorough investigation of excreted enzymes from this group of organisms has not been performed. Thermostable, hydrolytic enzymes are of biotechnical interest as robust tool for certain industrial and agroindustrial processes.

Eleven genera of the order *Thermotogales* has been isolated and described so far: *Defluviitoga*, one species, *Fervidobacterium*, six species, *Kosmotoga*, three species, *Marinitoga*, five species, *Mesotoga*, one species, *Mesoaciditoga*, one species, *Oceanotoga*, one species, *Geotoga*, two species, *Petrotoga*, six species, *Thermosipho*, seven species and *Thermotoga*, nine species. Their main characteristics will be described below. (Fig. 2.1).

Bhandari and Gupta (2014), has suggested to divide these organisms into three new orders: *Thermotogales*, *Kosmotogales* and *Petrotogales*. The *Thermotogales* order will then contain *Thermotogaceae* and *Fervidobacteriaceae*, the *Kosmotogae* order of *Kosmotogaceae*, the *Petrotogales* order of *Petrotogaceae*.

The family *Thermotogaceae* will consist of two genera: *Thermotoga* and the *Pseudothermotoga,* which means that the species *Thermotoga maritima*, *T. naphtophila*, *T. neapolitana* and *T. petrophila* will belong to the genus *Thermotoga*. *Pseudothermotoga elfii*, *Pse. hypogea*, *Pse. lettingae*, *Pse. subterrranea*, and *Pse. thermarum* will belong to the genus *Pseudothermotoga* with the same species description as below. (See 2.1.7.).

The family *Fervidobacteriaceae* will contain two genera: *Fervidobacterium* and *Thermosipho* and the family *Kosmotogaceae* will contain two genera *Kosmotoga* and *Mesotoga* with the same species description as below. (See 2.1.2, 2.1.6, 2.1.1 and 2.1.4.)

The family *Petrotogaceae* will contain six genera: *Defluviitoga, Petrotoga, Geotoga, Oceanotoga*, *Petrotoga* and *Marinitoga* with the same species description as below. (See 2.1.1, 2.1.3 and 2.1.5.). (Fig. 2.1).

The parameters given in the tables (Temp, pH and salt), are under optimal growth conditions.

2.1 Description of the different genera of the phylum *Thermotogae*

2.1.1 Description of the genera *Defluviitoga*, *Mesoaciditoga Mesotoga*, *Oceanotoga* and *Geotoga*

One species of the genus *Defluviitoga* has been isolated, *Defluviitoga tunisiensis* (Hania et al. 2012), which is a mesophilic, slightly halophilic, anaerobic, and chemo-organotrophic bacterium. *D. tunisiensis* can ferment lots of carbohydrates. (Table 2.1). Thiosulfate and elemental sulfur are reduced to H_2S. The bacterial cells are rod-shaped, and the end products of glucose fermentation are ethanate, H_2 and CO_2.

One species of the genus *Mesoaciditoga* has been isolated, *Mesoaciditoga lauensis* (Reysenbach et al. 2013), which is a moderately thermophilic, acidophilic, anaerobic and chemo-organotrophic bacterium. *M. lauensis* grows on carbohydrates and proteinaceous substances. (Table 2.1). The bacterial cells are rod-shaped or cocci, motile with multiple flagella and elemental sulfur is reduced to H_2S.

One species of the genus *Mesotoga* has been isolated, *Mesotoga prima* (Nesbø et al. 2012), which is a mesophilic, slightly aerotoleant, fermentative and chemo-organotrophic bacterium. *M. prima* grows on carbohydrates and proteinaceous substances, which is slightly stimulated by thiosulfate, sulfite and elemental sulfur. (Table 2.1). The bacterial cells are ovoid with cocci and pleomorphic forms at all growth stages. The major fermentation product is ethanoic acid.

One species of the genus *Oceanotoga* has been isolated, *Oceanotoga. teriensis* (Himali et al 2011), which is moderately thermophilic, and chemo-organotrophic bacterium. *O. teriensis* can ferment lots of carbohydrates and proteinaceous substances. (Table 2.1). Thiosulfate and elemental sulfur are reduced to H_2S. The bacterial cells are short rods in pairs or chains, motile with multiple flagella. The end products of glucose fermentation are H_2, CO_2 and ethanoic acid.

Two species of the genus *Geotoga* has been isolated, *Geotoga petrea* and *G. subterranea* (Davey et al. 1993), which are moderately thermophilic, obligate anaerobic and heterotrophic bacteria. Elemental sulfur is reduced to H_2S by both species. Grows on some carbohydrates. (Table 2.1). The bacterial cells are rod-shaped, single, in pairs or up to five cells within the sheath.

Table 2.1: Main characteristics of the genera *Defluviitoga*, *Mesotoga*, *Oceanotoga* and *Geotoga*

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
D. tunisiensis	55ºC	6.9	0.5%	Mesothermic and anaerobic whey digester	Yeast extract,arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, ribose, sucrose, xylose, microcrystalline cellulose, xylan, thiosulfate.
M. lauensis	60ºC	5.7	3.0%	Dee-sea hydrothermal vent	Yeast extract, peptone, maltose, sucrose, fructose, glucose, tryptone, starch, xylose.
M. prima	37ºC	7.5	4%	Sediments from Baltimore Harbour, MD, USA	Bacto peptone, tryptone, casamino acids, sucrose, lactose, maltose, mannose, fructose, galactose, ribose, xylose.
O. teriensis	55-58ºC	7.3-7.8	4-4.5%	Offshore oil-producing wells	Glucose, fructose, cello-biose, arabinose, raffinose rhamnose, sucrose,xylose, ribose, starch, ethanol, methanate, ethanate, brain-hearth infusion, yeast extract,bio-trypticase.
G. petrea	50ºC	6.5	3%	Oil-fields	Mannose, lactose, maltose, starch, maltodextrin, glucose, sucrose, galactose.
G. subterranean	45ºC	6.5	4%	Oil-fields	Mannose, lactose, maltose, starch, maltodextrin, glucose, sucrose, galactose, tryptone.

2.1.2 Description of the genus *Fervidobacterium*

Six species of the genus *Fervidobacterium* has been isolated, *Fervidobacterium changbaicum* (Cai et al. 2007), *F. gondwadense,* (Andrews et al. 1996), *F. islandicum* (Huber et al. 1990), *F. nodosum* (Patel et al. 1985), *F. pennavorans* (Friedrich and Antranikian 1996) and *F. riparium* (Podosokorskaya et al. 2011). All species are thermophilic, obligate anaerobic. The bacterial cells are rod-shaped, motile, and with terminal spheroids. The *Fervidobacterium* species are chemo-organotrophic or organotrophic and can grow on different carbohydrates but *F. riparium* also needs proteinaceous substances. (Table 2.2). The main end products of glucose fermentation are ethanate, ethanol and H_2, but *F. islandicum*, *F. nodosum,* and *F. riparium* also produced 2-hydroxy propanate. Elemental sulfur is reduced to H_2S by all species. *F. pennavorans* is able to degrade feathers by the enzyme keratinase.

Table 2.2: Main characteristics of the genus *Fervidobacterium*.

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
F. changbaicum	75-80^{o}C	7.5	0%	Volcanic hot spring	Glucose, lactose, D-fructose, sucrose, maltose, starch, sorbitol, cellobiose, trehalose*2H_2O, D-galactose, meso-erythritol, dulcitol, chrysanthanol, melibiose, pyruvate, glycerol.
F. gondwanense	65-68^{o}C	7.0	0.2%	Non-volcanically heated geothermal waters	Glucose, mannose, maltose, starch, amylopectin cellobiose, carboxymethyl cellulose, lactose, dextrin, fructose, xylose, galactose, pyruvate, casamino acids, gelatin, sorbose, ribose, raffinose, arabinose, dextran, xylan, chitin.
F. islandicum	65^{o}C	7.0	0%	Neutral hot spring	Yeast extract, pyruvate, ribose, glucose, maltose, raffinose, starch, cellulose.
F. nodosum	70^{o}C	7.0	0%	Slightly acidic, neutral and alkaline hot springs	Glucose, raffiose, galactose, mannose, fructose, sorbitol, arabinose, lactose, maltose, sucrose, pectin, glycerol, nitrate broth.
F. pennavorans	70^{o}C	6.5	0%	Hot spring	Starch, glycogen, pullulan, glucose, fructose, maltose, xylose, yeast extract.
F. riparium	65^{o}C	7.8	0%	Hot spring	Peptone, yeast extract, pyruvate, glucose, xylose, fructose, maltose, sucrose, cellobiose, starch, xylan, microcrystalline cellulose, amorphous cellulose, filter paper, birch sawdust.

2.1.3 Description of the genus *Marinitoga*

Five species of the genus *Marinitoga* have been isolated, *Marinitoga camini* (Wery et al. 2001), *M. hydrogenitolerans* (Postec et al. 2005), *M. litoralis* (Postec et al. 2010), *M, okinawensis* (Nunoura et al. 2007), and *M. piezophila* (Alain et al. 2002). All species are thermophilic and anaerobic. The bacterial cells are rod-shaped, and most of the species are motile with polar flagella. *M. hydrogenitolerans* is weakly mobile with only one polar flagellum. The *Marinitoga* species are chemo-organotrophic and can grow on carbohydrates and proteinaceous substances. (Table 2.3). *M. piezophila* also needs 40 MPa pressure for optimal growth. The end products of glucose fermentation for *M. camini* and *M. hydrogenitolerans* are CO_2, H_2 and ethanate. *M. hydrogenitolerans* also produces ethanol and methanate. *M. camini* also produces isopenthanoic acid and isobutanoic acid. Elemental sulfur is reduced to H_2S for all species and *M. hydrogenitolerans* and *M. litoralis* tolerate O_2 concentrations below 4%

Table2.3: Main characteristics of the genus *Marinitoga*.

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
M. camini	55°C	7.0	3.0%	Deep-sea hydrothermal Vent	Yeast extract, gluten,brain-heart infusion,peptone, tryptone, sucrose, glucose, fructose, maltose, cellobiose
M. hydrogenitolerans	60°C	6.0	3.0-4.0%	Black smoker chimney	Glucose, maltose, pyruvate, starch, glycogen, chitin, yeast extract, brain-heart infusion, peptone, casein.
M. litoralis	60°C	6.0	2.6%	Coastal thermal spring	Cellobiose, galactose, glucose, glycogen, lactose, maltose, ribose, starch, brain-heart infusion, casamino acids, casein, peptone, pyruvate, tryptone, yeast extract.
M. okinawensis	55-60°C	5.5-5.8	3.0-3.5%	Deep-sea hydrothermal field	Yeast extract, tryptone, peptone, starch, glucose, glycerol.
M. piezophila	65°C	6.0	3.0%	Deep-sea hydrothermal vent	Yeast extract, casein, casamino acids,peptone, tryptone, starch, D(-)-fructose, D(+)-glucose, D(+)-galactose, maltose, D(+)-cellobiose, D(-)-ribose, ethanate.

2.1.4 Description of the genus *Kosmotoga*

Three species of the genus *Kosmotoga* have been isolated, *Kosmotoga arenicorallina* (Nunoura et al. 2010), *K. olearia* (DiPippo et al. 2009), and *K. shengliensis* (reclassified from *Thermococcoides shengliensis*) (Nunoura et al. 2010, Feng et al. 2010). All species are thermophilic and anaerobic bacteria, but *K. olearia* tolerates O_2 concentrations below 15%. The *Kosmotoga* species are chemo-organotrophic and can grow on carbohydrates and proteinaceous substances. (Table 2.4). The end products of maltose fermentation are CO_2, H_2 and ethanate for *K. olearia*, and growth is enhanced by thiosulfate and sulfate without production of H_2S. The end products of glucose fermentation are ethanate, 2-hydroxy propanate, L-alanine, CO_2 and trace amounts of H_2 for *K. shengliensis*. The bacterial cells are cocci and/or short rods and are non-motile.

Table 2.4: Main characteristics of the genus *Kosmotoga*

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
K. arenicorallina	60ºC	7.1	3.0%	Submarine hot Spring	Yeast extract, glycerol, maltose, xylose.
K. olearia	65ºC	6.8	2.5-3.0%	Oil production fluid	Yeast extract, maltose, ribose, sucrose, starch, casamino acids, tryptone, pyruvate, fructose, galactose, mannose, raffinose, xylan, casein, peptone,
K. shengliensis	65ºC	7.0	1.5%	Oil production fluid	Yeast extract, glucose, ethanate, methanol, galactose, fructose, xylose, sucrose, maltose, sorbitol, lactose, xylan, arabinose, methanate, rhamnose, glycerol, pyruvate, starch, lactate, n-propyl alcohol.

2.1.5 Description of the genus *Petrotoga*

Six species of the genus *Petrotoga* have been isolated, *Petrotga halophila* (Miranda-Tello et al. 2007), *P. mexicana* (Miranda-Tello et al. 2004), *P. miotherna* (Davey et al. 1993), *P. mobilis* (Lien et al. 1998), *P. olearia* (L'Haridon et al. 2002) and *P. sibirica* (L'Haridon et al. 2002). All species are strictly anaerobic, moderately thermophilic and xylanolytic, except for *P. miotherma*. *P. halophila* is moderately halophilic and the bacterial cells are rod-shaped. The *Petrotoga* species are heterotrophic, fermentative bacteria, which can grow on carbohydrates and some proteinaceous substances. (Table 2.5). The end products of glucose fermentation for *P. halophila* and *P. mexicana* are ethanate, 2-hydroxypropanate, L-alanine, H_2 and CO_2. *P. halophila*, *P. miotherma* and *P. mexicana* are non-motile, but *P. mobilis*, *P. olearia* and *P. sibirica* are motile with a subpolar flagellum. Elemental sulfur is reduced to H_2S by all species of *Petrotoga*.

Table 2.5: Main characteristics of the genus *Petrotoga*.

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
P. halophila	60°C	6.7-7.2	4-6%	Offshore oil well	D-arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, rhamnose, ribose, starch, sucrose, xylose, xylan, pyruvate.
P. mexicana	55°C	6.6	3.0%	Oil-producing well	D-arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, raffinose, rhamnose, ribose, starch, sucrose, xylose, xylan, pyruvate.
P. miotherma	55°C	6.5	2.0%	Oil field	Yeast extract, mannose, starch, maltodextrin, glucose, lactose, sucrose, galactose, maltose, xylose.
P. mobilis	58-60°C	6.5-7.0	3.0-4.0%	Oil-production well	Starch, xylan, maltodextrin, maltose, cellobiose, sucrose, lactose, glucose, galactose, fructose, arabinose, ribose, rhamnose, yeast extract.
P. olearia	55°C	7.5	2.0%	Continental petroleum reservoir	Pyruvate, peptone, papaic digest of soybean meal, yeast extract, arabinose, xylose cellobiose, dextrin, sucrose, glucose, fructose, maltose, ribose, trehalose, xylan.
P. sibirica	55°C	7.5	1.0%	Continental petroleum reservoir	Yeast extract, sucrose, glucose, maltose, ribose, trehalose, xylan, pyruvate, peptone, galactose, papaic digest of soybean meal.

2.1.6 Description of the genus *Thermosipho*

Seven species of the genus *Thermosipho* have been isolated, *T. affectus* (Podosokorskaya et al. 2011), *T. africanus* (Huber et al. 1989), *T. atlanticus* (Urios et al. 2004), *T. geolei* (L'Haridon et al. 2001), *T. globiformans* (Kuwabara et al. 2011), *T. japonicus* (Takai et al.2000) and *T. melanisiensis* (Antoine et al. 1997). All species are thermophilic and obligate anaerobic bacteria, except *T. affectus*, which tolerates O_2 concentrations below 4%. The bacterial cells are rod-shaped, may form chains up to twelve cells and are non-motile. *T. globiformans* forms spheroids in early growth phase because of a defect in formation of peptidoglycan. The *Thermosipho* species are chemo-oranotrophic and grow on carbohydrates and proteinaceous substances. (Table 2.6). The end products of glucose fermentation for *T. affectus* and *T. africanus* are H_2, CO_2 and ethanate.

Table 2.6: Main characteristics of the genus *Thermosipho*.

Name	Temp	pH	Salt	Location	Growth substrates
T. affectus	70°C	6.6	2.0%	Mid-Atlantic Ridge hydrothermal vent	Yeast extract, beef extract, glucose, maltose, sucrose, dextrin, starch, microcrystalline cellulose, CMC, cellulose (filter paper).
T. africanus	75°C	7.2	0.11-3.6%	Marine hydrothermal vent	Yeast extract, peptone, tryptone, cysteine, D-glucose, maltose, starch, D-ribose, thiosulfate.
T. atlanticus	65°C	6.0	3.0%	Mid-Atlantic Ridge hydrothermal vent	Brain-heart infusion, starch, galactose, arabinose, glucose, trehalose, cellobiose, gelatin, peptone, yeast extract.
T. geolei	70°C	7.5	2.0-3.0%	Continental petroleum reservoir	Beef extract, peptone, papaic digest of soybean meal, yeast extract, glucose.
T. globiformans	68°C	6.8	2.5%	Hydrothermal vent	Yeast extract, tryptone, starch.
T. japonicas	72°C	7.2-7.6	4.0%	Deep-sea hydrothermal vent	Yeast extract, peptone, tryptone, casein, maltose, glucose, galactose, starch, sucrose, ribose.
T. melanesiensis	70°C	6.5	3.0%	Deep-sea hydrothermal vent	Yeast extract, brain-heart infusion, malt extract, tryptone, sucrose, starch, glucose, maltose, lactose, cellobiose, galactose,

2.1.7 Description of the genus *Thermotoga*

Nine species of the genus *Thermotoga* have been isolated, *T. elfii* (Ravot et al. 1995), *T. hypogea* (Fardeau et al. 1997), *T. lettingae* (Balk et al. 2002), *T. maritima* (Huber et al. 1986), *T. neapolitana* (Jannasch et al. 1988), *T. thermarum* (Windberger et al. 1989), *T. petrophila* (Takahata et al. 2001), *T. naphtophila* (Takahata et al, 2001), and T. *subterranea* (Jeanthon et al. 1995). All species are extremely thermophilic, strictly anaerobic and chemo-organotrophic bacteria. The bacterial cells are rod-shaped, and are motile with subpolar and/or lateral flagella except *T. neapolitana* and *T. subterranea*, which are non-motile. All *Thermotoga* species can grow on various kinds of carbohydrates and proteinaceous substances. (See Table 2.7). The end products of glucose fermentation (and xylose fermentation for *T. hypogea*), are ethanate, CO_2, H_2, and/or 2-hydroxypropanate, except *T. neapolitana*, *T. thermarum* and *T. subterranea*. Thiosulfate and/or elemental sulfur are reduced to H_2S.

Table 2.7: Main characteristics of the genus *Thermotoga*.

Name	**Temp**	**pH**	**Salt**	**Location**	**Growth substrates**
*T. elfii**	66°C	7.5	1.0%	African oil-producing Well	D-glucose, D-arabinose, D-fructose, lactose, maltose, D-mannose, D-ribose, sucrose, thiosulfate, yeast extract, bio-trypticase.
*T. hypogea**	70°C	7.3-7.4	0.2%	Oil-producing well	D-glucose, DL-fructose, D-galactose, DL-lactose, DL-maltose, D-mannose, D-sucrose, D-xylose, xylan, yeast extract, bio-trypticase.
*T. lettingae**	65°C	7.0	1.0%	Thermophilic anaerobic reactor	Yeast extract, peptone, bio-trypticase, gelatin, casamino acids, methanol, 2-hydroxy-propanate, pyruvate, glucose, fructose, galactose, mannose, xylose, lactose, maltose, sucrose, arabinose, ribose, cellobiose, rhamnose, glycerol, pectin, methylamines, starch, xylan, 2-oxoglutarate, serine.
T. maritima	80°C	7.0	2.7%	Geothermally heated sea floors	Glucose, ribose, xylose, galactose, sucrose, maltose, starch, glycogen, yeast extract, whole cell extract of bacteria.
T. neapolitana	80°C	7.0	0.35%	Shallow submarine hot springs	Ribose, xylose, glucose, mal-tose, sucrose, lactose, galactose, starch, glycogen.
*T. thermarum**	70°C	7.0	0.35%	African continental solfataric springs	Yeast extract, starch, glucose, maltose.
T. petrophila	80°C	7.0	1.0%	Kubiki oil reservoir	Yeast extract, peptone, glucose, galactose, fructose, ribose, arabinose, sucroses, lactose, maltose, starch, cellulose.
T. naphtophila	80°C	7.0	1.0%	Kubiki oil reservoir	Yeast extract, peptone, glucose, galactose, fructose, mannitol, ribose, arabinose, sucrose, lactose, maltose, starch.
*T. subterranea**	70°C	7.0	1.2%	Continental oil reservoir	Yeast extract, peptone, tryptone, casein, glucose, maltose.

* *Pseudotoga* has been suggested as a new genus name by Bhandari and Gupta (2014)

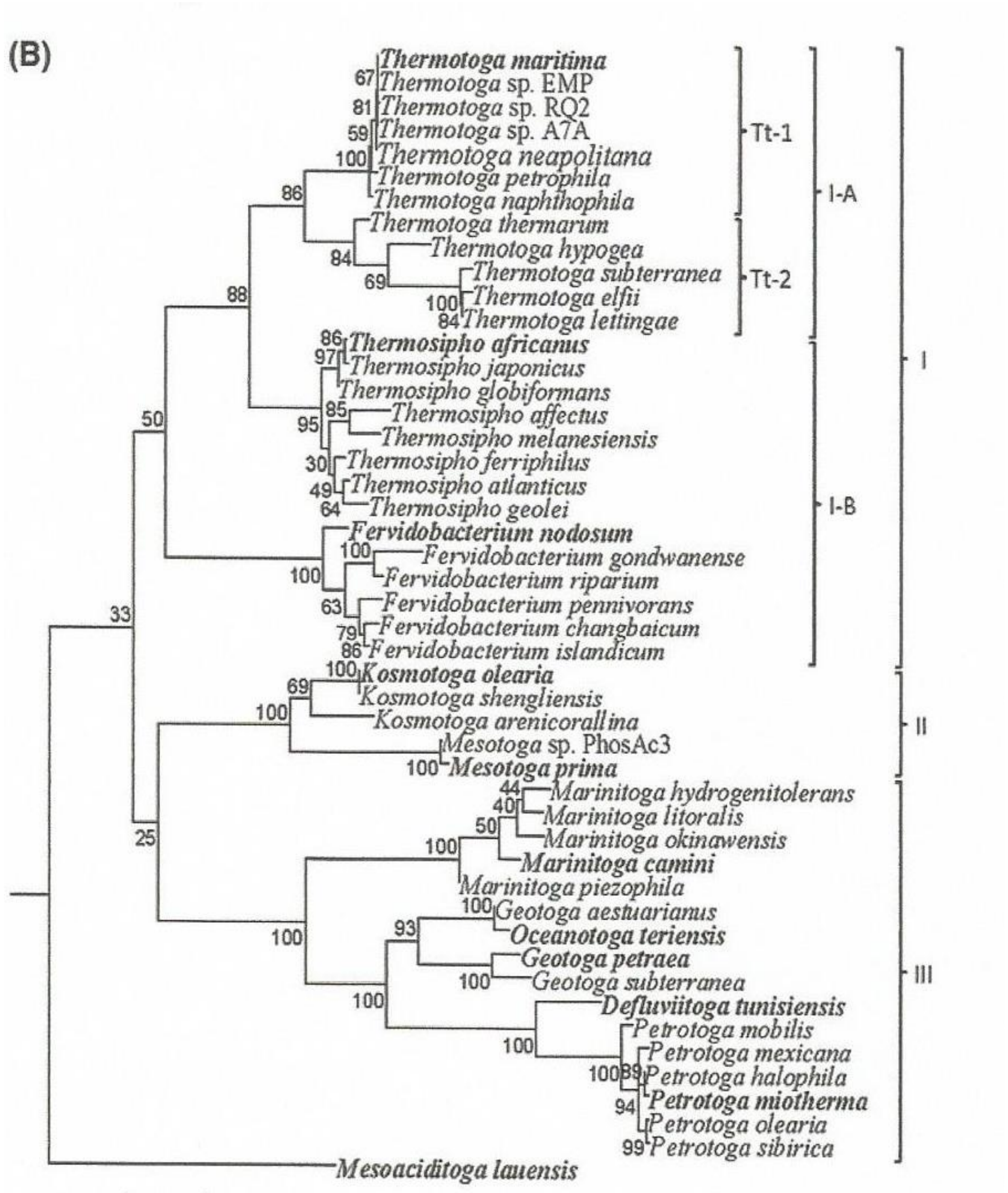

Fig. 2.1: A Neighbour-Joining phylogenetic tree for the eleven *Thermotogae* genera based upon 16S rRNA gene sequences. The species *Coprothermobacter proteolyticus* was used to root the tree, because the bacterium branches close to the *Thermotogae* species in the 16S rRNA gene tree according to Yarza et al.2010). (Bhandari and Gupta 2014).

2.2 How proteins are secreted into the medium

Proteins are secreted into the medium by the general secretory pathway, (GSP), also known as the Sec system. Proteins, which are transported by the Sec partway has an N-terminal sequence, a so-called signal peptide. It has three regions at the N-terminal end: a central hydrophobic region, and a recognition site for peptidases. The basic end has a positive charge at physiological pH and attaches to the membrane phospholipid end at the start of the transportation process. The central hydrophobic region inserts itself into the cytoplasmic membrane, which then can transport the main peptide. The recognition site on the peptide is remowed at the membrane during or after transport by a signal peptidase. (Fig. 2.2 and 2.3)

Proteins must remain unfolded when transported through the general secretory pathway, (GSP). This pathway can be divided into two other pathways: A signal recognition pathway, (SRP) and a Sec B pathway, dependent on how the peptides are kept unfolded during transport. A molecular chaperone, Sec B, is bound to the peptides, in the Sec pathway, and targeted to the cytoplasmic membrane for transportation through a protein-conducting channel, called the Sec Y complex. The N-terminal hydrophobic signal peptide is bound by the SRP, in the SRP pathway, at the start of translation. The SRP-bound translation machinery is targeted to the Sec Y complex for transportation, aided by the membrane-bound receptor, FtsY. The energy needed for transportation is provided by Sec A, an ATPase, which hydrolyses ATP. The N-terminal signal sequence is cleaved by the signal peptidase during the transportation process. (Fig. 2.2 and 2.3).

The outer membrane proteins are excreted through the Sec B pathway and the cytoplasmic membrane proteins are inserted into the membrane by the SRP pathway in Gram-negative bacteria. Other proteins, including periplasmic chaperones are also involved in the GSP pathway. (Kim and Gadd 2008).

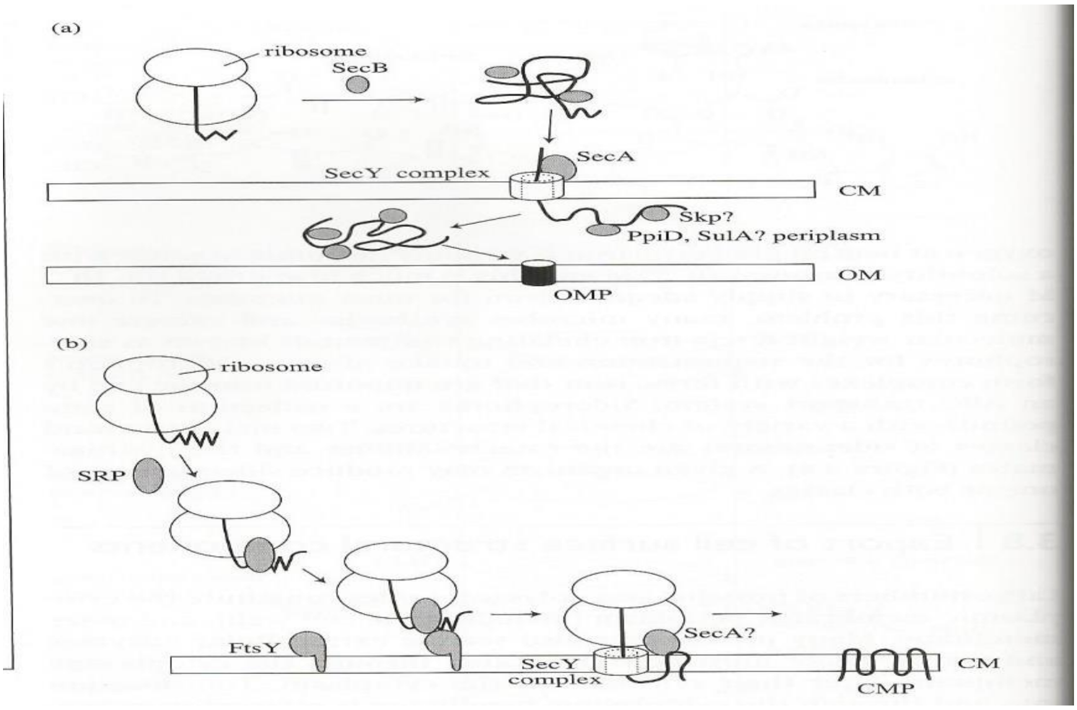

Fig. 2.2: Overview of the general secretory pathway. (Kim and Gadd 2008). See text above for details.

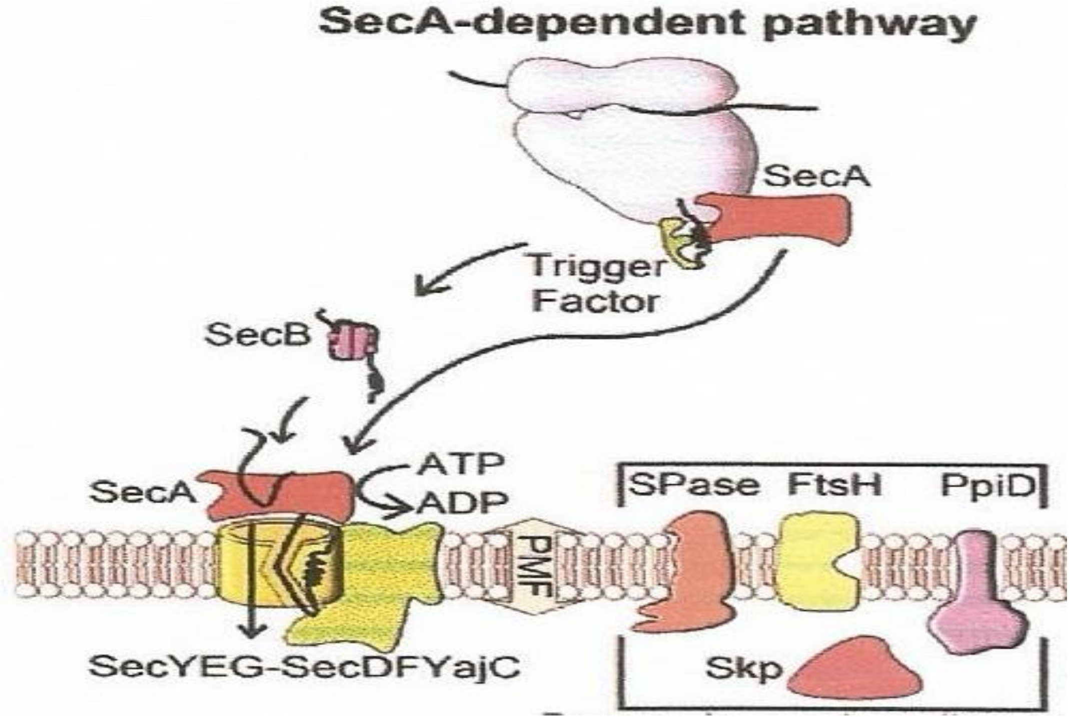

Fig. 2.3: Denks et al. (2014), has performed a detailed study on the SecA dependent pathway. This pathway is used by periplasmic proteins and other membrane proteins, which also contains a signal peptide. Chaperones like trigger factors and SecB keep the nascent protein in an unfolded state during its journey to the membrane. The precursor protein is then transferred to SecA, which is translocates through the SecYEG channel by hydrolysis of ATP. (This figure has been modified).

2.3 Gene transfer in the order *Thermatogales*

The order *Thermatogales* belongs to the second phylum of the domain Bacteria, and consists currently (2013) of eleven different genera. They are a quite diverse group of bacteria, ranging from mesophilic, moderately thermophilic and hyperthermophilic genera.

Four studies have been performed to examine if some of the species in the order *Thermotogales* has undergone lateral gene transfer from *Archaea* or other *Bacteria*:

Nelson et al. (1999) performed a study on the genome of *Thermotoga maritima*. The whole genome was sequenced and shows that *T. maritima* can use many different carbohydrates, both sugars and polysaccharides. The genome is 1,860,725 bp long, and consists of 1,877 predicted coding regions, 1,014 genes have known function and 863 genes have unknown function. 108 genes have orthologues in the genomes of other thermophilic bacteria and *Archaea*. The *T. maritima* genome has totally 1877 genes. 81 of the genes (24% of all genes) are similar to *Archaea*, and clustered in 15 regions of the genome. The size of these genes range from 4 kbp to 20 kbp. Nelson et al. (1999) concludes that lateral gene transfer may have occurred between *Thermotogales* and *Archaea*.

Nesbø et al. (2009) performed a study on the genome of *Thermosipho africanus*. The whole genome was sequenced and is 2,016,657 bp long. The genome has 2000 potential coding regions, 1913 genes are protein-coding open reading frames (ORFs), 30 genes are considered to be pseudogenes, and 57 genes encode RNA. The *T. africanus* genome is 155932 bp longer than the *T. maritima* genome, and has 36 more ORFs (1,913 compared to 1,877). 3.7% of the *T. africanus* ORF's are phylogenetically, closely related to *Archaea* and 8% of the ORF's are phylogenetically closely related to *Firmicutes*. Nesbø et al. (2009) concludes that lateral gene transfer may have occurred among *Thermotogales*, *Archaea* and *Firmicutes*

Zhaxybayeva et al. (2009) performed an extended study on the *Thermotogales* genomes, where the genomes of *Thermotoga petrophila*, *Thermotoga lettingae*, *Thermosipho melanesiensis* and *Fervidobacterium nodosum* were included together with the *Thermotoga maritima* genome.Ribosomal protein genes may place *Thermotogales* as a sister group to *Aquificales*. Most of the genes with sufficient phylogenetic signal maybe related to *Archaea* and *Firmicutes* also, especially the genus *Clostridia* (Zhaxybayeva et al. (2009) concludes that the order *Thermotogales* may belong to the phylum *Firmicutes* rather than the phylum *Thermotogae*.

Zhaxybayeva et al. (2012) performed a study on the *Mesotoga prima* genome. The whole genome was sequenced and is 2,974,299 bp long, and a plasmid, which is 1,724 bp long. The chromosome has 2,736 genes and 2,660 encodes proteins, while the rest are pseudogenes. A taxonomic distribution has revealed that 766 genes have been involved in lateral gene transfer with several bacterial groups, gene numbers in brackets: *Firmicutes,* (353), *Proteobacteria*, (121)*, Spirochaetes,* (53)*, Chloroflexi,* (34)*, Synergistetes,* (30), *Bacteroidetes,* (27), *Haloplasmatales,* (22), *Archaea*, (60), *Eukaryota*, (2), and 64 genes from 11 other groups. Zhaxybayeva et al. (2012) concludes that *M. prima* is related to *T. lettingae* because both species are often isolated from mesothermic bioreactors and fermenters.

2.4 Carbohydrate degradation in the genus *Thermotoga*

Three studies have been performed to examine how the different species in the genus *Thermotoga* degrade and ferment different carbohydrates. Chhabra et al. (2002) performed a study on how glycosyl hydrolases are regulated in *T. maritima*. *T. maritima* has six cellulases, four xylanases, four mannases, one laminarinase, six amylases, one pullulanase, five galactosidases and eleven other glycosyl hydrolases, which means it has the largest number of glycosyl hydrolases compared to archaea like *Pyrococcus furiosus*, *Pyrococcus horiskoshii*, *Aquifex aeolicus* and *Methanocaldococcus jannaschii*. To find out how the different glycosyl hydrolases are regulated, dependent on the carbohydrates added to the growth media, *T. maritima* was grown on a variety of carbohydrates like barley β-glucan, carboxymethyl cellulose (CMC), carob galactomannan, konjac glucomannan and potato starch. *T. maritima* grew on all carbohydrates with cell densities up to10^9 cells/mL. Chhabra et al. (2002) concluded that certain genes were expressed on all carbohydrates in the growth media, and other genes were expressed only when specific carbohydrates were present in the growth media.

Chhabra et al. (2003) continued the study from last year (2002), using cDNA microarray and analyses of mixed models to find out how the different carbohydrates were used by *T. maritima*. The genes, which responded to the different carbohydrates, were coordinately regulated. All glycoside hydrolases were down-regulated when glucose was added to the growth medium, but the genes were up-regulated or down-regulated dependent on which carbohydrate other than glucose added to the medium. Chhabra et al. (2003) concluded that *T. maritima* may adapt to different environments because it can grow on multiple carbohydrates.

Frock et al. (2012) performed a similar study where four *Thermotoga* species were included, *T. maritima*, *T. neapolitana*, *T. petrophila* and *Termotoga* sp. strain RQ2. They have 1,470 open reading frames (ORFs), i.e. 75% of their genomes in common. To examine how the different *Thermotoga* species use carbohydrates, each of them were grown in media with a mixture of glucose, xylose, arabinose fructose, mannose and galactose added. *T. maritima* and *T. neapolitana* preferred glucose and xylose over fructose and arabinose. *Thermotoga* sp. strain RQ2 also used glucose and xylose, but could also use fructose. *T. petrophila* used glucose less than the other species, because it lacked a glucose transporter, and used a xylose transporter for glucose transport into the cells. Frock et al. (2012) concluded that because the *Thermotoga* species use different carbohydrates, it is right to consider them to be separate species.

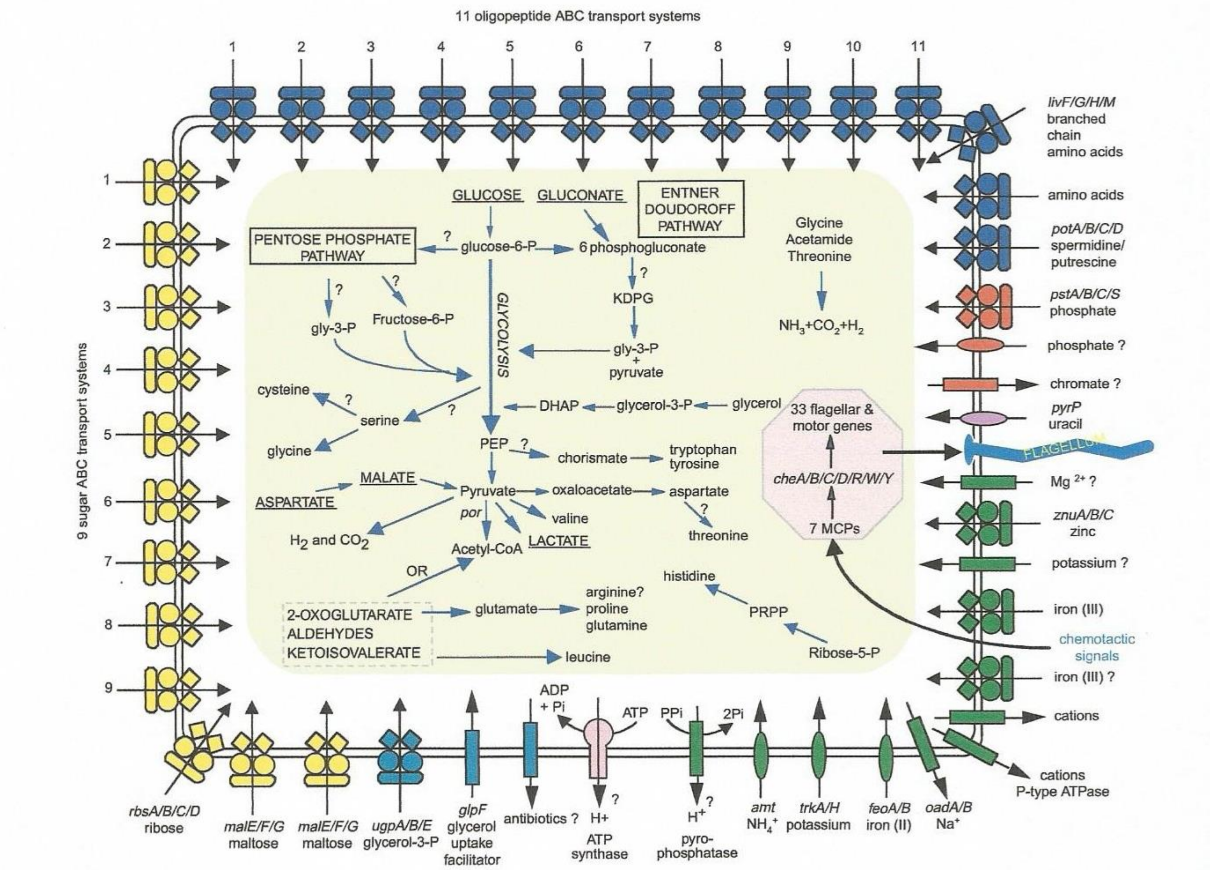

Fig. 2.4: An overview of the different metabolic pathways in *Thermotoga maritima.*Several pathways are used for metabolism of organic compounds, acids and aldehydes. The different transporters are coloured according to role category: Cations (green), anions (red), carbohydrates (yellow), amino acids, peptides, amines (dark blue), and other (light blue). Several pathways for glucose metabolism are used like glycolysis, Entner-Doudoroff pathway, and pentose-phosphate pathway. (Nelson et al. 1999).

2.5 Aim of the study

The main goal of this study was to identify extracellular proteins secreted from three members of the *Thermotogales* order, *Thermosipho africanus*, *Petrotoga mobilis* and *Kosmotoga olearia* based on proteomics analysis of culture supernatans.

Subgoals:

1. Production of active cultures on different substrates.
2. Concentration of proteins from culture supernatants using ammonium sulfate precipitation and ultrafiltration.
3. Analysis of the concentrated extracellular protein fraction supernatants using SDS-PAGE.
4. Identification of protein bands and total extracellular proteins using LC-MS.
5. Better understanding of the diversity of the secreted proteins in Thermotogales.

3 MATERIALS AND METHODS

3.1 Materials

3.1.1 Bacterial strains

DSM 21960	*Kosmotoga olearia*
DSM 10674	*Petrotoga mobilis*
DSM 13872	*Thermosipho africanus*
TCEL 2	*Thermotoga maritime*

3.1.2 Chemicals

Chemicals	**Supplier**
4-aminobenzosyre	Sigma-Aldrich
Ammonia chloride	Merck
APS	Sigma-Aldrich
Ammonium sufhate	Merck
D (+) Biotin	Sigma-Aldrich
Boric acid	Merck
Bovine serum albumin	Sigma-Aldrich
Butanol	Merck
Calcium chloride * $2H_2O$	Merck
Calcium di hydrogen phosphate	Merck
Carbon dioxide	Yara
Calcium-D (+) pantothenate	Sigma-Aldrich
Cobalt chloride * $6H_2O$	Merck
Coomassie Brilliant Blue G-250	BIO-RAD
Cupper (II) sulfate * $5H_2O$	Merck
Dextrin 20 (maltodextrin)	Fluka
Di sodium molybdate * $2H_2O$	Kebo Lab
Di potassium hydrogen phosphate	Sigma-Aldrich
Di sodium sulfide * $9H_2O$	Merck
Ethanol	Sigma-Aldrich
Ethanoic acid	Sigma-Aldrich
Ethanthiol	BIO-RAD
Glucose	Fluka/Sigma-Aldrich
Glycine	Sigma-Aldrich
Hydrochloric acid	Sigma-Aldrich
Iron (II) di ammonium sulfate * $7H_2O$	Merck
Iron (II) chloride * $4H_2O$	Fischer
Iron (II) sulfate * $7H_2O$	Sigma-Aldrich
Maltose	Sigma-Aldrich
Manganese chloride * $4H_2O$	Merck
Manganese sulfate * H_2O	Merck
Magnesium chloride ‘ $6H_2O$	Sigma-Aldrich
Magnesium sulfate * $7H_2O$	Fluka
Nickel chloride * 6 H_2O	Merck

Chemicals	Supplier
Nitriloethanoic acid	Sigma-Aldrich
Nicotine acid	Merck
Nitrogen	Yara
PIPES	Sigma Aldrich
Polyacrylamide bis acrylamide	BIO-RAD
Potassium aluminium sulfate *12H_2O	Merck
Potassium chloride	Sigma-Aldrich
Pyridoxamine * 2HCl	Sigma-Aldrich
Resazurin	Sigma-Aldrich
SDS	Sigma-Aldrich
Sodium chloride	Sigma-Aldrich
Sodium hydrogen carbonate	Merck
Sodium dithionite	Merck
Sodium thiosulfate * 5H_2O	Merck
TEMED	BIO-RAD
Thiamine dichloride	Sigma-Aldrich
TRIS	Fluka
Tryptone	Fluka
Xylan	Roth
Zinc sulfate * 7H_2O	Merck
Yeast extract	Merck

3.1.3 Instruments

Instruments	Description	Supplier
Biofuge 13	Centrifuge	Heraeus Sepatech
Centrifuge 5430R	Centrifuge	Eppendorf
Rotor 135-6-30	8 (50 mL) Falcon tubes and 4 (15 mL) Falcon tubes	Eppendorf
Cary 100 Conc	UV-VIS Spectrophotometer	Varian
UV Mini 1240	UV-VIS Spectrophotometer	Shimadzu

3.1.4 SDS-PAGE and Columns

Name	Supplier
Amersham ECL Gel 4-12%, 10 wells	GE Healthcare Amersham Life Sciences
Amersham ECL Gel 12%, 10 wellls	GE Healthcare Amersham Life Sciences
Amersham ECL Gel Running Buffer 10X	GE Healthcare Amersham Life Sciences
Amersham ECL Gel Box	GE Healthcare Amersham Life Sciences
Amicon Ultra -15 Centrifugal Filter Devices	Millipore
Bio-Rad Protein Assay	BIO-RAD
Full Range Rainbow Recombinant Protein Molecular Marker	GE Healthcare Amersham Life Sciences
PD-10 Columns Sephadex G-25M	Ge Healthcare Amersham Life Sciences
SDS-PAGE Standards Broad Range	BIO-RAD

3.2 Methods

3.2.1 Preparation of cultures with medium 718 and 1163.

Two different culture media were prepared according to protocol provided from DSMZ (www.dsmz.de), no.718 Petrotoga medium was used for cultivation of *Petrotoga mobilis* and *Thermosipho africanus*, no.1163 KTM medium was used for cultivation of *Kosmotoga olearia* (See Appendix 7.1.1 and 7.1.3). SL-10 Trace element solution (Widdel et al. 1983) and vitamin solution (Widdel &Pfenning 1981), were also prepared. SL-10 was used only for the first culture preparation. Trace element solution for no.141 Methanogenium medium (Appendix 7.1.4), was used for the next medium preparations.

The different chemicals were put into a 2 L Erlenmeyer flask, 1L MilliQ water was added, 1 mL Resazurin was pipetted into the Erlenmeyer flask, and a rubber stopper was screwed on the top. Three glass tubes was inserted into the stopper, one long, one short, and a small tube with screw cap. The long tube was also connected to a rubber tube with a Pasteur pipet. The short one just penetrates the stopper. Before autoclaving, alumina foil was folded around on the ends of both tubes to prevent contamination, and the screw cap on top is opened.

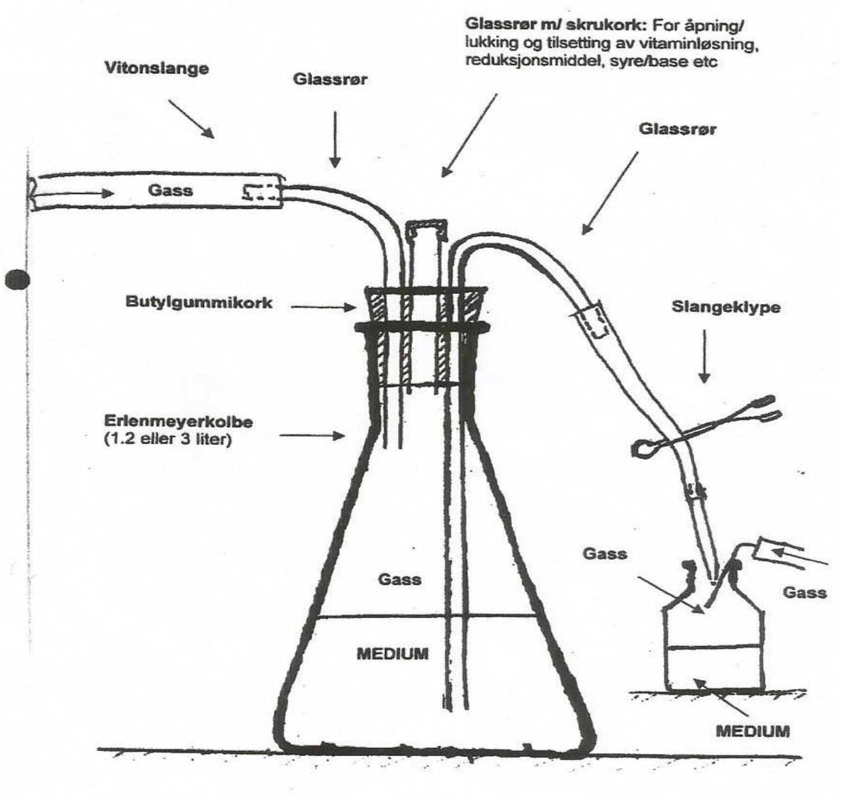

Fig. 3.1. A 2 L Erlenmeyer flask with a serum bottle using the Hungate Techniqe. (See above text, 3.2.1. for explanation).(MIK200, laboratory course).

The medium was autoclaved for 1,5 hours, the Erlenmeyer flask was put on ice, and placed on a magnetic stirrer, gas was let into the small tube (head space 80% N_2, 20% CO_2 for no. 718, *Petrotoga* medium, and 100% N_2 for no.1163, KTM medium). 12 mL 1M $NaHCO_3$ and 4 mL 0.5M Na_2S*9H_2O were added after removal of the ice and cooling of no.718 Petrotoga medium, to room temperature. (The screw cap was closed).

Sterile serum bottles had been prepared beforehand, and each serum bottle was flushed with N_2 gas before it was filled with medium. When it was 4/5 full, a sterile Balch stopper was put on top of the bottle, while the gas needle was removed, and an aluminium ring crimped on.

No. 718, Petrotoga medium is a basal medium for *Petrotoga mobilis*, *Petrotoga miotherma*, *Petrotoga mexicana*, *Petrotoga halophila* and *Thermosipho africanus*. No. 1163, KTM medium is a basal medium for *Kosmotoga olearia* and *Oceanotoga teriensis*. Each bacterial strain need special substrates, which are added to the medium before inoculation. (Appendix 7.1 and 7.2).

3.2.2 Cultivation of *Petrotoga mobilis, Thermosipho africanus, Kosmotoga olearia* and *Thermotoga maritima* TCEL 2.

Stock cultures were first cultivated in 50 mL serum bottles, and 0.8 mL 10% yeast extract, 0.8 mL vitamin solution were added to all bacterial cultures. *T. africanus* needed 0.4 mL 50% glucose, 0.8 mL tryptone and 0.8 mL 1M $Na_2S_2O_3$ as additional substrates. *P. mobilis* needed 0.4 mL 50% glucose as an additional substrate. *K. olearia* needed 2 mL maltose as an additional substrate.

New cultures were cultivated from stock cultures in 100 mL serum bottles, and 1.6 mL 10% yeast extract, 1.6 mL vitamin solution, were added to all bacterial cultures. *T. africanus* needed 0.8 mL 50% glucose, 1.6 mL 10% tryptone and 1.6 mL 1M $Na_2S_2O_3$ as an additional substrates. *P.mobilis* needed 0.8 mL 50% glucose as an additional substrate. *K. olearia* needed 4 mL 10% maltose as an additional substrate. 1 ml of each stock culture was inoculated to all cultures before incubation on 70°C, 55°C and 60°C respectively.

Petrotoga mobilis has also been cultivated on 10% maltodextrin and 10% xylan, respectively and *Thermotoga maritima* TCEL2 was cultivated on medium no.1163 with 1.25% Avicel cellulose to find out if these bacterial strains can grow on other carbohydrates than 50% glucose.

The cultures were harvested, and each of them was transferred into two 50 mL Falcon tubes and centrifuged at 7197 rct for 30 min at 4°C in an Eppendorf centrifuge 5430R with rotor 135-6-30. One supernatant from each culture was frozen and the other one was kept on 4°C until further use.

3.2.3 Ammonium sulfate precipitation

A saturated $(NH_4)_2SO_4$- solution was prepared. 22 g $(NH_4)_2SO_4$ was added to 50 mL chilled supernatant, (3.2.2), in a 50 mL Falcon tube, transferred to a 100 mL Erlenmeyer flask and kept on shaking overnight at 4°C. The precipitated supernatants were centrifuged at 3000 rct for 5 min at 4°C, transferred into new 50 mL Falcon tubes and kept at 4°C.100 mL 100 mM Tris-HCl buffer, pH 7.0 was prepared, and sterile filtered (0.45 µm) into a 100 mL sterile bottle. Two mL Tris-HCl buffer was added to each 50 mL Falcon tube to dissolve the salt. The Falcon tubes were set aside in the refrigerator overnight. The precipitate was dissolved as the Falcon tubes became room-tempered. A PD-10 desalting column was used for desalting, and the desalted solutions were transferred to Eppendorf tubes, and stored at 4°C overnight.

3.2.4 Ultrafiltration

The desalted solutions were centrifuged at 5000 rct for 10 min at 4°C in Ultra-15 centrifugal devices from Millipore, with a cut-off value of 10.000 kDa. The concentrated solutions were transferred to Eppendorf tubes. The frozen supernatants (3.2.2), were thawed on ice, and centrifuged at 5000 rct at 4°C in Ultra-15 centrifugal devices until the supernatants became concentrated, and then transferred to Eppendorf tubes, and frozen.

3.2.5. Bradford assay

To determine the amount of proteins in the concentrated supernatans, a Microassay procedure from Bio-Rad was performed and the absorbance was measured on a spectrophotometer, (Cory 100 from Varian) at 595 nm. A standard curve was constructed based on the concentrations of the BSA standards. (See Fig. 3.1).

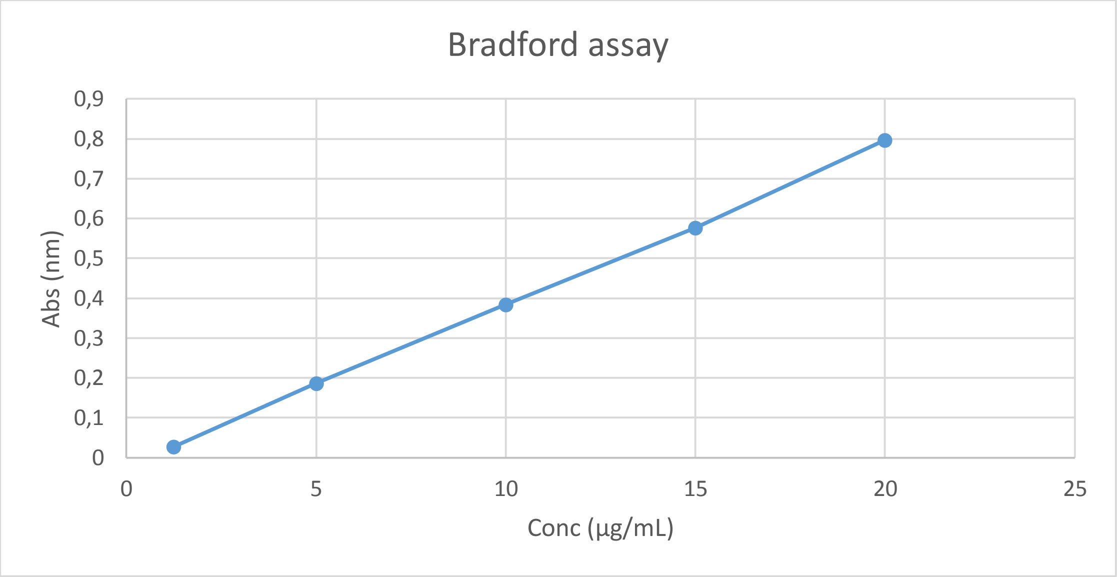

Fig: 3.1 Standard curve for the Bradford protein assay.

3.2.6. Visualisation of proteins by SDS-PAGE, protocols and preparations

SDS-PAGE protocols

12% Running gel	**4% Stacking gel**
3.35 mL MQ H_2O	6.1 mL MQ H_2O
2.5 mL 1.5 M Tris HCl pH 8.8	2.5 mL 1.5 mL Tris HCl pH 6.8
0.1 mL 10% SDS	0.1 mL 10% SDS
4.0 mL 30% acrylamide	1.3 mL acrylamide
0.05 mL APS	0.05 mL APS
0.005 mL TEMED	0.01 mL TEMED

10 mL final volume for both gels.

10X TGS-buffer

0.25 M, 30.285 g Tris

1.92 M, 144,192 g glycine

1%, 10 g SDS

1 L MQ H_2O

Staining solution	**Destaining solution**
10% ethanoic acid	10% ethanoic acid
40% ethanol	20% ethanol
0.1% Coomassie Brilliant Blue R-250	in 700 mL MQ H_2O

Preparation of the SDS-PAGE

Two glass slides were prepared by washing with ethanol and assembled. Both gels were prepared according to the protocols mentioned above. The running gel was first prepared and pipetted between the glass slides with a Pasteur pipet. A small volume of butanol was pipetted on the top to give a smooth surface, and the gel was left to polymerise for one hour. The butanol layer was then rinsed off with distilled water, and a comb was put on top. The stacking gel was then prepared and pipetted on the top with a Pasteur pipet. More stacking gel was pipetted on both sides of the comb to avoid drying of the gel, which was left to polymerise for another hour.

The samples were prepared like this: 1 µL Broad Range Standard, 15 µL sample buffer and 1 µL ethanethiol was added to an Eppendorf tube. 2µL pellet, 18 µL sample buffer and 1 µL ethanethiol was added to Eppendorf tubes. 10 µL concentrated supernatant, 10 µL sample buffer and 1 µL ethanethiol was added to Eppendorf tubes. The samples were boiled at 95°C for 5 min to let the proteins denature.

The gel was then transferred to the electrophoresis chamber and 1X TGS-buffer was filled into the chamber. The newly prepared samples was pipetted into the wells of the gel and the gel was run for 45 min at 190 V. After the run was completed, the gel was put into a box filled with Coomassie staining solution and stained for one hour, and the gel was then put into another box filled with destaining solution and destained for one hour. The gel was stored in a box filled with distilled water overnight, and photographed with the Gel Doc system.

ECL gels from Amersham GE Healthcare was also used. They were already prepared in a gel cassette ready for use, and run under the same conditions as the manually prepared gels, but the voltage was set to 160 V instead of 190 V. Each gel was run in a gel box, and the samples were pipetted into the wells in the same manner as for agarose gels. Both gradient gels 4-12% and homogenous gels 12% were used, but the homogenous gels gave the best results.

The concentrated supernatants (50µL), and excised gel bands were sent to the Proteomic Unit of the University of Bergen, Department of Biomedicine (PROBE) laboratory for proteomics analysis. LTQ Orbitrap MS, (mass spectrometer) was used for the analysis, and Peptide Shaker, a search tool, was used for visualisation of the proteins and peptides. (Olav Mjåvatten, personal communication). The accession numbers for each protein in the UniProt database were found in Peptide Shaker.

The MS2Quant value is defined as a spectrum counting quantification index. (http://genesis.ugent.be/files/costore/practicals/bioinformatics-for-proteomics/4-Peptide).

4 RESULTS

4.1 SDS-PAGE identification of protein bands

Supernatants of cultured *T.africanus, P. mobilis* and *K. olearia* were both ammonium sulfate precipitated and ultrafiltered (Materials and Methods, 3.2).

Table 4.1: Protein concentrations in concentrated supernatants, (µg/mL)

Ta-P	*Pm*-P	*Ta*-F	*Pm*-F	*Ko*-F
70.76	11.40	207.39	173.50	271.50

These results, (Table 4.1), were used to calculate the amount of sample, to be pipetted into the wells of an SDS-PAGE gel, which was run to visualise the protein bands, (Fig. 4.1).

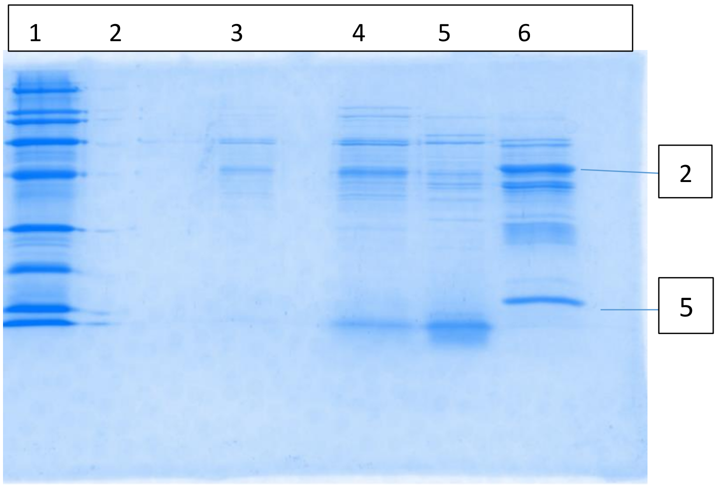

Fig: 4.1: SDS-PAGE analysis of extracellular proteins: (Gel no. 1): Lane 1: MWS, Broad Range, (Bio-Rad). Lane 2: *Ta*-P. Lane 3: *Pm*-P. Lane 4: *Ta*-F. Lane 5: *Pm*-F. Lane 6: *Ko*-F. Two bands in lane 6 are labelled 2 and 5 respectively. The initials P and F, means ammonium sulfate precipitated and ultrafiltered.

The protein bands from ammonium sulftate precipitated cultures were very weak, but the protein bands from ultrafiltered supernatants were strong. The protein bands from *Ko*-F had another band patterns compared to those from *Ta*-F and *Pm*-F. Two of the protein bands, *Ko*-2 and *Ko*-5 have been subjected to proteomics analyses.

Sample 1, *Kosmotoga olearia*, band 2

854 proteins were detected in the band and 94 of them were confident. The MS2 Quant value varied from 0.04 to 0.00. Most of the proteins had 54% confidence except for the last ones, no. 89 to 94, which had 51% confidence. Proteins with signal peptides are listed below. See Appendix (Table 7.1), for proteins without signal peptides.

Table 4.2: Protein identification of *K. olearia*, band 2.

Accession	Description	Coverage	Peptides
C5CEX4	Uncharacterised protein	45.74%	169
C5CDP1	Alkaline phosphatase	58.82%	44
C5CHI91	Extracellular solute-binding protein	68.46%	43
C5CIG7	Extracellular solute-binding protein	75.77%	42
C5CGM6	5'-nucleotidase domain protein	41.85%	33
C5CER0	Extracellular solute-binding protein	36.60%	25
C5CEG1	Extracellular solute-binding protein	34.78%	23
C5CF24	Protease Do	43.94%	23
C5CEB0	Glycoside hydrolase	17.80%	21
C5CDX4	Von Willebrand factor, type A	27.40%	21
C5CHP8	Extracellular solute-binding protein	34.33%	20
C5CIC7	Nucleic acid-binding OB fold tRNA/helicase type	39.88%	20
C5CDU3	Carboxyl-terminal protease	43.24%	17
C5CIJ8	PHP domain protein	23.15%	17
C5CI22	Uncharacterised protein	33.71%	16
C5CIK8	Extracellular solute-binding protein	42.13%	15
C5CG42	Peptidase S41	21.78%	15
C5CE13	Extracellular solute-binding protein	20.64%	13
C5CI23	Peptidase C1A papain	29.71%	12
C5CEF1	S-layer domain protein	28.26%	12
C5CF06	Uncharacterised protein	23.60%	12
C5CIU6	S-layer domain protein	27.96%	12
C5CFJ1	Extracellular solute-binding protein	25.48%	11
C5CFR6	Uncharacterised protein	9.26%	8
C5CE80	Basic membrane lipoprotein	18.75%	7
C5CD84	Extracellular solute-binding protein	7.24%	6
C5CGG1	Type II and III secretion system protein	0.30%	6
C5CDX5	M6 family metalloprotease domain protein	1.91%	5
C5CDY5	Extracellular solute-binding protein	16.16%	5
C5CH29	Extracellular solute-binding protein	13.33%	4
C5CGS6	Uncharacterised protein	6.83%	4
C5CET4	Uncharacterised protein	7.35%	4
C5CIC9	Uncharacterised protein	12.22%	3
C5CER4	Basic membrane lipoprotein	2.64%	3
C5CFZ5	Basic membrane lipoprotein	7.65%	3
C5CH54	Extracellular solute-binding protein	2.45%	3
C5C103	Uncharacterised protein	4.41%	3
C5CI67	Peptidase C11 clostripain	4.63%	2

Accession	Description	Coverage	Peptides
C5CG44	Peptidase S41	4.61%	2
C5CE21	Uncharacterised protein	11.55%	4
C5CFE7	Pyrrolo-quinoline-quinone	1.33%	3
C5CH84	Alpha amylase catalytic region	3.02%	4
C5CG51	Uncharacterised protein	3.61%	2

One uncharacterised proteins is strongly dominating and have 169 identified peptides. 2 proteases, 2 S-layer domain proteins, 3 basic lipoproteins, 4 peptidases, 10 uncharacterised proteins, and 12 extracellular solute-binding proteins are identified in this band as well. With the most abondant groups being the extracellular solute-binding proteins and the uncharacterised proteins.

The dominating uncharacterised protein (C5CEX4) probably makes up the major fraction of this band. Although it does not contain any conserved functional motifs it appers to be a major secreted protein in *K. olearia*. It has homologs in a number of *Thermotogales* species, but not in *P. mobilis*.

Sample 2, *Kosmotoga olearia* band 5

524 proteins were detcted in the band and 82 of them were confident. The MS2 Quant value varied from 0.73 to 0.00. Most of the proteins had 100% confidence except for the last ones, no. 83 and 84, which had 96% confidence. Proteins with signal peptides are listed below. See Appendix (Table 7.2), for proteins without signal peptides.

Table 4.3: Protein identification of *K. olearia* band 5.

Accession	Description	Coverage	Peptides
C5CDF3	Uncharacterised protein	47.73%	42
C5CEX4	Uncharacterised protein	48-85%	28
C5CG51	Uncharacterised protein	47.87%	17
C5CGM9	5’-nucleotidase domain protein	16.02%	14
C5CIC9	Uncharacterised protein	36.65%	12
C5CER4	Basic membrane lipoprotein	14.96%	11
C5CI36	5’-nucleotidase domain protein	13.74%	11
C5CG42	Peptidase S41	12.18%	10
C5CIG7	Extracellular solute-binding protein	17.58%	10
C5CDY5	Extracellular solute-binding protein	18.43%	10
C5CHK4	Uncharacterised protein	23.61%	9
C5CIC8	Nuclease (SNase domain protein)	25.09%	9
C5CDF1	Alkaline phosphatase	14.71%	8
C5CEB0	Glycoside hydrolase family 57	5.24%	8
C5CE80	Basic membrane lipoprotein	18.75%	8
C5CIC7	Nucleic acid-binding OB fold tRNA helicase type	13.16%	8

Accession	Description	Coverage	Peptides
C5CD84	Extracellular solute-binding protein	15.25%	7
C5CFG4	Uncharacterised protein	18.12%	7
C5CI22	Uncharacterised protein	10.11%	5
C5CEZ5	Uncharacterised protein	19.50%	5
C5CFE8	Pullulanase, type I	2.56%	4
C5CGC4	OstA family protein	4.95%	4
C5CEZ1	Periplasmic-binding protein, LacI transcriptional regulator	13.70%	4
C5CF27	Periplasmic-binding protein	15.15%	4
C5CI23	Peptidase C1A papain	4.71%	4
CECIK8	Extracellular solute-binding protein	10.65%	4
C5CJ31	Basic membrane lipoprotein	3.33%	4
C5CGZ5	Basic membrane lipoprotein	13.03%	4
C5CEG1	Extracellular binding protein	5.04%	3
C5CE35	Uncharacterised protein	8.33%	3
CECEE7	PEGA domain protein	0.53%	3
CECER0	Extracellular solute-binding protein	3.92%	3
C5CDW8	Uncharacterised protein	5.66%	3
CECED1	PEGA domain protein	17.24%	3
C5CGP3	PEGA domain protein	5.87%	3
C5CH84	Alpha amylase catalytic region	0.90%	3
C5CIB4	Uncharacterised protein	8.26%	3
C5CIK4	Uncharacterised protein	17.24%	3
C5CG28	Peptidase S41	5.31%	2
C5CDK6	Uncharacterised protein	6.10%	2
C5CDX4	Von Willebrand factor, type A	3.01%	2
C5CFR6	Uncharacterised protein	2.71%	2
C5CFZ4	Uncharacterised protein	11.68%	2
C5CGF7	Uncharacterised protein	7.78%	2
C5CGQ1	Uncharacterised protein	7.43%	2
C5CI25	Extracellular solute-binding protein	2.45%	2
C5CDX6	Uncharacterised protein	5.26%	1
C5CIB5	Extracellular solute-binding protein	5.00%	1

The most dominating protein in this band is an uncharacterised protein (C5CDF3), which have 42 identified peptides. 2 5’Nucleotidase domin proteins, 2 peptidases, 3 PEGA domain proteins, 4 basic lipoproteins, 8 extracellular solute-binding proteins and 17 additional uncharacterised proteins are identified in this band as well. The uncharacterised proteins and the extracellular solute-binding proteins are the major proteins in this protein band.

These two protein bands (no.2 and no.5), was compared with each other to find out if they had any proteins in common and 20 proteins were found, including the major protein (C5CDX4) in band 2, indicationg a partional degradation because band 5 represents more extracellular proteins the major proteins in the supernatants

The major protein in band 5 has an MW of 19.4 kDa and is unique to K. olearia, but contains also another C5CDF3 homolog with about 35% amino acid sequence identity. The strong secretion of several uncharacterised proteins without any conserved domain or motif indicating a range of new and interesting functions in this organism.

4.2: SDS-PAGE identification in *P. mobilis* grown on different substrates

P. mobilis were cultivated using maltodextrin and xylan as growth substrates to examine if other proteins different from *P. mobilis* cultivated on glucose would be secreted into the growth media. *P. mobilis* were subcultured twice to replace the former substrate. *K. olearia* medium with growth substrates added, was also incubated and ultrafiltered, to find out if proteins from yeast extract would give background on the gels. The bacterial initials with Gluk, Malt or Xyl added, means glucose, maltodextrin and xylan. The Microassay procedure (Materials and methods, 3.2.5), was used for measuring protein concentrations and the results were like this (µg/mL): *Pm*-Malt: 261.1872, *Pm*-Xyl: 225.9606, *Ko*-KTR: 151.6502. A SDS-PAGE was run to visualise the proteins (Fig. 4.2).

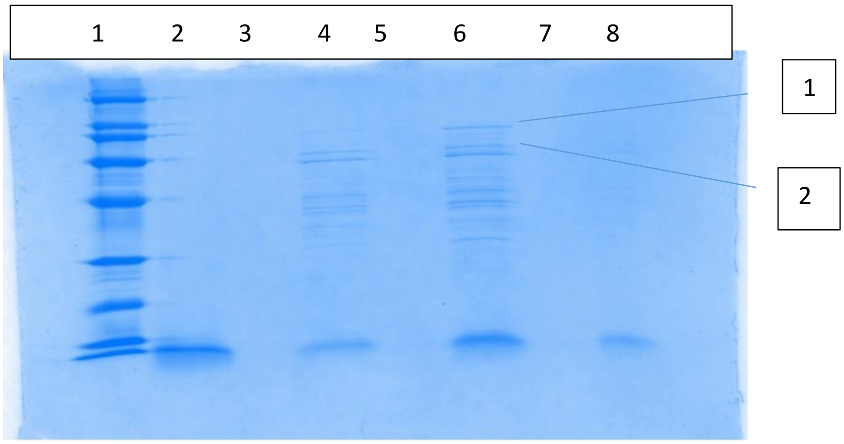

Fig: 4.2: SDS-PAGE analysis of culture concentrated supernatants of P. mobilis, grown on various substrates, (Gel no. 2). Lane 1: MWS, Broad Range (Bio Rad). Lane 2: *Ko*-Ktr. Lane 4: *Pm*-Gluk. Lane 7: *Pm*-Malt. Lane 8: Pm-Xyl. Two bands in lane 6 are labelled 1 and 2.

According to Fig. 2, lane 2, the only detected proteins and peptides from the yeast extract were migrating with the electrophoretic front, and thus yeast extract is unlikely to represent anly background of gel bands.

The protein bands from *Pm*-Gluk and *Pm*-Malt were almost equally strong, and the protein bands from *Pm*-Xyl were very weak. To examine the protein contents in *Pm*-Malt, two protein bands in lane 6 were excised from the gel, as indicated by “1” and “2” in the figure, were subjected to proteomics analyses.

Pmm1, *Petrotoga mobilis*, grown on maltodextrin, band 1

513 proteins were detected in the band, 31 of them were confident. The MS2 Quant value varied from 0.02 to 0.00. Most of the proteins had 57% confidence except for the last ones, no. 30 and 31, which had 45% and 46% confidence. See Appendix (Table 7.3), for proteins without signal peptides.

Table 4.4: Protein identification of *P. mobilis*, grown on maltodextrin, band 1.

Accession	Description	Coverage	Peptides
A9BI16	Alpha amylase catalytic region	19.09%	30
A9BHS2	Surface antigen (D15)	7.70%	15
A9BJD5	Metallophosphoesterase	14.26%	13
A9BGR7	Polysaccharide export protein	5.30%	10

An alpha amylase is the most dominating protein and have 30 identified peptides. This band probably represents an amylase, which is secreted in the growth medium, because the bacterial culture was grown with maltodextrin as a growth substrate. It is probably regulated by the presence of this substrate as this band is not seen (or very weakly) in the glucose-grown culture.

Pmm2, *Petrotoga mobilis*, grown on maltodextrin, band 2

578 protein were detected in the band, 28 of them were confident. The MS2Quant value varied from 0.04 to 0.00. Most of the proteins had 100% confidence except for the last one, no. 28, which had 87% confidence. Proteins with signal peptide are listed below. See Appendix (Table 7.4), for proteins without signal peptides.

Table 4.5: Protein identification of *P. mobilis* grown on maltodextrin, band 2.

Accession	Description	Coverage	Peptides
A9BGI1	Extracellular solute-binding protein	62.86%	79
A9BX05	5’-nucleotidase domain protein	42.38%	44
A9BGN1	Extracellular solute-binding protein	25.28%	29
A9BI19	Alpha amylase catalytic region	21.86%	15
A9BII1	Ppic-type peptidyl-prolyl cis-trans isomerase	21.86%	15
A9BES7	Uncharacterised protein, precursor	17.34%	14

Three proteins are dominating with 79, 44, and 29 peptides. 2 extracellular solute-binding proteins, the other proteins are only one of each. Alpha amylase constitures a fraction in this band, (no. 2). No proteins were shared between the two ban

4.3: Total protemics analysis of secreted proteins

To get an overview of all the secreted proteins from the three species, standard cultues of *T. africanus*, *P. mobilis* and *K. olearia*, as well as *T. maritima* TCEL2, grown on AVICEL (microcrystalline cellulose),were subjected to proteomics analysis. First, cell pellets and supernatant concentrates for each bacteria were analysed by SDS-PAGE to visualise the proteins (Fig. 4.3), to find out whether the extracellular fractions were unique of if these proteins could be a prodcuct of partial leakage from the cells. The protein profiles from *T. africanus*, *P. mobilis* and *K. olearia* were quite different with only a few common bands. These bands could of course represent different proteins. The supernatant of *T. maritima* TCEL2 yielded a very faint pattern, which seems to be very similar to the pelleted fraction.

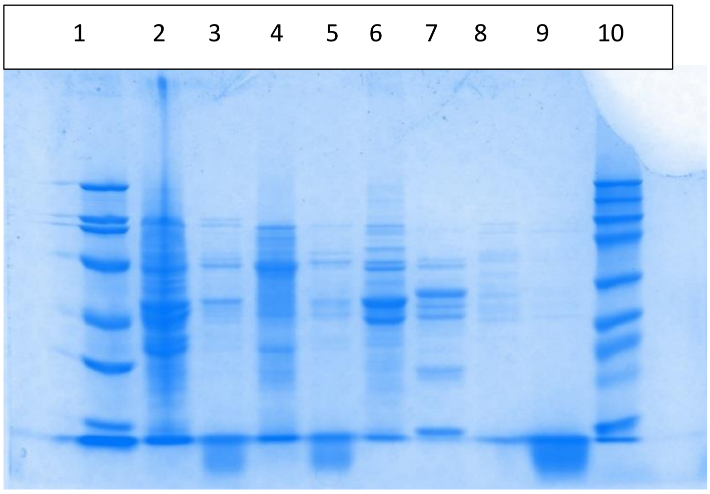

Fig: 4.3: SDS-PAGE protein identification, **(**Gel no.4). MWS, Broad Range (Bio-Rad). Lane 2: *Ta*-P. Lane 3: *Ta*-S. Lane 4: *Pm*-P. Lane 5: *Pm*-S. Lane 6: *Ko*-P. Lane 7: *Ko*-S. Lane 8: *Tm* TCEL2-P. Lane 9: *Tm* TCEL2-S. Lane 10: Full Range Rainbow Amersham Marker.

The concentrated supernatants were and labelled *Ta*-S, *Pm*-S, and *Ko*-S, and subjected to proteomics analyses. (*Tm*-TCEL2 was not subjected to proteomics analysis).

<u>*Taf, Thermosipho africanus*</u>

1878 proteins were detected in the supernatant, 114 of them were confident. The MS2Quant value varied from 0.04 to 0.00. Most of the proteins had 100% confidence, except for the last ones, no.108 to 114, which had 98% confidence. Surprisingly, none of the confident identified proteins had signal peptides, and are only listed in the Appendix (Table 7.5). This indicates that there is a strong leakage of intracellular proteins for this species during growth.

Pmo, Petrotoga mobilis

1556 proteins were detected in the supernatant, 410 of them were confident. The MS2Quant value varied from 0.07 to 0.00. Most of the proteins had 100% confidence, except for the last ones, no. 398 to no. 410, and the confidence varied from 82% to 73%. Proteins with signal peptides are listed below. See Appendix (Table 7.6), for proteins without signal peptides.

Table 4.6: Total protein identification of the *P.mobilis* culture supernatant.

Accession	**Description**	**Coverage**	**Peptides**
A9BFA7	Extracellular solute-binding protein	58.31%	32
A9BIV2	Periplasmic binding protein	61.98%	32
A9BJC0	5'-nucleotidase domain protein	27.62%	31
A9BI83	Basic membrane lipoprotein	50.55%	24
A9BH08	Basic membrane lipoprotein	43.44%	24
A9BI16	Alpha amylase catalytic region	14.08%	23
A9BEP8	Extracellular solute-binding protein	34.14%	23
A9BID9	Extracellular solute-binding protein	39.28%	22
A9BI61	Extracellular solute-binding protein	51.03%	21
A9BJC1	5'-nucleotidase domain protein	36.58%	21
A9BH85	Uncharacterised protein	40.65%	20
A9BEN7	Uncharacterised protein	26.94%	20
A9BGR7	Polysaccharide export protein	13.73%	20
A9BJ79	FAD-dependent pyridine nucleotide-disulfide oxidoreductase	23.21%	19
A9BGN1	Extracellular solute-binding protein	11.35%	18
A9BH89	Pyruvate flavodoxin/ferredoxin oxidoreductase, domain protein	37.28%	17
A9BFA1	Extracellular solute-binding protein	37.38%	17
A9BEQ2	Protease Do	29.80%	17
A9BJD5	Metallophosphoesterase	7.79%	17
A9BEQ9	Periplasmic binding protein	45.21%	16
A9BII1	Ppic-type peptidyl-prolyl cis-trans isomerase	26.16%	16
A9BJW8	Periplasmic binding protein	32.88%	16
A9BGC6	Extracellular solute-binding protein	37.99%	15
A9BES8	S-layer domain protein	37.11%	15
A9BHD5	Carboxyl-terminal protease	27.23%	13
A9BHD2	Outer membrane protein-like protein	38.74%	13
A9BFP8	Extracellular solute-binding protein	18.64%	13
A9BGI1	Extracellular solute-binding protein	16.52%	13
A9BGL1	Uncharacterised protein	23.56%	13
A9BH87	Ppic-type peptidyl-prolyl cis-trans isomerase	3.15%	13
A9BH12	Extracellular solute-binding protein	19.95%	12
A9BJS1	Peptidglucan-binding, LysM	2.68%	12
A9BJF3	Periplasmic binding protein, LacI transcriptional regulator	27.12%	12
A9BFM8	Substrate-binding region of ABC-type glycine betaine transport system	9.09%	12

Accession	Description	Coverage	Peptides
A9BHJ4	ABC-type nitrate/sulfonate/bicarbonate transport system, periplasmic-like protein	31.49%	11
A9BF08	Flagellar P-ring protein	17.07%	11
A9BFN9	Extracellular solute-binding protein	11.67%	11
A9BH45	Dipeptidyl aminopeptidase/acyclaminoacylpeptidase-like protein	12.55%	11
A9BJT3	Extracellular solute-binding protein	13.78%	11
A9BES7	Uncharacterised protein	11.94%	10
A9BHD0	Uncharacterised protein	42.73%	10
A9BH16	Extracellular solute-binding protein	3.18%	10
A9BIB5	Outer membrane , Skp (OmpH)	56.25%	9
A9BIG9	Protein tyrosine phosphatase	35.33%	9
A9BIH8	Extracellular solute-binding protein	4.25%	9
A9BG91	Substrate-binding region of ABC-type glycine betaine transport system	25.85%	8
A9BGU7	Uncharacterised protein	40.00%	8
A9BF09	Flagellar basal body, L-ring protein-like protein	18.01%	8
A9BJ69	Uncharacterised protein	7.66%	8
A9BF57	Uncharacterised solute-binding protein, Pmob-0379	3.12%	7
A9BJ86	Extracellular solute-binding protein	7.32%	6
A9BI11	Uncharacterised protein	5.08%	6
A9BHS2	Surface antigen (D15)	1.86%	5
A9BI27	S-layer domain protein	16.06%	5
A9BHX1	Peptidase M32B	7.03%	5
A9BI84	Uncharacterised protein	5.14%	4
A9BHK6	Phosphoesterase PA-phosphatase-related	4.88%	2
A9BEP1	OstA family protein	13.94%	1
A9BJQ3	Monosaccharide-transporting ATPase	4.15%	1
A9BG31	Extracellular solute-binding protein	2.80%	10

Three proteins, an extracellular solute-binding protein, a periplasmic-binding protein and a protein carrying a 5’-nucleotidase domain are dominating with 32-31 identified peptides. 2 basic lipoproteins, 2 oxidoreductases, 3 periplasmic-binding proteins, 4 proteases/ amino-peptidase//peptidase, 9 uncharacterised proteins and 16 extracellular solute-binding proteins are identified. Among these groups, the uncharacterised proteins and extracellular proteins are the major proteins in the supernatant. The large number of extraellular solute-binding proteins and uncharacterised proteins indicates a large variety of extracellular biochemical functions probably involved in nutrient uptake functions.

Kol, Kosmotoga olearia

1581 proteins were detected in the supernatant, 250 of them were confident. The MS2Quant ones, from no. 202 to no. 250, and the confidence varied from 97% to 83%. Proteins with signal peptides are listed below. See Appendix (Table 7.7), for proteins without signal peptides.

Table 4.7: Total protein identification of *K. olearia* culture supernatant.

Accession	Description	Coverage	Peptides
C5CEX4	Uncharacterised protein	83.87%	245
C5CER4	Basic membrane lipoprotein	89.44%	149
C5CF27	Periplasmic binding protein	80.13%	104
C5CD84	Extracellular solute-binding protein	73.90%	94
C5CDF3	Uncharacterised protein	85.80%	72
C5CGM9	5’-nucleotidase domain protein	63.29%	71
C5CI36	5’-nucleotidase domain protein	62.31%	70
C5CFG4	Uncharacterised protein	49.38%	65
C5CEB0	Glycoside hydrolase family 57	28.88%	54
C5CHI9	Extracellular solute-binding protein	61.45%	54
C5CEE7	PEGA domain protein	26.21%	54
C5CI23	Peptidase C1A papain	55.33%	54
C5CIG7	Extracellular solute-binding protein	76.96%	45
C5CDU3	Carboxyl-terminal protease	66.58%	44
C5CF24	Protease Do	55.19%	42
C5CI58	Extracellular solute-binding protein	64.72%	41
C5CG44	Peptidase S41	61.06%	40
C5CE27	Fibronectin, type III domain protein	7.49%	37
C5CIK8	Extracellular solute-binding protein	53.27%	36
C5CG51	Uncharacterised protein	69.51%	34
C5CFE7	Pyrrolo-quinoline quinone	8.65%	34
C5CGC8	Uncharacterised protein	54.49%	32
C5CDX4	Von Willebrand factor, type A	38.49%	32
C5CFE3	Pullulanase, type I	13.84%	31
C5CIG1	Fibronectin, type III domain protein	0.80%	31
C5CFR6	Uncharacterised protein	50.34%	27
C5CEG1	Extracellular solute-binding protein	35.38%	27
C5CFZ4	Uncharacterised protein	56.20%	26
C5CI66	Uncharacterised protein	52.44%	25
C5CI22	Uncharacterised protein	39.78%	25
C5CDW8	Uncharacterised protein	57.08%	25
C5CFE1	Extracellular solute-binding protein	30.50%	24
C5CGK2	Extracellular solute-binding protein	37.68%	23
C5CEI9	Uncharacterised protein	3.12%	23
C5CE80	Basic membrane lipoprotein	55.11%	22
C5CDY5	Extracellular solute-binding protein	51.77%	22
C5CEE5	Uncharacterised protein	11.03%	22
C5CIJ8	PHP domain protein	10.19%	22
C5CFJ1	Extracellular solute-binding protein	46.39%	21
C5CG42	Peptidase S41	27.73%	21
C5CD64	Uncharacterised protein	35.91%	19
C5CH18	Von Willebrand factor, type A	26.78%	19
C5CE35	Uncharacterised protein	40.83%	19

Accession	**Description**	**Coverage**	**Peptides**
C5CGG1	Type II and III secretion system protein	2.13%	19
C5CE21	Uncharacterised protein	32.34%	17
C5CIC7	Nucleic acid binding OB fold, tRNA helicase type	18.07%	17
C5CIB4	Uncharacterised protein	44.78%	16
C5CIL9	Extracellular solute-binding protein	27.60%	16
C5CIC9	Uncharacterised protein	50.23%	15
C5CEZ5	Uncharacterised protein	62.50%	15
C5CGC4	OstA family protein	43.69%	15
C5CFG3	Ppic-type peptide-prolyl, cis-trans isomerase	3.25%	15
C5CIC8	Nuclease (SNase domain protein)	41.94%	15
C5CJ03	Extracellular solute-binding protein	29.10%	15
C5CHG6	Phosphofructokinase	11.98%	15
C5CDX5	M18 family metalloprotease domain protein	2.55%	14
C5CER0	Extracellular solute-binding protein	10.46%	14
C5CG81	Extracellular solute-binding protein	10.98%	14
C5CH29	Extracellular solute-binding protein	2.89%	14
C5CD36	Membrane protein insertase, YidC	11.24%	12
C5CF94	Uncharacterised protein	17.30%	12
C5CI67	Peptidase C11 clostripain	6.33%	12
C5CIU6	S-layer domain protein	18.71%	11
C5CGW5	Outer membrane chaperone Skp (OmpH)	51.52%	11
C5CH54	Extracellular solute-binding protein	5.39%	11
C5CIP2	Uncharacterised protein	26.44%	11
C5CET4	Uncharacterised protein	11.83%	11
C5CDP1	Alkaline phosphatase	12.90%	11
C5CF06	Uncharacterised protein	13.71%	11
C5CGP3	PEGA domain protein	14.80%	11
C5CEF1	S-layer domain protein	13.29%	10
C5CEF2	Uncharacterised protein	10.58%	10
C5CGZ5	Basic membrane protein	7.65%	8
C5CFG15	Uncharacterised protein	20.07%	8
C5CDK6	Uncharacterised protein	18.29%	5
C5CGS6	Uncharacterised protein	10.56%	5
C5CEZ1	Periplasmic binding protein/LacI transcriptional regulator	16.10&	5
C5CHW2	Uncharacterised protein	9.36%	5
C5CFG0	Uncharacterised protein	24.57%	4
C5CI12	Uncharacterised protein	14.35%	9
C5CJ31	Basic membrane lipoprotein	6.67%	10
C5CIL6	Uncharacterised protein	3.42%	25
C5CF91	Uncharacterised protein	17.10%	5
C5CHP8	Extracellular solute-binding protein	7.98%	17
C5CFE2	Uncharacterised protein	3.11%	7
C5CIY4	Extracellular solute-binding protein	2.73%	6
C5CIB5	Extracellular solute-binding protein	5.00%	6
C5CIT3	Thioredoxin-related protein-like protein	5.78%	6
C5CG07	Uncharacterised protein	5.75%	7

One uncharacterised protein is dominating and have 245 peptides. 2 5’-nucleotidase domain proteins, 2 fibronectin, type III domain proteins, 2 von Willeband factor type A, 3 proteases/metallprotease, 4 basic lipoproteins, 19 extracellular solute-binding proteins and 32 uncharacterised proteins are identified. Among these groups, the uncharacterised proteins and the extracellular solute-binding proteins are the major proteins in the supernatant. Von Willebrand factor is known for playing a major role in the human coagulation cascade system, but the role of this homolog in *K.olearia* is unknown. The large number of annotated proteins as well as hypothetical extracellular proteins in the culture supernatant of *K. olearia* indicates an extensive extracellular activity for this bacterium.

4.4: Identification of total proteins in culture supernatants *T. africanus, P. mobilis, K. olearia and T. maritima* TCEL2, after sterile filtration

Due to the large numbers of proteins detected in supernatants lacking signal peptides, and belonging to groups of typical cytoplasmatic proteins Supernantans from *T. africanus*, *P. mobilis*, *K. olearia* and *T. maritima* TCEL2 were sterile filtered, before ultrafiltration, to remove cells possibly remaining in the supernatants (Materials and methods, 3.2.2). *T. maritima* TCEL2 was also included in this analysis after it had been cultivated on AVICEL, (microcrystalline cellulose). *T. maritima* was sucultured twice to replace the former substrate. A SDS-PAGE was run to visualise the proteins (Fig. 4.4).

The *K. olearia* culture partly lysed during the incubation. The reason for this is unclear, but it may be caused by a viral infection. Several attempts to grow this species to high density failed, and it was no longer possible to reproduce the same protein profile as before. The protein yield was low and a band representing a high MW protein band (labelled “V” in Fig. 4.4), and which was not observed in previous gels, was excised and sent for analysis. The results gave no indication of a virus-derived protein, but hits to high MW proteins from *K. olearia* (Table 4.11).

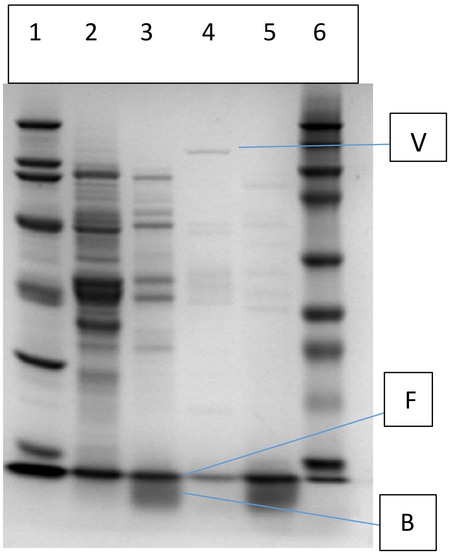

Fig: 4.4: SDS-PAGE protein identification (Gel no. 5). Lane 1: MWS, Broad Range (Bio-Rad). Lane 2: *Ta*-S. Lane 3: *Pm*-S. Lane 4: *Ko*-S. Lane 5: *Tm* TCEL2-S. Lane 6: Full Range Rainbow Amersham Marker. Three of the bands are labelled, B, F, (in lane 3), and V, (in lane 4).

T. maritima TCEL2 also yielded a very low amount of proteins, while *T. africanus* and *P. mobilis* gave high yields. For all cultures, a distinctive MW band, (labelled "F"), was seen in a position representing the electrophoresis front. Another more diffuse band, even lower in the gel was also frequently observed, (labelled "B"). To find out whether these two bands represents heavily degraded proteins originating from the respective bacteria, they were also excised and analysed. The concentrated supernatants, labelled *Ta*-S, *Pm*-S, Ko-S and *Tm* TCEL2-S, were also analysed.

The results showed a match with a large range of P. mobilis proteins of different MW's, indicating that they are peptides derived from degradation of larger proteins (Tables 4.8 and 4.9).

<u>*Pm*-B, *Petrotoga mobilis*, bottom band, sterile filtered</u>

1076 protein were detected in the band, 57 of them were doubtful, the MS2Quant value varied from 0.08 to 0.00. Most of the proteins had 100% confidence except for the last ones, from no. 51 to no. 57, and the confidence varied from 98%, to 87% confidence. Proteins with signal peptides are listed below. See Appendix (Table 7.8), for proteins without signal peptides.

Table 4.8: Protein identification of *P. mobilis*, bottom band.

Accession	**Description**	**Coverage**	**Peptides**
A9BF09	Flagellar basal body L-ring protein-like protein	5.69%	11
A9BFA7	Extracellular solute-binding protein	2.98%	9
A9BIG9	Protein tyrosine phosphatase	7.33%	8
A9BG52	Basic membrane lipoprotein	21.93%	6
A9BJB5	Outer membrane chaperone, Skp (OmpH)	20.62%	2

The first protein, flagellar basal body L-ring-like protein, is dominating and has 11 peptides.

Pm-F, *Petrotoga mobilis*, front band, sterile filtered

1321 proteins were detected in the band, 257 of them were doubtful, the MS2 Quant value varied from 0.21 to 0.00. Most of the proteins had 100% confidence, except for the last ones, from no. 248 to 257, and the confidence varied from 96% to 95%. Proteins with signal peptides are listed below. See Appendix (Table 7.9), for proteins without signal peptides.

Table 4.9: Protein identification of *P. mobilis*, front band.

Accession	Description	Coverage	Peptides
A9BIG9	Protein tyrosine phosphatase	79.33%	30
A9BFA7	Extracellular solute-binding protein	12.16%	12
A9BI83	Basic membrane lipoprotein	9.56%	10
A9BJB5	Outer membrane chaperone Skp(OmpH)	56.88%	9
A9BJZ2	Lytic transglycosylase catalytic	39.11%	8
A9BGI1	Extracellular solute-binding protein	6.13%	7
A8BID5	Metallophosphoesterase	5.44%	7
A9BIH8	Extracellular solute-binding protein	3.50%	6
A9BEP8	Extracellular solute-binding protein	1.94%	6
A9BGN1	Extracellular solute-binding protein	4.05%	6
A9BH89	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	3.60%	6
A9BGU4	Uncharacterised protein	10.03%	5
A9BH08	Basic membrane lipoporotein	3.83%	5
A9BID9	Extracellular solute-binding protein	2.17%	5
A9BF09	Flagellar basal body L-ring protein-like protein	18.96%	4
A9BHX1	Peptidase M23B	12.23%	4
A9BJW8	Periplasmic solute-binding protein	3.73%	4
A9BGU7	Uncharacterised protein	32.00%	3
A9BHD5	Carboxyl-terminal protease	2.11%	3
A9BG15	Uncharacterised protein	5.33%	2
A9BG31	Extracellular solute-binding protein	4.90%	2
A9BG94	Lipoprotein	4.36%	2
A9BJ08	Uncharacterised protein	12.59%	2
A9BGU8	Uncharacterised protein	10.00%	1
A9BEP2	Uncharacterised protein	9.36%	6
A9BJ79	FAD-dependent pyridine nucleotide-disulfide oxidoreductase	4.69%	8

Protein tyrosine phosphatese is dominating and have 30 peptides. 2 basic lipoproteisn, 2 oxidoreductases, 6 uncharacterised proteins and 8 extracellular solute-binding proteins are identified. Among these groups, the uncharacterised proteins and the extracellular proteins are the major proteins in this protein band.

These two bands, labelled B and F, were compared to each other to find if they shared any proteins and 5 common proteins were identified.

Ko-V, *Kosmotoga olearia*, virus band? Sterile filtered

1285 proteins were detected in the band, 12 of them were doubtful. The MS2 Quant value varied from 0.01 to 0.00. Most of the proteins had 100% confidence, except for the last ones, from no. 10 to no. 12, and the confidence varied from 87% to 74%. Proteins with signal peptides were listed below. See Appendix (Table 7.10), for proteins without signal peptides.

Table 4.11: Protein identification of *K. olearia*, virus band.

Accession	Description	Coverage	Peptides
C5CEB0	Glycoside hydrolase, family 57	8.79%	55
C5CEI9	Uncharacterised protein	10.85%	46
C5CIG1	Fibronectin, type III domain protein	1.59%	38
C5CEE5	Uncharacterised protein	2.86%	25
C5CF24	Protease Do	17.97%	23
C5CEX4	Uncharacterised protein	4.84%	8

Glycoside hydrolase is dominating and has 55 peptides. 3 uncharacterised proteins are the major proteins in this protein band.

Taf-S, *Thermosipho africanus*, sterile filtered

1441 proteins were detected in the supernatant, 427 of them were doubtful. The MS2 Quant value varied from 0.59 to 0.00. Most of the proteins had 100% confidence, except for the last ones, from no. 414 to no. 427, and the confidence varied from 99% to 79%. Surprisingly, none of the proteins had signal peptides, and are therefore only listed in Appendix (Table 7.11). This indicates that there is a strong leakage of intracellular proteins for this species during growth.

Pmo-S, *Petrotoga mobilis*, sterile filtered

1551 protein were detected in the supernatant, 540 of them were doubtful. The MS2 Quant value varied from 0.11 to 0.00. Most of the proteins had 100% confidence, except for the last ones, from no. 528 to no. 540, and the confidence varied from 99% to 53%. Proteins with signal peptides are listed below. See Appendix (Table 7.12), for proteins without signal peptides.

Table 4.12: Total protein identification of *P. mobilis*.

Accession	Description	Coverage	Peptides
A9BH89	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	49.36%	29
A9BFA7	Extracellular solute-binding protein, family 1	54.84%	25
A9BEP8	Extracellular solute-binding protein, family 1	45.04%	23
A9BJC0	5’-nucleotidase domain protein	25.24%	22
A9BGN1	Extracellular solute-binding protein, family 5	27.39%	20
A9BJC1	5’-nucleotidase domain protein	30.22%	18
A9BI16	Alpha amylase catalytic region	15.04%	18
A9BGI1	Extracellular solute-binding protein	17.38%	15
A9BGR7	Polysaccharide export protein	17.47%	15
A9BI11	Uncharacterised protein	19.13%	15
A9BGC6	Extracellular solute-binding protein	20.59%	13
A9BJD5	Metallophosphoesterase	13.82%	13
A9BHD5	Carboxyl-terminal protease	14.79%	13
A9BHJ4	ABC-type nitrate/sulfonate/bicarbonate transport system, periplasmic component-like protein	35.71%	12
A9BEQ9	Periplasmic-binding protein	45.21%	12
A9BII1	Ppic-type peptidyl-prolyl cis-trans isomerase	16.13%	12
A9BGL1	Uncharacterised protein	20.16%	11
A9BES8	S-layer domain protein	22.33%	11
A9BES7	Uncharacterised protein	15.99%	10
A9BFP8	Extracellular solute-binding protein	3.20%	10
A9BH85	Uncharacterised protein	20.55%	9
A9BHN6	Uncharacterised protein	1.90%	9
A9BJW8	Periplasmic solute-binding protein	23.73%	9
A9BH08	Basic membrane lipoprotein	7.10%	9
A9BIH8	Extracellular solute-binding protein	8.50%	9
A9BH45	Dipeptidyl aminopeptidase/acylaminoacyl-peptidase-like protein	8.11%	9
A9BIY2	Extracellular solute-binding protein	9.98%	9
A9BEN7	Uncharacterised protein	10.85%	8
A9BF08	Flagellar P-ring protein	16.46%	8
A9BFA1	Extracellular solute-binding protein	15.29%	8
A9BG31	Extracellular solute-binding protein	10.26%	7
A9BIG9	Protein tyrosine phosphatase	35.33%	7
A9BIV2	Periplasmic-binding protein	17.37%	7

Accession	Description	Coverage	Peptides
A9BJB5	Outer membrane chaperone Skp (OmpH)	45.62%	6
A9BHD2	Outer membrane protein-like protein	18.02%	6
A9BJP5	Basic membrane lipoprotein	14.50%	5
A9BG91	Substrate-binding region of ABC-type glycine/beatine transport system	99.39%	5
A9BH00	Uncharacterised protein	15.86%	5
A9BF57	Uncharacterised solute-binding protein, Pmob-0379	36.20%	5
A9BI61	Uncharacterised protein	4.53%	4
A9BJ69	Uncharacterised protein	7.66%	4
A9BF09	Flagellar basal body L-ring protein-like protein	18.01%	4
A9BIX1	Peptidase M32B	8.56%	4
A9BHS2	Surface antigen	2.92%	3
A9BHK6	Phosphoesterase PA-phosphatase-related	4.88%	3
A9BFU9	Extracellular solute-binding protein	7.55%	2
A9BG07	Uncharacterised protein	2.29%	1
A9BJ86	Extracellular solute-binding protein	10.30%	6

One pyruvate flavodoxin/ferredoxin oxidoreductase, domain proteinis the most dominating protein and have 29 peptides. 2 basic lipoproteins, 2 aminopeptidase/peptidase, 3 periplasmic-binding proteins, 10 uncharacterised proteins and 11 extracellular solute-binding proteins are identified. Among these groups, the uncharacterised proteins and the extracellular proteins are the major proteins in the supernatant.

The non-sterile filtered and sterile filtered supernatants were compared to each other to find common proteins. 31 common proteins were found. Among them were 7 extracellular solute-binding proteins and 8 uncharacterised proteins the major common proteins.

Kol-S, *Kosmotoga olearia*, sterile filtered

1661 proteins were detected in the supernatant, 142 of them were doubtful. The MS2Quant value varied from 0.09 to 0.00. The proteins had 100% confidence, except for the last ones, from no. 126 to no.142, and the confidence varied from 99% to 65%. Proteins with signal peptides are listed below. See Appendix (Table 7.13), for proteins without signal peptides.

Table 4.13: Total protein identification of *K. olearia.*

Accession	Description	Coverage	Peptides
C5CIK8	Extracellular solute-binding protein	41.16%	41
C5CI36	5’-nucleotidase domain protein	32.33%	34
C5CEB0	Glycoside hydrolase, family 57	4.28%	34
C5CER0	Extracellular solute-binding protein	5.56%	24
C5CE19	Uncharacterised protein	0.94%	23

Accession	Description	Coverage	Peptides
C5CEX4	Uncharacterised protein	25.35%	21
C5CIJ8	PHP domain protein	1.85%	21
C5CG44	Peptidase S41	25.81%	20
C5CD84	Extracellular solute-binding protein	18.60%	20
C5CGM9	5’-nucleotidase domain protein	22.52%	20
C5CER4	Basic membrane lipoprotein	33.43%	18
C5CDP1	Alkaline phosphatase	11.54%	18
C5CIG7	Extracellular solute-binding protein	20.67%	16
CECF24	Protease Do	2.81%	16
C5CI23	Peptidase C1A papain	16.19%	15
C5CG42	Peptidase S41	11.48%	15
C5CGK2	Extracellular solute-binding protein	15.02%	14
C5CGC8	Uncharacterised protein	14.24%	11
C5CF27	Periplasmic-binding protein	29.29%	10
C5CI58	Extracellular solute-binding protein	7.16%	9
C5CHP8	Extracellular solute-binding protein	5.39%	8
C5CFG4	Uncharacterised protein	8.44%	7
C5CDE3	Uncharacterised protein	35.23%	6
CECE21	Uncharacterised protein	9.24%	6
C5CG51	Uncharacterised protein	25.90%	6
C5CHG6	Phosphofructokinase	2.69%	4

One extracellular solute-binding protein is the most dominating protein and have 41 peptides. 2 5’- nucleotidase domain proteins 3 peptidases, 7 uncharacterised proteins and 7 extracellular solute-binding proteins are identified. Among these groups, the extracellular solute-binding proteins and the uncharacterised proteins are the major proteins in the supernatant.

The non-sterile filtered and sterile filtered supernatants were compared to each other to find common proteins. 23 common proteins were found. Among these were 6 extracellular proteins, 6 uncharacterised proteins, 3 peptidases the major common proteins.

Tma-S, *Thermotoga maritima* TCEL2, grown on AVICEL, sterile filtered

4209 protein were detected in the supernatant, 68 of them were doubtful. The MS2Quant valuevaried from 0.06 to 0.00. All proteins had 16% coverage. Proteins with signal peptides are listed below. See Appendix (Table 7.14), for proteins without signal peptides.

Table 4.14: Total protein identification of *T. maritima* TCEL2.

Accession	Description	Coverage	Peptides
A5IJJ1	UDP-glucose-6-dehydrogenase	7.90%	3
B1L857	Competence protein, ComE, helix-hairpin-helix	7.73%	2
A5IJW1	4-phytase	1.95%	1

5 DISCUSSION AND FURTHER PERSPECTIVES

5.1 Discussion

Four different bacteria have been cultivated in this study. *Thermosipho africanus*, *Petrotoga mobilis*, *Kosmotoga olearia* and *Thermotoga maritima* TCEL2. *P. mobilis* was also cultivated on maltodextrin and xylan to find out if it could thrive on those substrates instead of glucose. *T. maritima* TCEL2 was cultivated on AVICEL, microcrystalline cellulose, for the same purpose as mentioned above.

First, both ammonium sulfate precipitation and ultrafiltration was used, but the bands on the gel (Fig, 4.1, lane 2 and 3), from the precipitated cultures were very weak. It was decided to continue this study, and only use ultrafiltration.

The protein band patterns of *T. africanus* and *P. mobilis* are quite similar, but the protein band patterns of *K. olearia* are different. Two of the most prominent bands from the *K. olearia* lane (Fig. 4.1.), labelled *Ko*-2 and *Ko*-5 was subjected to proteomics analysis. The main protein in these bands (band 2 and 5, in Fig. 4.1), were uncharacterised, (hypothetical), proteins without known function. Several additional uncharacterised proteins, as well as a large number of extracellular solute-binding proteins and hydrolytic enzymes, were also detected but in lower abundance.

As it had been shown that *P. mobilis* can use maltodextrin and xylan for growth, it was of interest to find out if additional proteins, e.g. hydrolases, were secreted during growth on these polymeric substances. *P. mobilis* did not grow well on xylan, and gave very weak bands on the gel (Fig. 4.2, lane 8). It grew however well on maltodextrin and gave strong bands on the gel. The uppermost band (Fig. 4.2, lane 6), seemed to be unique (or at least more intense), as compared to growth on glucose (Fig. 4.2, lane 4). This band, labelled *Pm*-m1 and another prominent band, labelled *Pm*-m2 were subjected to proteomics analysis. Out of four confidently identified proteins with signal peptides, one was an alpha amylase (EC 3.2.1.1.). This indicates a regulatory role for the substrate maltodextrin in expression and secretion of this enzyme. Homologs of this protein is widespread in the *Thermotogales* order. Another, quite different alpha amylase was identified in the second band. This enzyme also shares significant homology with neopullanase (EC 3.2.1.135.), also termed alpha amylase II.

Total concentrated supernatants of *T. africanus*, *P. mobilis* and *K.olearia* were also analysed. Although the SDS-PAGE profile of the extracellular fraction of *T. africanus* proteins looks quite different from the cell pellet fraction (Fig. 4.3, lanes 2 and 3), no proteins with signal peptides could be found among the 114 confidently identified proteins. To eliminate the possibility that this could be caused by cells of *T. africanus* possibly remaining in the supernatant, samples were also sterile filtered prior to proteomics analysis. For *T. africanus* the result was, however, the same with a large amount of identified typical intracellular proteins and no proteins with signal peptides. This is mostly due to a heavy leakage of cytoplasmic proteins from the actively growing cells. The reason for this is unclear, but one explanation could be presence of actively propagating viruses (phages), or a high degree of spontaneous lysis.

P. mobilis gave more consistant results having 60 and 49 proteins with signal peptides in the non-sterile filtered and sterile filtered supernatants, respectively. Sterile filtration reduced the total number of possible proteins, most of which are typically intracellular proteins from 1556 to 1551, which is insignificant. Removal of cells by two, repeated centrifugation therefore seems to be an adequate method. Sterile filtration removed a number of the signal peptide-contaning proteins, which had been adsorbed to the filter.

For *P. mobilis*, there was no clear dominating extracellular proteins, but a total of 22 extracellular solute-binding/periplasmic-binding proteins, were identified along with 10 uncaracterised proteins and 7 hydrolytic enzymes as well as several other transport system and cell and outer membrane components.

When *K. olearia* was cultivated to make new culture supernatants for sterile filtering, it started to grow poorly and it looked like the culture partly lysed before reaching high density. It was not possible to reproduce stable growth pattern as before. The protein profile was quite different from the previous one (without sterile filtration), where the organism grew well (Fig. 4.4, lane 4 versus Fig. 4.3, lane 7), and the protein yields was also lower than before. The number of identified proteins with signal peptides was also lower, 26 proteins in the sterile filtered fraction versus 90 in the non-sterile filtered fraction. We therefore focused on the result from the former fraction. This fraction contained several major proteins belonging to the uncharacterised group (Table 4.7), as well as periplasmic and extracellular binding proteins. The three most dominating uncharacterised proteins (C5CEX4, C5CDF3 and C5CGF4), are unique to *K. olearia,* but the relatively high level of secretion and presence of conserved signal peptides indicates that they play importnant extracellular roles. The MW range from 19.4 to 48.3 kDa. The total number of uncharacterised secreted proteins is 33. 19 different extracellular solute-binding proteins were identified, belonging to carbohydrate-, oligo/dipeptide and amino acid-binding families, and 12 different hydrolases. This demonstrates a very high potentional for nutrient utilisation and uptake, as well as a great potentional for exploring of new extracellular functions.

The analysis of the concentrated supernatant from *T. maritima* TCEL2 (Fig. 4.4, lane 5), did not give meaningful results, but mostly typical intracellular proteins hits and very few with proteins having predicted signal peptides. This is probably because the strain grew very poorly, if at all, and the protein yield also was very low. The genome sequence of this strain is not yet publicly available, and the MASCOT searches were done, using other *Thermotogales* organisms. This may also explain the poor result.

The reason why the *K.olearia* band in Fig. 4.4, lane 4, was labelled with a "V" is that it was a unique band, which had not been detected in the previous gels, and it could possibly originate from a virus, since this culture did not grow well, and seemed to partly lyse during cultivation. The protein analysis resulted however in hits against mostly high-MW proteins from *K. olearia*. Except from C5CF24, the identified proteins in Table 4.11 have MW above 142kDa. There was no sign of any unusual or virus-related proteins.

Proteins with signal peptides are secreted by the general secretory pathway also known as the Sec pathway, (Introduction, 2.3). Bendtsen et al. (2005) have reviewed the literature describing the so-called "non-classical" protein secretion in bacteria, i.e. proteins lacking known signal peptides but found in the spent medium. Many of these proteins are typical intracellular proteins, e.g. 50S and 30S ribosomal proteins, pyruvate dehydrogenase, superoxide dismutase and translation elongation factors.The mechanisms behind non-classical protein secretion are unknown according to Bendtsen et al. (2005).

A total of 160 proteins without predicted signal peptide, were confidently identified in the spent medium of *K. olearia*. For *P. mobilis*, the large amount of typical intracellular proteins in the extracellular fraction seems to be due to some sort of unspecific leakage from the cells, while for *K. olearia* a more specific set of proteins, were detected in the secretome, including an iron-containing alcohol dehydrogenase, pyruvate dikinase and a Hsp60 (chaperonin), all identified by more than 50 peptides. For *P. mobilis*, the pyruvate dikinase is also a top hit with 93 identified peptides, indicating a selective secretion of this protein. The extracellular *K.*

olearia proteins also included nine 50S ribosomal proteins (L2, L4, L9, L7/12, L13, L20, L22, L23 and L25), and three 30S ribosomal proteins (S5, S10 and S19).

Boetzkes et al. (2012) have performed a secretome analysis of three *Clostridium diffficile* strains. Proteomics analysis identified 107 different proteins witout signal peptide in the supernatant, representing the major fraction of extracellular proteins.The patterns of the extracellular and intracellular protein fractions were not similar, indicating a selective release of these proteins, possibly using an unknown secretion system. Release of proteins due to partial cell lysis, cannot, however, be completely ruled out. Only a few highly specific intracellular proteins were identified. These types of so-called "non-classically" secreted proteins are sometimes referred to as "moon-ligthing" proteins, which means they have two or more functions (Boetzkes et al. 2012, Wang et al. 2013).

Wang et al. (2013) have writeen a review, which discusses how non-classically secreted proteins might be released into the medium. They discuss different views on the release of non-classically secreted proteins, and that cell lysis might not be the only cause of such release of proteins. These proteins could be secreted by new and unknown pathways, which have to be studied in details.

5.2 Further perspectives

The large number of extracellular proteins identified in culture supernatants of *P. mobilis* and *K. olearia*, including solute-binding proteins, hydrolytic enzymes, as well as previously uncharacterised proteins, makes these organisms interesting experimental systems for exoproteomic and biochemical studies. The large number of abundantly secreted uncharacterised proteins from *K. olearia* would be particularly interesting for further investigations, with potetional for identification of new functions or protein families. The following approaches are suggested to follow up this work:

1. Cloning and expression in *Escherichia coli*, of the genes encoding the uncharacterised proteins, followed by purification of recombinant product.
2. X-ray structural determination of the purified proteins to look for known structural functions and folds. This might give some clues for function.
3. Biochemical testing of hydrolytic activities using a number of polymeric substances. This could reveal new types of hydrolytic enzymes.

4. To perform knock-out experiments of the genes, followed up by search for phenotypic characteristics of the mutants, e.g. changes in substrate utilisation, growth rate and other characteristics.

The large number of apparently secreted proteins without any predicted signal peptide is also an interesting challenge. Whether these proteins are transported by an alternative secretory pathway (sometimes referred to as “Non-classical secretory pathway”), and have any biological function in the extracellular environment is not known. An approach to try to solve these questions, could be to compare and search in these proteins for common features, such as special amino acid composition, secondary structures, size, structural disorder, etc.

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7 APPENDIX

7.1 Protocols

7.1.1 718. Petrotoga medium from DSMZ

KCl	0.335 g
$MgCl_2$ x 6 H_2O	4.000 g
$MgSO_4$ x $7H_2O$	3.450 g
NH_4Cl	0.250 g
$CaCl_2$ x $2H_2O$	0.140 g
K_2HPO_4	0.140 g
NaCl	18.000 g
Trace element solution	10.000 mL
$Fe(NH_4)(SO_4)_2$ x $7H_2O$	2.000 mg
Resazurin	1.000 ml
$NaHCO_3$	1.000 g
Na_2S	0.500 g/L
Distilled water	1000.000 mL

Dissolve reagents (except bicarbonate and sulphide), boil medium for 1 min, then cool to room temperature under 80% N_2 + 20% CO_2 gas atmosphere. Add bicarbonate, dispense medium under same gas atmosphere in culture vessels and autoclave. After autoclaving, add sulphide and substrates as listed below from sterile stock solutions. Prior to inoculation adjust pH to 6.5-6.7, if necessary.

For DSM 10674 (*P. mobilis)*:

Yeast extract	0.2 g/L
Glucose	5.0 g/L
Vitamin solution (MIK 200)	10.0 mL/L

For DSM 10691 (*P. miotherma*):

Yeast extract	1.0 g/L
Trypticase	1.0 g/L
Glucose	5.0 g/L
Vitamin solution	10.0 mL/L

For DSM 13674 (*T. africanus*):

Yeast extract	1.0 g/L
Trypticase	1.0 g/L
Glucose	5.0 g/L
$Na_2S_2O_3$ x 5 H_2O	5.0 g/L
Vitamin solution	10.0 mL/L

For DSM 14811 (*P. mexicana*):

Yeast extract	0.2 g/L
$Na_2S_2O_3$ x $5H_2O$	2.0 g/L
Glucose	5.0 g/L
Vitamin solution	10.0 mL/L

For DSM 16923 (*P. halophila*):

Supplement medium prior to autoclaving with 50 g/L NaCl

Then add from sterile anoxic stock solutions:

Yeast extract	2.0 g/L
Glucose	5.0 g/L

7.1.2 Vitamin solution (MIK200 laboratory exercise 1)

4-aminobenzoic acid	8 mg/L
(D+) Biotin	2 mg/L
Nicotinic acid	20 mg/L
Ca-D(+) pantothenate	10 mg/L
Pyridoxamine x 2HCl	30 mg/L
Thiamine dichloride	20 mg/L

7.1.3 1163 KTM medium from DSMZ

NaCl	30.00 g
PIPES	6.70 g
KCl	0.33 g
$MgCl_2 \times 2H_2O$	0.90 g
$MgSO_4 \times 7H_2O$	1.40 g
NH_4Cl	0.25 g
$CaCl_2 \times H_2O$	0.14 g
KH_2PO_4	0.45 g
Yeast extract	5.00 g
Trace element solutions	10.00 mL
Resazurin	0.50 mg
Vitamin solution	10.00 mL
Maltose	5.00 g
Cysteine-HCl	0.50 g
Distilled water	1000.00 mL

Dissolve ingredients except vitamins and maltose. Prepare medium anoxically under 100% N_2, gas-atmosphere, adjust pH to 6.8 with NaOH, dispense in vials retaining anoxic conditions and autoclave. Add maltose and vitamins after autoclaving from anoxic stock solutions prepared under N_2 and sterilized by filtration.

For DSM 15011 (*Oceanotoga* sp.) and DSM 24906 (*O. teriensis*), replace maltose with glucose and adjust pH to 7.3-7.5.

For DSM 24739 (*Mesotoga prima*), replace PIPES with HEPES and adjust pH to 7.5 prior to autoclaving. Replace maltose with 5 g/L xylose and supplement medium with 2.5 g/L Na-thiosulfate added from an anoxic sterile stock solution.

7.1.4 141 Methanogenium medium, Vitamin solution from DSMZ

Nitrilotriacetic acid	1.50 g
$MgSO_4$ x $7H_2O$	3.00 g
$MnSO_4$ x H_2O	0.50 g
NaCl	1.00 g
$FeSO_4$ x $7H_2O$	0.10 g
$CoSO_4$ x $7H_2O$	0.18 g
$CaCl_2$ x $2H_2O$	0.10 g
$ZnSO_4$ $7H_2O$	0.18 g
$CuSO_4$ $5H_2O$	0.01 g
$KAl(SO_4)_2$ x $12H_2O$	0.02 g
H_3BO_3	0.01 g
Na_2MoO_4 x $2H_2O$	0.01 g
$NiCl_2$ x $6H_2O$	0.03 g
Na_2SeO_3 x $5H_2O$	0.30 g
Distilled water	1000.00 mL

First dissolve nitrilotriacetic acid and adjust p-h to 6.5 with KOH, then add minerals. Final pH 7.0 (with KOH).

7.1.5 SL-10 Trace Elements Solution

Milli-Q H_2O	800.000 mL
$FeCl_2$ x $4H_2O$	1.500 g
7.7 M HCl	10.000 mL
$CoCl_2$ x $6H_2O$	0.006 g
$MnCl_2$ x $4H_2O$	0.036 g
$ZnCl_2$	0.024 g
H_3BO_3	0.002 g
Na_2MnO_4 x $2H_2O$	0.190 g
$NiCl_2$ x $6H_2O$	0.100 g
$CuCl_2$ x $2H_2O$	0.070 g

Complete volume with Milli-Q water to 1000.000 mL

Use iron contaminated beaker, stir bar, and disposable pipettes. Protect solution from light. Put HCl in a beaker, add $FeCl_2$. Allow to mix and dissolve completely, add 800 mL milli-Q water into a beaker with a stir bar, add all other ingredients. Allow to mix and dissolve completely, adjust pH to 6.0, and complete volume with milli-Q water in a graduated cylinder.

7.2 Proteins without signal peptides

Table 7.1: Protein identification of *Kosmotoga olearia*, band 2

Accession	Description	Coverage	Peptides
C5CG15	Iron-containing alcohol dehydrogenase	63.93%	29
C5CDZ3	Glutamate dehydrogenase	46.52%	21
C5CHK9	Pyrrole quinoline quinone	55.81%	21
C5CET3	Pyrimidine nucleoside phosphorylase	38.95%	20
C5CHR8	Amidohydrolase	32.37%	18
C5CFP3	Enolase	40.19%	17
C5CE76	Alpha amylase, catalytic region	19.40%	17
C5CFT9	Transglutaminase domain protein	25.78%	16
C5CI61	Pyridoxal-5’-phosphate dependent protein, beta subunit	35.79%	16
C5CFX5	Uncharacterised protein	37.77%	14
C5CHH3	Dipeptidase	28.73%	14
C5CHS1	M20 DapE family protein, YgeY	38.10%	13
C5CEN6	Probable 2.3-bisphosphoglycerate-dependent phosphoglycerate mutase	31.75%	13
B5M6L7	60kDa chaperonin	12.24%	11
C5CFT5	Pyruvate phosphodikinase	9.52%	10
C5CJ07	Amidohydrolase	42.89%	10
C5CHP6	Cytosine deaminase and related metal-dependent hydrolase-like proteins	12.62%	10
C5CIH0	Glucose-6-phosphate isomerase	24.06%	9
C5CHM3	Phosphoglycerate kinase	21.73%	8
C5CGT4	Glycogen synthase	19.79%	8
C5CHD5	Phosphopentomutase	15.17%	7
C5CGR6	Elongating factor Tu	16.79%	7
C5CIA1	Pyridoxal phosphate dependent acyltransferase	15.78%	7
C5CIR2	Aminotransferase class V	15.53%	7
B5M6P0	Acetate kinase	14.63%	6
C5CF45	Probable glycine dehydrogenase, decarboxylating subunit 1	11.14%	6
C5CGG8	DNA polymerase III, subunit beta	14.99%	6
C5CDT7	Pyruvate flavodoxin/ferredoxin oxidoreductase	8.23%	6
C5CEA8	Serine hydroxymethyltransferase	14.45%	6
C5CF36	Protein translocase, subunit secD	2.89%	6
C5CD80	Glycosyl transferase group 1	16.59%	5
C5CHS5	Amidohydrolase	10.83%	5
C5CHS7	Threonine synthase	2.15%	5
C5CDG7	Glutamyl-tRNA (Gln) amidotransferase subunit 1	4.63%	4
C5CDK1	D-Lysine 56-aminomutase alpha subunit	1.92%	4
C5CDT2	Histidine-tRNA ligase	3.34%	4
C5CHS4	Amidohydrolase	2.20%	4
C5CFV9	Hydroxypyruvate reductase	3.61%	3
C5CG30	Peptidase S41	9.90%	3
C5CDF3	Uncharacterised protein	24.43%	3

Accession	Description	Coverage	Peptides
C5CEE6	Von Willebrand factor type A	3.59%	3
C5CG75	Quinolinate phosphoribosyl transferase	5.54%	3
C5CIX1	PhoH family protein	6.06%	3
C5CD68	ABC transporter related	6.37%	2
C5CIX5	HAD superfamily (Subfamily IIIA) phosphatase	1.71%	2
C5CHF6	Peptidase U62 modulator of DNA gyrase	7.66%	2
C5CGM0	Transketolase domain protein	1.89%	1
C5CGM7	Glucose-1-phosphate adenylyltransferase	2.84%	1
C5CDJ9	Pyridoxal-5'-phosphate dependent protein beta subunit	2.11%	1
C5CDG4	Carboxypeptidase Taq	2.20%	5

Table 7.2: Protein identification of *Kosmotoga olearia*, band 5.

Accession	Description	Coverage	Peptides
C5CG15	Iron-containing alcohol dehydrogenase	19.90%	11
C5CHK9	Pyrrolo-quinoline quinone	17.68%	9
C5CHM3	Phosphoglycerate kinase	14.57%	7
C5CDQ1	Methylmalonyl-CoA epimerase	54.07%	7
C5CFY5	Ferredoxin hydrogenase	1.88%	7
C5CHV3	Uncharacterised protein	15.58%	7
C5CHB0	Ribose-5-phosphate isomerase B	20.53%	6
C5CE42	OsmC family protein	22.46%	5
C5CGD5	Uncharacterised protein	7.24%	5
C5CG18	Peptide deformylase	21.95%	5
C5CFU7	OsmC family protein	12.93%	5
C5CDZ3	Glutamate dehydrogenase	7.91%	4
C5CD34	Cupin 2 conserved barrel domain protein	12.61%	3
C5CPF5	Elongating factor Ts	5.50%	3
C5CEB8	50S ribosomal protein, L19	15.65%	3
C5CHM2	Glycerol dehyde-3-phosphate dehydrogenase type 1	9.97%	3
C5CG28	Peptidase S41	5.31%	2
C5CD59	Trk-A-N domain protein	15.33%	2
C5CFQ4	Cytidine deaminase	8.83%	2
C5CG30	Peptidase S41	5.94%	2
C5CGM3	Nucleoside diphosphate kinase	11.56%	2
C5CH05	MaoC domain protein dehydratase	19.42%	2
C5CH11	Beta-hydroxyacyl-(Acyl-carrier-protein) dehydratase FabA/FabZ	9.35%	2
C5CIG5	Leucine-tRNA ligase	2.15%	2
C5CD95	Single-stranded DNA-binding protein	13.33%	2
C5CDT8	Thiamine pyrophosphatase domain protein, TPP-binding	36.02%	2
C5CGB5	Rubrerythrin	5.61%	2
C5CIN3	Desulfoferredoxin ferrous iron-binding region	12.71%	2
C5CF24	Uncharacterised protein	4.66%	1
C5CH09	Thioesterase superfamily protein	8.46%	1
C5CEEF	Von Willebrand factor type A	2.26%	1

Accession	Description	Coverage	Peptides
C5CF51	10 kDa chaperonin	11.24%	1
C5CFE4	Molybdopterin-binding domain protein	7.10%	1
C5CGR6	Elongating factor Tu	2.26%	1
C5CHC6	Uncharacterised protein	4.74%	1
C5CHG7	30S ribosomal protein S13	5.00%	2
C5CGN6	D-tyrosyl-tRNA /Tyr) deacyclase	8.11%	1

Table 7.3: Protein identification of *Petrotoga mobilis*, grown on maltodextrin, band 1.

Accession	Description	Coverage	Peptides
A9BEZ5	Pyruvate phosphate dikinase	58.35%	120
A9BGX9	Alphaglucan phosphorylase	48.19%	70
A9BI29	Isoleucine-tRNA ligase	43.96%	57
A9BF22	Leucine-tRNA ligase	37.08%	49
A9BGY0	Ribonucleoside diphosphate reductase	30.12%	42
A9BIJ7	Valine-tRNA ligase	32.27%	42
A9BFA3	Kijobiose phosphorylase	29.62%	35
A9BHA8	Elongating factor G	29.93%	34
A9BJ21	Peptidase S16 Ion-dominating protein	24.05%	33
A9BJE0	Alanine-tRNA ligase	21.46%	32
A9BJK9	Small GTP-binding protein	32.90%	32
A9BFH1	Phenylalanine-tRNA ligase beta subunit	22.96%	30
A9BFA2	Phosphopyruvate decarboxylase-related protein	18.86%	16
A9BIT7	Xanthine dehydrogenase	11.37%	13
A9BF41	ATPase AAA-2 domain protein	4.22%	12
A9BFV7	Short-chain dehydrogenase/reductase SDR	11.16%	11
A9BHL6	D-Lysine 56-aminomutase alpha subunit	8.06%	11
A9BF71	Flagellar hook-associated protein	8.44%	9
A9BHK4	60kDa chaperonin	11.11%	9
A9BH06	Glutamate synthase (NADPH homotetramer)	7.63%	7
A9BIT6	Aspartate/ornithine carbamoyltransferase, carbamoyl-P- binding	5.65%	7
A9BG95	Beta-lactamase domain protein	4.76%	4
A9BJ29	Glycoside hydrolase family 3 domain protein	1.54%	4
A9BG77	WD-40 repeat protein	3.50%	3
A9BJF0	Transposase IS204/IS1096/IS1165 family protein	1.59%	3
A9BI31	PAS7PAC sensor signal transduction histidine kinase	1.94%	13
A9BEU8	DNA polymerase	0.89%	8

Table 7.4: Protein identification of *Petrotoga mobilis*, grown on maltodextrin, band 2

Accession	Description	Coverage	Peptides
A9BHK4	60kDa chaperonin	61.67%	49
A9BIW3	Phosphoglucomutase/phosphomannomutase alpha/beta alpha domain	45.93%	39
A9BHJE	Hydrogenase Fe-only	32.51%	38
A9BIG1	Transketolase domain protein	35.18%	36

Accession	**Description**	**Coverage**	**Peptides**
A9BEZ5	Pyruvate phosphate dikinase	16.03%	35
A9BEV1	Hydrogenase Fe-only	33.91%	33
A9BI52	Chaperone protein DnaK	39.83%	32
A9BII4	Phosphoglycerate kinase, Triose-phosphate isomerase	41.98%	30
A9BI96	Peptidase M3A and M3B thimet/oligopeptidase	33.56%	29
A9BJZ1	CTP synthase	26.19%	27
A9BI24	Aspartate-tRNA ligase	24.87%	26
A9BHU9	Uncharacterised protein	19.83%	23
A9BH53	Methylmalonyl-CoA mutase, large subunit	26.12%	22
A9BHF0	Threonine-tRNA ligase	10.00%	16
A9BIV8	Formate-tetrahydrofolate ligase	14.98%	16
A9BFM4	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	11.58%	14
A9BGX7	NAD^+ synthetase	10.43%	13
A9BGA8	Proline-tRNA ligase	1.72%	12
A9BGR9	Uncharacterised protein	4.15%	7
A9BGV4	Glutamine-fructose-6-phosphate aminotransferase (isomerising)	3.76%	7
A9BG70	Peptidase M28	1.22%	4
A9BIP1	2,5-didehydrogluconate reductase	4.38%	6

Table 7.5: Total protein identification of *Thermosipho africanus*

Accession	**Description**	**Coverage**	**Peptides**
B7IFM2	Pyruvate phosphate dikinase	33.71%	66
B7IFK4	Alcohol dehydrogenase	62.13%	54
B7IHU4	Elongating factor Tu	59.75%	52
B7IDJ1	Translation elongating factor G	31.24%	42
B7IDE9	Ferritin	8.14%	36
B7IHF2	2-oxogluterate oxidoreductase, alpha subunit	3.12%	34
B7IFZ5	Pyruvate synthase subunit PorD	24.55%	33
B7IFA6	60kDa chaperonin	27.46%	33
B7IH48	Indolepyruvate ferredoxin oxidoreductase , alpha subunit	9.81%	32
B7IFW8	Leucine-tRNA ligase	3.76%	30
B7IDG8	Gap glyceraldehyde-3-phosphate dehydrogenase, type I	48.19%	29
B7IFN4	Enolase	47.79%	28
B7IEA0	Valine-tRNA ligase	1.92%	26
B7IGN4	Fe-hydrogenase, subunit beta	1.60%	26
B7IGN5	Hydrogenase-1	4.41%	26
B7IG24	Glutamate dehydrogenase	24.59%	24
B7IDD0	Chaperone protein	19.73%	24
B7IF30	Urocanate hydrolase	10.02%	22
B7ICV4	Uncharacterised protein	35.08%	21
B7IGB0	Rubrerythrin	14.29%	21
B7IGK6	Uncharacterised protein	16.00%	21
B7IFZ6	Pyruvate/ketoisovalerate oxidoreductase, common subunit gamma	42.41%	21

Accession	Description	Coverage	Peptides
B7IGE5	Maltose ABC transporter, periplasmic maltose-binding protein	16.88%	20
B7IDI0	Ribosome recycling factor	38.92%	20
B7IFZ4	Pyruvate synthase subunt PorA	17.09%	20
B7ICR4	50s ribosomal protein, L77L12	18.75%	20
B7IDZ6	3-oxoacyl-(Acyl-carrier-protein) synthase 2	16.42%	19
B7IF24	Probable glycine dehydrogenase (decarboxylating) subunit 2	4.38%	18
B7IDU0	Methenyltetrahydrofolate cyclohydrolase	14.49%	16
B7IEZ7	Pyridoxal biosynthesis lyase, PdxS	20.21%	16
B7IH22	6-phosphofructokinase	3.80%	16
B7IHP8	Conserved oxidoreductase	10.21%	15
B7IFB8	Glutamate-tRNA ligase	7.82%	15
B7IFH0	2-oxoglutarate oxidoreductase, gamma subunit	5.59%	15
B7ICR8	Flagellin	8.53%	15
K2PJF6	Uncharacterised protein	4.64%	14
B7IDF1	Desulfoferredoxin	28.57%	14
B7ID58	8-amino-7-oxononanate synthase	20.97%	14
B7IDG7	Phosphoglycerate kinase	8.21%	14
B7IFZ3	Pyruvate oxidoreductase, beta subunit	8.31%	14
B7IDH4	Fba fructose-1,6-bisphosphate aldolase, class II	16.35%	14
B7IDR1	2’-3’-cyclic-nucleotide 2’-phosphodiesterase	4.50%	14
B7IF92	Iron (III) transporter, periplasmic-binding	25.34%	13
B7IGT2	Transcriptional regulating protein, ResD	35.29%	13
B7IDP1	Lipopolysaccharide biosynthesis protein	5.75%	13
B7IFB0	TldD protein	1.95%	13
B7IFH8	Aminotransferase, class I	11.44%	13
B7IHS0	Oligopeptidase F	4.32%	13
B7IEF9	Uncharacterised protein	13.10%	13
B7IDT9	Enzyme of the cupin superfamily	12.36%	12
B7IEU5	Udk phosphoribulokinase family protein	1.61%	12
B7IG23	Acyl carrier protein	42.11%	12
B7IFT5	Tail-specific protease	5.21%	12
B7IHF7	Nicotinate-nucleotide dimethylbenzimidazole phosphoribosyltransferase	8.72%	11
B7IHQ9	Outer membrane protein, Omp 85 family, putative	2.35%	11
B7IFH5	Probable butyrate kinase	5.79%	11
B7IFH6	Phosphate acetyltransferase	13.20%	10
B7IFI0	Soluble hydrogenase 42kDa subunit	17.89%	10
B7IHC1	Phosphoglucomutase/phosphomannomutase family protein	1.92%	10
B7ID59	L-threonine-3-dehydrogenase	11.08%	10
B7IHE6	Serine hydroxylmethyltransferase	3.77%	10
B7IHX5	Trigger factor	8.97%	10
B7IHL4	Dihydropteroate synthase	5.07%	10
B7IGU6	Uncharacterised protein	8.33%	10
B7IH65	Cationic outer membrane protein	12.96%	10
B7ICT0	Asparigine-tRNA ligase	9.05%	9

Accession	Description	Coverage	Peptides
B7IEV2	S-adenosylmethionine synthase	4.81%	9
B7IFR9	Endoglucanase M	8.68%	9
B7IGU1	Trehalose/maltose-binding protein	1.93%	9
K2VQH4	Glyoxalase/bleomycin resistance protein/dioxygenase	19.26%	9
B7ICR1	Amidohydrolase family protein	2.11%	9
B7IF29	Succinyl-diaminopimelate desuccinylase	2.95%|	9
B7IGC5	Glycerol kinase	2.25%	8
B7IGN3	Fe-hydrogenase, gamma subunit	11.46%	8
B7IDT4	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	5.22%	8
B7IHS8	Putative chemotaxis protein, CheY-like protein	20.83%	8
B7IDJ9	Flagellar basal body associated protein, FliL	7.47%	8
B7IFA7	10kDa chaperonin	44.44%	7
B7IDQ8	Uncharacterised protein	3.48%	7
B7IFQ1	Uncharacterised protein	9.19%	7
B7IHC7	Uncharacterised protein	1.98%	7
B7IHW5	50S ribosomal protein, L15	13.61%	7
B7IHE8	Prepilin-type N-cleavage/methylation domain protein	22.70%	6
B7IDZ8	Putative enoyl-(Acyl-carrier-protein) reductase II	8.06%	6
B7IF63	Phosphate acetyltransferase	9.52%	6
B7IFI7	Stage V sporulating protein S	29.89%	6
B7IFZ0	Purine-binding chemotaxis protein	26.49%	6
B7IH58	Uncharacterised protein	35.14%	5
B7IDK5	CBS domain protein	11.50%	5
B7IES9	Exopolyphosphate family protein	5.18%	5
B7IEY2	Adenine phosphoribosyltransferase	8.82%	5
B7IFY6	Orotidine 5’-phosphate decarboxylase	4.93%	5
B7IH23	NAD-dependent butanol dehydrogenase a	6.53%	5
B7IE71	Uncharacterised protein	6.17%	5
B7IHV4	50S ribosomal protein, L29	21.21%	5
B7IEY3	Glucose-6-phosphate isomerase	2.90%	4
B7IG99	50S ribosomal protein, L1	6.47%	4
B7IGW9	Polysaccharide report protein, putative	1.78%	4
B7IDE8	Nitric oxide reductase	2.58%	4
B7IHM4	Uncharacterised protein	7.28%	4
B7IHU7	50S ribosomal protein, L4	8.14%	4
B7ICJ8	Extradiol ring-cleavage dioxygenase, class III enzyme, subunit B	16.76%	3
B7IGH6	DNA-binding protein, HU1	17.39%	3
B7ICS3	Endoribonuclease L-PSP	8.87%	3
B7IHV2	30S ribosomal protein, S3	4.78%	3
B7ICY6	Methylmalonyl-CoA epimerase	23.19%	2
B7IFM6	50S ribosomal protein, L25	4.61%	1
B7ICT6	DNA polymerase III, subunit beta	7.36%	12
B7IEP7	Transketolase	1.61%	19
B7IGA9	Glycyl-tRNA synthetase, beta subunit	3.92%	17
B7IH51	Phosphate propanoyltransferase	8.67%	6
B7IHP7	Metal-accepting chemotaxis protein 4	1.69%	8

Accession	Description	Coverage	Peptides
B7IFL2	K^+ channel, beta subunit	2.50%	21
B7IFW7	Basic membrane protein, putative	2.10%	15

Table 7.6: Total protein identification of *Petrotoga mobilis*

Accession	Description	Coverage	Peptides
A9BHI4	NADH flavin oxidoreductase	12.65%	93
A9BEZ5	Pyruvate phosphate dikinase	42.89%	75
A9BGX9	Alpha-glucan phosphorylase	13.57%	44
A9BI29	Isoleucine-tRNA ligase	9.36%	39
A9BHF0	Threonine-tRNA ligase	16.72%	38
A9BI22	Carboxypeptidase Taq	20.84%	36
A9BFV2	NADH dehydrogenase (quinone)	12.78%	36
A9BHA7	Elongating factor Tu	51.38%	35
A9BFU5	Heavy metal transport/detoxification protein	21.74%	33
A9BHK4	60kDa chaperonin	38.15%	33
A9BIS7	Enolase	58.56%	32
A9BF90	Glycoside hydrolase family 4	52.48%	31
A9BHA8	Elongating factor G	23.31%	31
A9BII5	Glyceraldehyde-3-phosphate dehydrogenase, type I	46.16%	30
A9BFG8	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase, subunit B	10.06%	30
A9BHU9	Uncharacterised protein	12.27%	29
A9BIV8	Formate-tetrahydrofolate ligase	31.95%	28
A9BJ81	Glycerol kinase	2.61%	28
A9BG71	Alpha-glucosidase	5.22%	28
A9BIU0	Nucleotide sugar dehydrogenase	38.16%	27
A9BJ12	Flagellin domain protein	41.49%	27
A9BFA2	Phsophopyruvate decarboxylase-related protein	31.51%	27
A9BFV1	Hydrogenase. Fe-only	17.99%	27
A9BJ00	Alpha amylase, catalytic region	18.16%	27
A9BII4	Phosphoglycerate kinase, Triose-phosphate isomerase	33.59%	26
A9BFM9	Alpha amylase, catalytic subdomain	22.93%	26
A9BH06	Glutamate synthase	4.28%	26
A9BJ57	D-isomer specific 2-hydroxyacid dehydrogenase, NAD-binding	53.33%	25
A9BHJ5	Hydrogenase, Fe-only	14.59%	25
A9BFU2	Sulfate adenylyltransferase	12.24%	25
A9BHL0	Peptidase U62 modulator of DNA gyrase	17.87%	25
A9BI52	Chaperone protein, DnaK	32.00%	24
A9BGY0	Ribonucleoside diphosphate reductase	11.55%	24
A9BJK9	Small GTP-binding protein	16.67%	24
A9BIG1	Transketolase domain protein	26.31%	24
A9BGA8	Proline-tRNA ligase	14.26%	24
A9BGA5	Methionine-tRNA ligase	8.99%	24
A9BGH1	1,4-alpha-glucan branching enzyme, GlgB	1.65%	24
A9BIW3	Phosphoglucomutase/phosphomannomutase alpha/beta alpha domain	30.85%	23

Accession	Description	Coverage	Peptides
A9BIB0	Trigger factor	23.70%	23
A9BHJ6	NADH dehydrogenase (quinone)	32.43%	22
A9BK!6	3-oxoacyl-(acyl-carrier-protein) synthase 2	19.51%	22
A9BQ68	Glycogen synthase	13.87%	22
A9BJ39	Urocanate hydratase	1.78%	22
A9BFC1	Lysine-tRNA ligase	11.955%	21
A9BJ05	Histidine-tRNA ligase	19.24	21
A9BJE0	Alanine-tRNA ligase	2.63%	21
A9BFH1	Phenylalanine-tRNA ligase	1.85%	21
A9BIP1	2,5-didehydroglucanate reductase	41.97%	20
A9BI96	Peptidase M3A and M3B thimet/oligopeptidase F	10.62%	20
A9BF22	Leucine-tRNA ligase	7.13%	20
A9BG95	Beta-lactamase domain protein	30.08%	20
A9BEV7	Rubrerythrin	41.67%	20
A9BH73	Polyribonucleotide nucleotidyltransferase	13.81%	20
A9BIJ7	Valine-tRNA ligase	6.41%	20
A9BI24	Aspartate-tRNA ligase	8.18%	19
A9BGN9	Glutamate dehydrogenase	16.24%	19
A9BH53	Metylmalonyl-CoA mutase, large subunit	11.81%	19
A9BJ80	FAD-dependent oxidoreductase	21.26%	18
A9BFW9	M18 family aminopeptidase	33.05%	18
A9BGW5	Phosphoglucomutase/phosphomannomutase alpha/beta alpha domain	21.47%	18
A9BHF5	Alkyl hydroxyperoxidase-like protein, AhpD family	15.94%	18
A9BG26	Uncharacterised protein	29.72%	18
A9BGT2	Protein GrpE	8.97%	18
A9BJA6	Tryptophanase	2.60%	18
A9BJZ9	Imidazole glycerolphosphate synthase, subunit HisF	59.52%	17
A9BK02	Iron-containing alcohol dehydrogenase	24.23%	17
A9BEX9	Uncharacterised protein	25.36%	17
A9BGL0	8-amino-7oxononanoate synthase	40.97%	17
A9BIK8	Serine hydroxymethyltransferase	11.82%	17
A9BFL6	Basic membrane lipoprotein	1.60%	17
A9BHL1	Peptidase U62 modulator of DNA gyrase	23.42%	17
A9BFA3	Kojibiose phosphorylase	8.52%	17
A9BHL4	Pyridoxal-5-phosphatase-dependent protein, beta subunit	6.57%	17
A9BI57	Arginine-tRNA ligase	4.37%	17
A9BHX2	S-adenosylmethionine synthase	28.68%	17
A9BHU7	Asparigine-tRNA ligase	15.21%	17
A9BK01	Histidinol-phosphate aminotransferase	42.12%	16
A9BH70	Glutaredoxin-like domain protein	44.84%	16
A9BGK8	Amidohydrolase	23.12%	16
A9BJK5	Two-component transcriptional regulator, winged helix family	27.69%	16
A9BGT8	Glutamate-tRNA ligase 2	19.26%	16
A9BJB2	Glutamate-tRNA ligase 1	19.70%	16

A9BJ45	Peptidae S9 prolyl oligopeptidase, active site domain protein	4.65%	16
A9BHV4	Adenylsuccinate synthase	11.90%	16
A9BI68	Aldo/keto reductase	10.08%	16
A9BI98	Arginine biosynthesis bifunctional protein, ArgJ	2.70%	16
A9BID2	Pyruvate kinase	20.92%	16
A9BJ56	Response regulator receiver protein	42.15%	15
A9BG66	Acetylornithine deacetylase or succinyldiaminopimelate desuccinylase	26.78%	15
A9BJ201	Adenylsuccinate lyase	2.31%	15
A9BGW7	t-RNA pseudouridine synthase A	11.43%	15
A9BEX3	Serine-tRNA ligase	22.17%	15
A9BHG2	Uncharacterised protein	8.29%	14
A9BET9	Ornithine carbamoyltransferase, catabolic	27.05%	14
A9BG29	Glucose-6-phosphate isomerase	28.45%	14
A9BFV0	Glycoside hydrolase, family 38	4.01%	14
A9BIK9	Putative transcriptional regulator, GntR family	3.87%	14
A9BHJ7	NADH dehydrogenase, (ubiquinone) 24kDa subunit	26.62%	14
A9BJN9	Glucose-1-phosphate adenylyltransferase	2.63%	14
A9BFT0	Basic membrane lipoprotein	35.71%	13
A9BI63	Phosphoribosylaminoimidazole carboxamide formyltransferase	19.84%	13
A9BIT2	Pyridoxal-5’-phosphate-dependent protein, beta subunit	13.11%	13
A9BIG4	Acetate kinase	8.23%	13
A9BGT6	Cytidine deaminase	9.09%	13
A9BH57	Carboxyl transferase	9.46%	13
A9BJG3	Fructose-1,6-bisphosphate aldolase, class II	29.49%	13
A9BJ35	Rubrerythrin	12.43%	13
A9BIM0	Cysteine synthase	47.64%	13
A9BGW0	Ribosomal L11 methyltransferase	4.61%	13
A9BH43	Acetamidase/Formamidase	15.46	13
A9BIK7	Adenosyl/homocysteinase	20.34%	13
A9BGG5	DegV family protein	9.59%	13
A9BHK0	PfkB domain protein	26.33%	13
A9BH91	Pyruvate/ketoisovalerate oxidoreductase, gamma subunit	54.97%	12
A9BG61	Uncharacterised protein	55.91%	12
A9BGX5	Sucrose-phosphate synthase	14.41%	12
A9BHK7	Probable transaldolase	37.56%	12
A9BHV1	Phosphodiesterase, MJ0936 family	17.72%	12
A9BIV9	Bifunctional protein, FolD	37.00%	12
A9BI78	Uncharacterised protein	21.19%	12
A9BFL1	6-phosphogluconate dehydrogenase, decarboxylating	15.47%	12
A9BIZ4	Glutamine synthase	17.46%	12
A9BJQ6	Gammaglutamyltransferase	2.81%	12
A9BFE4	PfkB domain protein	22.19%	12
A9BJP3	Glycerolphosphoryldiester phosphodiesterase	30.93%	12
A9BG28	Adenine phosphoribosyltransferase	31.21%	11

Accession	Description	Coverage	Peptides
A9BEU2	Glycosyltransferase family 2	18.42%	11
A9BHF1	Peptide chain release factor 1	4.21	11
A9BHW0	Purine nucleoside phosphorylase	40.36%	11
A9BFW2	O-acetylhomoserine/O-acetylserine sulfhydrylase	10.02%	11
A9BGS7	UDP-N-acetylmuromoylalanine-D-glutamate ligase	45.70%	11
A9BFM6	Alkyl hydroxyperoxide/Thiol-specific antioxidant/Mal allergen	43.70%	11
A9BIU4	(2Fe-2S)-binding domain protein	8.54%	11
A9BJY2	Uncharacterised protein	19.33%	11
A9BF11	Flagellar basal-body rod protein, FlgG	9.96%	11
A9BFM4	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	7.02%	11
A9BH88	Thiamine pyrophosphate domain protein, TPP-binding	19.69%	11
A9BJ51	Alpha amylase catalytic region	5.96%	11
A9BER7	GMP synthase, (glutamine hydrolysing)	4.54%	11
A9BEX7	Flagellar hook-length control protein	2.52%	11
A9BF70	Flagellar hook-associated protein 3	3.02%	11
A9BGZ9	L-lactate dehydrogenase	18.89%	11
A9BX9	Bifunctional purine biosynthesis protein, PurH	2.36%	11
A9BK15	DegV family protein	15.96%	11
A9BJX2	6-phsophofructokinase	15.09%	11
A9BK09	Formiminotransferasee, cyclodeaminase	22.93%	10
A9BJZ5	Hstidinol dehydrogenase	10.13%	10
A9BG84	Peptidase M42 family protein	19.76%	10
A9BGT9	Cysteine-tRNA ligase	2.35%	10
A9BHC5	Metylthioribose-1-phosphate isomerase 2	15.47%	10
A9BHJ9	Homoserine dehydrogenase	10.21%	10
A9BI62	t-RNA synthetase, class II (D, K and N)	13.48%	10
A9BJN3	GTPase-like protein	9.32%	10
A9BF34	50S ribosomal protein, L7/L12	21.03%	10
A9BFG0	Cell wall hydrolase/autolysin	7.69%	10
A9BHA4	50S ribosomal protein, L4	11.76%	10
A9BJU1	Oxidoreductase domain protein	13.17%	10
A9BF66	CheW protein	21.43%	10
A9BFK8	GCN-5-related N-acetyltransferase	7.86%	10
A9BFR6	Alpha/beta hydrolase fold	5.32%	10
A9BGS5	Aminotransferase, class I and II	8.83%	10
A9BHT7	Nicotinic acid phosphoribosyltransferase-like protein	11.24%	10
A9BIC7	Aminomethyltransferase	5.67%	10
A9BJN0	Pseudouridine-5’-phosphate glycosidase	10.13%	10
A9BJ15	Probable endonuclease 4	10.80%	10
A9BK13	2-nitropropane dioxygenase, NDP	30.60%	9
A9BJT9	DegT/Dnr/EryC/StrS aminotransferase	9.02%	9
A9BGR9	Uncharacterised protein	13.29%	9
A9BJT8	Transcriptional regulator, MarR family	12.09%	9
A9BEU0	Carbamate kinase	13.42%	9
A9BF30	Tryptophanyl-tRNA synthetase	9.54%	9
A9BHP9	Phospho-2-dehydro-3-oxyheptonate aldolase	6.78%	9

Accession	Description	Coverage	Peptides
A9BHV9	Hypoxanthine phosphoribosyltransferase	54.12%	9
A9BIC5	Histone family protein, DNA-binding protein	30.00%	9
A9BIJ9	AMMERCR1 domain protein	37.57%	9
A9BFZ1	30S ribosomal protein, S4	20.85%	9
A9BHL7	DNA polymerase III, subunit beta	17.30%	9
A9BIU8	Dihydroorotate dehydrogenase family protein	10.47%	9
A9BEM3	Linocin-M18 bacteriocin protein	19.25%	9
A9BEN0	Deoxyribose-phosphate aldolase	21.69%	9
A9BH01	Nitroreductase	12.04%	9
A9BHA2	50S ribosomal protein, L2	16.79%	9
A9BHJ8	Threonine synthase	6.50%	9
A9BIR0	D-isomer-specific 2-hydroxyacid dehydrogenase	16.25%	9
A9BIT3	Threonine synthase	4.99%	9
A9BIT7	Xanthine synthase	3.29%	9
A9BFV7	Short-chain dehydrogenase/reductase, SDR	16.33%	9
A9BG82	Peptidase M42 family protein	9.83%	9
A9BGK1	Isochorismatase hydrolase	6.97%	9
A9BHA5	50S ribosomal protein, L3	20.19%	9
A9BIC8	Probable glycine dehydrogenase, (decarboxylating), subunit 1	3.35%	9
A9BIE3	Phosphate-specific transport system accessory protein, PhoU	5.45%	9
A9BIS5	Elongating factor Ts	15.23%	9
A9BJ43	Glycine cleavage system H protein	65.52%	8
A9BEN1	Cold-shock DNA-binding domain protein	81.54%	8
A9BHL2	Dihydropicolinate reductase	20.71%	8
A9BHK3	10kDa chaperonin	53.93%	8
A9BER6	Phosphoglycerate mutase	7.83%	8
A9BGE8	dDTP-4-dehydrorhamnose reductase	10.74%	8
A9BHY1	Response regulator receiver protein	25.21%	8
A9BIA4	N-acetyl gamma-glutamyl-phosphate reductase	5.22%	8
A9BJA5	Endoribonuclease L-PSP	39.37%	8
A9BFV3	NADH dehydrogenase (quinone)	12.10%	8
A9BIB5	Alpha amylase catalytic region	.2.82%	8
A9BIX4	Type III pantothenate kinase	4.71%	8
A9BJ18	Gamma-glutamyl phosphate reductase	3.12%	8
A9BJZ4	ATP phosphoribosyltransferase	40.00%	8
A9BEZ1	Aminotransferase, class I and II	6.48%	8
A9BFC0	Transcription elongating factor, GreA	5.66%	8
A9BFG4	Thiamine pyrophosphate protein domain protein, TPP-binding	3.97%	8
A9BHV3	Carbohydrate kinase, YjeF-related protein	2.65%	8
A9BIU6	Aldehyde oxidase and xanthine dehydrogenase, molybdopterin-binding	7.55%	8
A9BFB2	Stage V sporulating protein S	43.33%	7
A9BI87	Flagellar basal body rod protein, FlgB	7.52%	7
A9BFZ7	Adenylate kinase	29.77%	7
A9BGT7	NUDIX hydrolase	18.75%	7

Accession	Description	Coverage	Peptides
A9BH78	Phosphoesterase, recJ domain protein	9.55%	7
A9BIY8	Uracil phosphoribosyltransferase	30.14%	7
A9BJ01	Glucose-1-phosphate thymidyltransferase	12.04%	7
A9BK05	Phosphoribosyl-ATP diphosphatase	25.23%	7
A9BEQ8	Stage V sporulating protein S	30.30%	7
A9BFU6	Appr-1-processing domain protein	30.26%	7
A9BFN4	Putative phosphohistidine phosphatase, SixA	23.60%	7
A9BHU5	Alanine racemase domain protein	12.99%	7
A9BJD6	Ribosome-recycling factor	32.62%	7
A9BEW2	PEBP family protein	8.97%	7
A9BFW0	Acetate kinase	6.23%	7
A9BG77	WD-40 repeat protein	1.38%	7
A9BHV6	Glutamate formimidoyltransferase	12.13%	7
A9BI89	Flagellar hook-basal body complex proten, FliE	29.29%	7
A9BIL1	Phosphate butyryltransferase	4.67%	7
A9BIU9	Adenine deaminase	5.09%	7
A9BEN2	Elongating factor P	4.32%	7
A9BF98	Alkyl hydroxyperoxide reductase, Thiol-specific antioxidant/Mal allergen	32.72%	7
A9BH59	Biotin/lipoyl attachment domain-containing protein	19.87%	7
A9BK08	Uncharacterised protein	16.39%	7
A9BF38	Transcription termination/antitermination protein, NusG	4.24%	6
A9BGG0	Uncharacterised protein	23.68%	6
A9BIJ2	50S ribosomal protein, L25	28.31%	6
A9BF96	Carohydrate kinase, FGGY	1.86%	6
A9BFS9	Beta-lactamase domain protein	23.74%	6
A9BIC9	Probable glycine dehydrogenase, (decarboxylating) subunit 2	9.00%	6
A9BJR6	Transferase hexapeptide repeat-containing protein	14.36%	6
A9BK11	Short-chain dehydrogenase/reductase, SDR	16.53%	6
A9BEQ1	Phosphoglucosamine mutase	4.09%	6
A9BFA4	Beta-phosphoglucomutase	23.83%	6
A9BFX5	ATP synthase, subunit alpha	3.37%	6
A9BGK3	Tertatricopeptide, TPR-2 repeat protein	2.51%	6
A9BGW3	Uncharacterised protein	6.13%	6
A9BI30	Response regulator receiver protein	38.66%	6
A9BIA8	Isocitrate dehydrogenase (NAD(+))	8.76%	6
A9BIZ9	Inisitol-phosphate phosphatase	14.34%	6
A9BJ52	HAD superfamily hydrolase, subfamily IA, variant 1	5.98%	6
A9BJK7	Pyrimidine-nucleoside phosphorylase	5.44%	6
A9BJU7	Uncharacterised protein	15.75%	6
A9BJZ8	1-(5-phosphoribosyl)-5((5-phosphorbosylamino)-methylideneamino)-imidazole-4-carboxamide isomerase	14.96%	6
A9BFZ6	Methionineaminopeptidase	4.40%	6
A9BHK1	Aldo/keto reductase	20.26%	6
A9BHR1	ROK family protein	7.59%	6

A9BIL6	Thiamine pyrophosphate protein, domain protein, TPP-binding	5.58%	6
A9BJ25	Xylulokinase	2.65%	6
A9BJZ7	Imidazole glycerol phosphate synthase, subunit HisH	15.31%	6
A9BJ61	Uncharacterised protein	24.24%	5
A9BJY4	ATP-dependent Clp protease, proteolytic subunit	14.63%	5
A9BGZ6	Beta-lactamase domain protein	19.72%	5
A9BJZ3	ATP phosphoribosyltransferase, predicted regulatory subunit	5.75%	5
A9BES4	Csf-like hydrolase	14.80%	5
A9BAA9	30S ribosomal protein, S7	18.06%	5
A9BHR8	4-hydroxy-tetrahydrodipicolinate synthase	12.33%	5
A9BIX1	ABC transporter related	3.83%	5
A9BER5	2-phosphosulfolactate phosphorylase	8.15%	5
A9BEW9	Uncharacterised protein	4.08%	5
A9BF99	Ferroxidase	14.72%	5
A9BFB0	PhoH family protein	9.11%	5
A9BH60	Trk-A-N domain protein	3.18%	5
A9BG01	30S ribosomal protein, S5	16.38%	5
A9BH65	50S ribosomal protein, L9	22.82%	5
A9BH95	50S ribosomal protein, L14	9.02%	5
A9BHA0	50S ribosomal protein, L22	9.40%	5
A9BII3	GNC5-related N-acetyltransferase	13.17%	5
A9BII6	Nucleotide-associated protein, Pmob-1446	10.16%	5
A9BIU3	Molybdopterin dehydrogenase, FAD-binding	4.92%	5
A9BIZ0	Also/keto reductase	4.03%	5
A9BIL2	Probable butyrate kinase	9.58%	4
A9BI17	Sigma 54 modulation protein, ribosomal protein, S30EA	23.62%	4
A9BI88	Flagellar basal body rod protein, FlgC	17.86%	4
A9BJU5	HAD superfamily hydrolase, subfamily IA, variant 3	10.61%	4
A9BK06	Probable nicotinate-nucleotide adenylyltransferase	15.22%	4
A9BEZ9	UFP0109 protein, Pmob-0321	51.32%	4
A9BF74	Probable peroxiredoxin	12.96%	4
A9BFL8	HAD superfamily hydrolase, subfamily IA, variant 3	4.98%	4
A9BFU1	Adenylyl-sulfate kinase	6.97%	4
A9BFZ9	50S ribosomal protein, L15	24.50%	4
A9BH56	Methylmalonyl-CoA epimerase	17.16%	4
A9BJB3	Uncharacterised protein	13.22%	4
A9BEQ3	Orotate phosphoribosyltransferase	6.12%	4
A9BEV1	2’-5’-RNA ligase	5.45%	4
A9BF49	MOSC domain-containing protein	38.89%	4
A9BF76	Methenyl tetrahydrofolate cyclohydrolase-like protein	10.88%	4
A9BGV5	Ribosomal silencing factor, RefS	7.83%	4
A9BH33	Ribosomal maturation factor, RimP	8.92%	4
A9BIK4	Maf-like protein, Pmob-14641	7.89%	4
A9BIX5	Sua5/YciO/YrdC/YwlC family protein	3.78%	4
A9BEW1	Uncharacterised protein	27.44%	3
A9BGK9	Alcohol dehydrogenase, GroES domain protein	8.00%	3

Accession	Description	Coverage	Peptides
A9BFU0	50S ribosomal protein, L20	17.36%	3
A9BH62	30S ribosomal protein, S6	7.20%	3
A9BH77	Purine nucleoside phosphorylase	8.52%	3
A9BH97	50S ribosomal protein, L29	36.36%	3
A9BJG8	CoA-binding domain protein	15.86%	3
A9BET4	Uncharacterised protein	17.33%	3
A9BFY9	50S ribosomal protein, L17	10.94%	3
A9BG00	50S ribosomal protein, L30	19.67%	3
A9BG02	50S ribosomal protein, L18	7.38%	3
A9BGL6	Macrophage migration inhibitory factor, family protein	8.04%	3
A9BHF7	Phosphate propanoyltransferase	4.59%	3
A9BIM5	Peptidase M24	5.08%	3
A9BIZ1	HAD superfamily hydrolase, subfamily IIA,	5.42%	3
A9BH99	30S ribosomal protein, S3	9.05%	2
A9BF03	50S ribosomal protein, L19	8.40%	2
A9BF69	Flagellar assembly factor, FliW	9.55%	2
A9BFV4	NADH dehydrogenase (ubiquinone) 24kDa subunit	12.43%	2
A9BF62	MoaD family protein	13.19%	2
A9BGE0	Uncharacterised protein	19.70%	2
A9BH75	Metal-dependent phosphorylase	4.05%	2
A9BHD4	Uncharacterised protein	12.36%	2
A9BJG5	Ferripyochelin-binding protein, (Fbp)	5.36%	2
A9BJG6	PHP domain protein	5.99%	2
A9BJZ0	Acyl carrier protein	14.12%	2
A9BGA6	YcfA family protein	17.57%	2
A9BIE9	D-tyrosyl-tRNA (Tyr) deacyclase	11.63%	1
A9BJY6	Uncharacterised protein	10.00%	1
A9BI32	ATPase AAA-2 domain protein	0.00%	42
A9BHK9	Hydroxylalanine reductase	0.00%	26
A9BIG7	PAS modulation sigma 54 specific transcriptional regulator, Fis family	4.59%	9
A9BFA0	Methyl-accepting chemotaxis sensory transducer	0.00%	29
A9BF71	Flagellar hook-associated protein, FlgK	1.34%	19
A9BJ21	Peptidase Ion domain protein	1.39%	11
A9HIU0	Phage SPO1 DNA polymerase-related protein	16.37%	8
A9BK12	Malonyl-CoA-acyl carrier protein, transacyclase	10.65%	4
A9BFX3	ATP synthase, subunit beta	7.49%	12
A9BHZ6	DEAD/DEAH-box helicase domain protein	0.00%	22
A9BF47	Alpha amylase catalytic region	1.82%	19
A9BIT4	M20 DapE familyprotein, YgeY	3.00%	12
A9BJF8	Methylthioribose kinase	3.21%	11
A9BGR4	CoA substrate-specific enzyme activase	0.00%	26
A9BJ03	DegT/DnrJ/EryC/StrS aminotransferase	3.60%	14
A9BFL9	ATP-dependent zinc metalloprotease, FtsH1	2.45%	13
A9BFD9	DNA topoisomerase	0.00%	16

Table 7.7: Total protein identification of *Kosmotoga olearia*

Accession	**Description**	**Coverage**	**Peptides**
C5CG15	Iron-containing alcohol dehydrogenase	77.11%	99
C5CFT5	Pyruvate phosphate dikinase	51.02%	75
C5CE76	Alpha amylase, catalytic region	6.98%	69
B5M6L7	60kDa chaperonin	69.02%	52
C5CHK9	Pyrrolo-quinoline quinone	52.02%	45
C5CEP1	ABC-type nitrate/sulfonate/bicarbonate transport system, periplasmic components-like protein	79.42%	42
C5CFP3	Enolase	78.74%	41
C5CHA1	Isoleucine-tRNA ligase	14.12%	36
C5CIR2	Aminotransferase, class V	52.89%	32
C5CIR7	NADH dehydrogenase (quinone)	2.67%	31
C5CGU7	Probable transcriptional/regulatory protein, Kole-1935	17.86%	30
C5CHM3	Phosphoglycerate kinase	54.57%	30
C5CIG4	Hydrogenase, Fe-only	4.97%	29
C5CET5	Translation silencing factor G	32.17%	29
C5CHR8	Amidohydrolase	9.76%	28
C5CDT6	Pyruvate ferredoxin/flavodoxin oxidoreductase, delta subunit	38.78%	26
C5CEI1	Iron-containing alcohol dehydrogenase	57.97%	25
C5CI61	Pyridoxal-5’-phosphate-dependent protein, beta subunit	48.37%	25
C5CIR3	D-isomer specific 2-hydroxyacid dehydrogenase, NAD-binding	50.49%	23
C5CDT7	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	54.76%	23
C5CGD9	DNA-directed RNA polymerase,subunit beta	4.18%	23
C5CFT9	Transglutaminase domain protein	27.11%	22
C5CI65	Leucine-tRNA ligase	20.86%	22
C5CGM0	Transketolase domain protein	27.13%	21
C5CDU0	Phosphoribulokinase/uridine kinase, family protein	12.09%	21
C5CHS1	M20/SDapE family protein, YgeY	37.34%	21
C5CET1	Two-component transcriptional factor regulator, winged helix family	40.59%	20
C5CGG8	DNA polymerase III, subunit beta	42.51%	20
C5CFF7	Aldo/keto reductase	17.50%	20
C5CHV3	Uncharacterised protein	51.71%	18
C5CHS6	Dihydroorotate dehydrogenase, family protein	32.96%	18
C5CIA0	L-threonine-3-dehydrogenase	16.81%	18
C5CIR0	Putative transcriptional regulator ,GntR family	14.15%	18
B5M6P0	Acetate kinase	18.78%	18
C5CFX5	Uncharacterised protein	25.91%	17
C5CI62	Aldehyde oxidase and xanthine dehydrogenase, molybdopterin-binding	16.06%	17
C5CH14	Malonyl-CoA-acyl-carrier protein, transacyclase	40.32%	17
C5CD80	Glycosyl transferase, group 1	25.12%	16

Accession	**Description**	**Coverage**	**Peptides**
C5CD86	Peptidase U61 LD-carboxypeptidase A	54.93%	16
C5CIU5	Asparigine-tRNA ligase	14.12%	16
B5M6L6	Pyruvate kinase	26.06%	16
C5CFN7	Uncharacterised protein	4.24%	16
C5CIQ7	Probable butyrate kinase	19.56%	16
C5CHM2	Glyceraldehyde-3-phosphate dehydrogenase, type I	41.69%	15
C5CGR6	Elongating factor Tu	23.31%	15
C5CD54	Probable endonuclease 4	18.75%	15
C5CHF7	Peptidase U62 modulator of DNA gyrase	8.64%	15
C5CDT5	Pyruvate/keto/isovalerate oxidoreductase, gamma subunit	56.08%	14
C5CEN6	Phosphonopyruvate decarboxylase-related protein	38.75%	14
C5CHP6	Cytosine deaminase and related metal-dependent hydrolase-like protein	25.93%	14
C5CHB0	Ribose-5-phosphate isomerase B	15.89%	14
C5CDZ4	Translation initiating factor, IF-2	7.34%	14
C5CEA8	Serine hydroxymethyltransferase	9.24%	14
C5CGB5	Rubrerythrin	43.88%	13
C5CI57	Aldehyde oxidase and xanthine dehydrogenase, molybdopterin-binding	12.58%	13
C5CGH4	DNA-directed RNA polymerase, subunit alpha	13.87%	13
C5CHA7	Methionine-tRNA ligase	10.33%	12
C5CDQ4	Methylmalonyl-CoA mutase, large subunit	8.75%	12
C5CIQ4	Pyruvate flavodoxin/ferrredoxin oxidoreductase domain protein	14.37%	12
C5CE22	Carbamate kinase	25.00%	11
C5CFS0	50S ribosomal protein, L25	36.74%	11
C5CDK1	D-lysine 56-aminomutase, alpha subunit	5.34%	11
C5CIA1	Pyridoxal/phosphate-dependent acyltransferase	8.40%	11
C5CFY2	Putative PAS7PAC sensor protein	54.60%	10
C5CFH6	6-phosphofructokinase	14.71%	10
C5CFQ0	Purine nucleoside phosphorylase	29.72%	10
C5CHE0	3-oxoacyl-(acyl-carrier-protein) reductase 2	21.77%	10
C5CFC0	Nucleotide sugar dehydrogenase	3.69%	10
C5CDQ1	Methylmalonyl-CoA epimerase	50.37%	9
C5CEN8	Ferric uptake regulator, Fur family	12.84%	9
C5CGI5	30S ribosomal protein, S5	14.97%	9
C5CGE0	50S ribosomal protein, L7/L12	46.08%	9
C5CGT7	CBS domain-containing protein	7.88%	9
C5CIK7	Trascriptional regulator, LacI family	13.98%	9
C5CH16	3-oxoacyl-(acyl-carrier-protein) synthase 2	4.90%	9
C5CIQ8	Phosphate butyryltransferase	22.00%	9
C5CIQ9	Probable butyrate kinase	29.49%	8
C5CD17	PfkB domain protein	34.48%	8
C5CI44	Probable peroxiredoxin	22.43%	8
C5CFQ4	Cytidine deaminase	62.12%	8
C5CHM4	Triosephosphate isomerase	40.16%	8

C5CDT8	Thiamine pyrophosphate protein domain protein, TPP-binding	22.22%	8
C5CF44	Probable glycine dehydrogenase (decarboxylating) subunit 2	9.11%	8
C5CF51	10kDa chaperonin	62.92%	8
C5CIP5	M18 family aminopeptidase	19.87%	8
C5CE42	OsmC family protein	16.67%	8
C5CG86	Alanine racemase domain protein	11.93%	7
B5M6P1	Fructose-bisphosphate aldolase (fragment)	36.96%	7
C5CF34	Ribosome-recycling factor	15.14%	7
B5M6M7	Conserved protein	9.39%	7
C5CES3	2’-5’- tRNA ligase	20.19%	7
C5CGF3	DNA ligase	2.08%	7
C5CGQ9	50S ribosomal protein, L22	9.15%	7
C5CIN3	Desulfoferredoxin ferrous iron-binding region	74.58%	6
C5CI55	Molybdopterin dehydrogenase, FAD-binding	11.87%	6
C5CID6	Ferroxidase	7.98%	6
C5CDJ7	Dihyroorotate reductase	9.71%	6
C5CEA6	Methylthioribose-1-phosphate isomerase	5.19%	6
C5CE45	Probable glycine dehydrogenase (decarboxylating) subunit 1	4.68%	6
C5CIA6	Histidine triad (HIT) protein	31.25%	6
C5CFU7	OsmC family protein	29.25%	5
C5CG18	Peptide deformylase	32.93%	5
C5CFT8	Mandelate racemase/muconate, lactonisng protein	16.33%	5
C5CGR3	50S ribosomal protein, L4	7.52%	5
C5CIH1	Adenine phosphoribosyltransferase	28.82%	5
C5CIR6	NAD-reducing hydrogenase, subunit B	16.00%	5
C5CEZ0	Ribokinase	10.00%	5
C5CDH1	Histone family protein, DNA-binding protein	40.00%	4
C5CF18	CBS domain-containing membrane protein	15.97%	4
C5CI56	(2S-2Fe)-binding domain protein	10.83%	4
C5CFG8	Deoxyribose-phosphate aldolase	11.84%	4
C5CIQ3	Thiamine pyrophosphate domain protein, TPP-binding	15.54%	3
C5CFH5	AMMERCR1 domain protein	19.65%	3
C5CHR6	Cold-shock DNA-binding domain protein	39.39%	3
C5CI65	Endoribonuclease L-PSP	8.66%	3
C5CDN8	Histone family protein, DNA-binding protein	13.33%	2
C5CH08	Uncharacterised protein	7.66%	2
C5CD93	50S ribosomal protein, L9	10.60%	2
C5CDI1	50S ribosomal protein, L13	7.47%	2
C5CDW1	Alcohol dehydrogenase, GroES domain protein	7.52%	2
C5CH15	Acyl carrier protein	31.58%	2
C5CI92	Elongating factor P	14.05%	2
C5CFQ2	Glutamate-tRNA ligase	9.11%	15
C5CDZ3	Glutamate dehydrogenase	9.83%	14
C5CDL9	Beta-lactamase domain protein	8.84%	7
C5CHF3	Uncharacterised protein	4.74%	11
C5CEC6	Purine nucleoside phosphorylase	8.21%	3

Accession	Description	Coverage	Peptides
C5CEC5	Phosphoesterase, RecJ domain protein	6.44%	26
C5CFY5	Ferredoxin hydrogenase	6.28%	22
C5CFP9	Proline-tRNA ligase	3.96%	35
C5CIA4	Uncharacterised protein	35.09%	32
C5CIX5	Phosphate propanoyltransferase	23.35%	10
C5CD66	S-adenosylmethionine synthase	6.85%	17
C5CGP2	L-lactate dehydrogenase	6.50%	7
C5CH13	Enoyl-(acyl-carrier-protein) reductase II	11.71%	6
C5CHY7	DNA methylase N-4/N-6 domain protein	4.80%	2
C5CEN4	Iron-containing alcohol dehydrogenase	6.20%	10
C5CHB7	Uncharacterised protein	11.57%	6
C5CE41	50S ribosomal protein, L20	17.80%	5
C5CH11	Beta hydroxyacyl-(acyl-carrier-protein) dehydrogenase FabA/FabZ	20.86%	7
C5CGT8	Hydrolase of HD superfamily-like protein	6.65%	4
C5CEP5	Elongating factor Ts	5.00%	18
C5CGR2	50S ribosomal protein, L23	25.00%	4
C5CFG4	Molybdopterin-binding domain protein	14.84%	2
C5CH30	Oxidoreductase domain protein	6.09%	13
C5CIL2	Alpha amylase catalytic region	2.06%	11
C5CD88	Glutaredoxin-like domain protein	9.77%	4
C5CI54	Molydopterin dehydrogenase, FAD-binding	2.83%	22
C5CGR5	30S ribosomal protein, S10	9.80%	1
C5CEE2	Cold-shock DNA-binding domain protein	14.93%	6
C5CD95	Single-stranded DNA-binding protein	7.93%	5
C5CGR1	50S ribosomal protein, L2	4.36%	14
C5CIU7	Aspartate-tRNA ligase	1.69%	19
C5CGR0	30S ribosomal protein, S19	8.51%	3
C5CDT2	Histidine-tRNA ligase	2.63%	12
C5CGT0	Glucose-1,6-bisphosphate synthase	2.37%	7
C5CH00	Uncharacterised protein	4.04%	4
C5CEQ5	Primosomal protein N	1.71%	14
C5CDQ6	Carboxyl transferase	2.51%	6
C5CEL3	Uncharacterised protein	4.63%	11

Table 7.8: Protein identification of *Petrotoga mobilis*, bottom band, sterile filtered

Accession	Description	Coverage	Peptides
A9BH90	Pyruvate ferredoxin/flavodoxin oxidoreducase, beta subunit	10.00%	28
A9BHF5	Alkyl hydroxyperoxidase protein, AhpD family	39.86%	25
A9BFU5	Heavy metal transport/detoxification protein	21.74%	20
A9BEZ5	Pyruvate phosphate dikinase	1.24%	17
A9BFA2	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	10.92%	13
A9BJG3	Fructose-1,6-bisphosphate aldolase, class II	9.94%	12
A9BFV2	NADH dehydrogenase (quinone)	2.47%	11
A9BG84	Peptidase M42 family protein	3.29%	10

Accession	**Description**	**Coverage**	**Peptides**
A9BHA7	Elongating factor, Tu	3.76%	10
A9BII5	Glyceraldehyde-3-phospate dehydrogenase, type I	9.88%	8
A9BJ43	Glycine cleavage system H protein	74.14%	8
A9BIS7	Enolase	2.08%	8
A9BJG8	CoA-binding domain protein	7.59%	8
A9BHX0	RNA-binding protein, Hfq	28.75%	7
A9BJ07	50S ribosomal protein, L31	18.31%	7
A9BJ40	Nucleotide sugar dehydrogenase	9.66%	7
A9BI30	Response regulator receiver protein	26.89%	7
A9BI89	Flagellar hook-basal body complex protein	37.37%	6
A9BG52	Uncharacterised protein	21.93%	6
A9BHL1	Peptidase U62 modulator of DNA gyrase	2.93%	6
A9BF99	Ferroxidase	15.34%	6
A9BHY1	Response regulator receiver protein	17.65%	6
A9BGL6	Macrophage migration inhibitory factor family protein	20.54%	5
A9BJZ0	Acyl carrier protein	24.71%	5
A9BFW9	M18 family aminopeptidase	4.66%	5
A9BH97	50S ribosomal protein, L29	39.39%	5
A9BHK3	10kDa chaperonin	14.61%	5
A9BK08	Uncharacterised protein	16.39%	5
A9BEQ8	Stage V sporulating protein S	30.30%	4
A9BFB2	Stage V sporulating protein S	43.33%	4
A9BHA3	50S ribosomal protein, L23	21.43%	4
A9BIE3	Phosphate-specific transport system accessory protein	5.45%	4
A9BF68	Carbon storage regulator CsrA	13.64%	4
A9BH91	Pyruvate/ketoisovalerate oxidoreductase, gamma subunit	5.76%	4
A9BHA6	30S ribosomal protein, S10	9.71%	3
A9BEN1	Cold-shock DNA-binding domain protein	41.54%	3
A9BES3	Uncharacterised protein	8.57%	3
A9BEZ9	UPF 0109 protein, Pmob-0321	13.16%	3
A9BF34	50S ribosomal protein, L7/12	19.53%	2
A9BH54	Cobalamin B-12-binding domain protein	9.02%	2
A9BF90	Glycoside hydrolase family 4	3.31%	2
A9BGA6	YcfA family protein	17.57%	2
A9BHL5	Uncharacterised protein	7.03%	2
A9BEY0	Flagellar FlbD family protein	24.59%	1
A9BF37	50S ribosomal protein, L11	14.18%	5
A9BFD0	Peptidase S58 DmpA	3.45%	5
A9BF78	Uncharacterised protein	0.00%	6
A9BI34	Desulfoferredoxin ferrous iron-binding region	11.11%	5
A9BHY8	GTP-binding signal recognition particle. SRP54, G-dominating	4.34%	2
A9BFT4	Uncharacterised protein	8.00%	1
A9BIZ6	Glutamine synthase, NADH/NADPH, small subunit	0.00%	21
A9BJA5	Endoribonuclease L-PSP	0.00%	5

Table 7.9: Protein identification of *Petrotoga mobilis*, front band, sterile filtered

Accession	**Description**	**Coverage**	**Peptides**
A9BHF5	Alkyl hydrolase-like protein, AhpD family	44.93%	32
A9BJG3	Fructose-1,6-bisphosphate aldolase, class III	34.15%	26
A9BII4	Phosphoglycerate kinase, triose-phosphate isomerase	15.57%	22
A9BFY2	NADH dehydrogenase (quinone)	12.16%	22
A9BFV1	Hydrogenase, Fe-only	11.07%	21
A9BGA5	Methionine-tRNA ligase	13.18%	21
A9BGA2	Sugar-phosphate isomerase, RpiB/LacA/LacB family	77.08%	20
A9BFW9	M18 family aminopeptidase	31.14%	20
A9BEZ5	Pyruvate phosphate dikinase	8.24%	20
A9BFM6	Alkyl hydroxyperoxidase reductase/Thiol-specific antioxidant/Mal allergen	84.11%	19
A9BG04	30S ribosomal protein, S8	62.69%	19
A9BH90	Pyruvate ferredoxin/flavodoxin oxidoreductase, delta subunit	79.00%	19
A9BK02	Iron-containing alcohol dehydrogenase	22.16%	18
A9BHA7	Elongating factor Tu	22.31%	18
A9BFE3	Putative signal transduction protein with CBS domain	67.76%	17
A9BIW3	Phosphoglucomutase/phosphomannomutase, alpha/beta alpha subunit	10.72%	17
A9BFU5	Heavy metal transport/detoxification protein	21.74%	17
A9BFA2	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	28.04%	16
A9BGW5	Phosphoglucomutase/phosphomannomutase, alpha/beta alpha domain	12.16%	16
A9BHU9	Uncharacterised protein	9.41%	16
A9BH56	Methylmalonyl-CoA epimerase	74.63%	15
A9BET9	Ornithine carbamoyltransferase, catabolic	8.51%	15
A9BJ22	Carboxypeptidase, Taq	3.61%	15
A9BI22	OsmC family protein	55.07%	14
A9BIE9	D-tyrosyl-tRNA (Tyr) diacyclase	66.67%	14
A9BJU7	Uncharacterised protein	55.16%	14
A9BIE3	Phosphate-specific transport system accessory protein	45.91%	14
A9BIS7	Enolase	16.20%	14
A9BF03	50S ribosomal protein, L19	46.22%	13
A9BEW2	PEBP family protein	61.54%	13
A9BGU0	CheW domain protein	29.20%	13
A9BFK8	GCN-related N-acetyltransferase	56.43%	13
A9BK16	3-oxoacyl- (acyl-carrier-protein) synthase 2	6.83%	13
A9BGT6	Cytidine deaminase	51.52%	13
A9BIV8	Formate tetrahydrofolate ligase	5.60%	13
A9BHK4	60kDa chaperonin	6.30%	13
A9BHY1	Response regulator receiver protein	81.51%	12
A9BI34	Desulfoferredoxin ferrous iron-binding region	56.41%	12
A9BJA5	Endoribonuclease L-PSP	48.82%	12
A9BII5	Glyceraldehyde-3-phosphate dehydrogenase, type I	35.93%	12
A9BEV7	Rubrerythrin	44.64%	12

Accession	**Description**	**Coverage**	**Peptides**
A9BHA6	30S ribosomal protein, S10	58.25%	12
A9BIS5	Elongating factor Ts	12.18%	12
A9BF90	Glycoside hydrolase family 4	26.03%	12
A9BGL6	Macrophage migration inhibitory factor, family protein	43.75%	11
A9BHA9	30S ribosomal protein, S7	59.35%	11
A9BG85	Methylglyoxal synthase	50.00%	11
A9BHC0	50S ribosomal protein, L21	54.81%	11
A9BF67	Cupin 2 conserved barrel domain protein	78.69%	11
A9BIU4	(2Fe-2S)-binding domain protein	31.71%	11
A9BJ61	Uncharacterised protein	62.12%	11
A9BJ56	Response regulator receiver protein	23.30%	11
A9BJN7	Nucleoside diphosphate kinase	41.89%	11
A9BJ58	tRNA (guanine-N-(7))-methyltransferase	91.67%	11
A9BF49	MOSC domain-containing protein	56.94%	11
A9BE22	Leucine-tRNA ligase	1.33%	11
A9BHL1	Peptidase U62 modulator of DNA gyrase	4.50%	11
A9BI30	Response regulator receiver protein	70.59%	10
A9BEN1	Cold-shock DNA-binding domain protein	83.08%	10
A9BH91	Pyruvate/ketoisovalerate oxidoreductase, gamma subunit	45.55%	10
A9BHK3	10kDa chaperonin	49.44%	10
A9BJG8	CoA-binding domain protein	46.21%	10
A9BF34	50S ribosomal protein, L7/L12	37.50%	10
A9BFZ3	30S ribosomal protein, S13	66.29%	10
A9BET8	Uncharacterised protein	62.67%	10
A9BFV4	NADH dehydrogenase (ubiquinone) 24kDa subunit	49.11%	10
A9BF98	Alkyl dehydrogenase reductase/Thiol-specific antigen/Mal allergen	53.70%	10
A9BFT3	Uncharacterised protein	51.16%	10
A9BH62	30S ribosomal protein, S6	56.00%	10
A9BJY1	N-utilising substance protein B homolog	38.41%	10
A9BJU0	Nucleotide sugar dehydrogenase	23.68%	10
A9BJG5	Ferripyochelin-binding protein	35.71%	10
A9BFW0	Acetate kinase	6.23%	10
A9BG52	Uncharacterised protein	42.11%	10
A9BG84	Peptidase M42 family protein	18.26%	10
A9BIZ4	Glutamine synthetase	2.27%	10
A9BJ96	Transposase IS204/IS1001/IS1096/IS1165 family protein	5.88%	10
A9BFV3	NADH dehydrogenase (quinone)	21.77%	9
A9BF35	50S ribosomal protein, L10	34.83%	9
A9BFT4	Uncharacterised protein	56.67%	9
A9BF37	50S ribosomal protein, L11	11.35%	9
A9BES0	Peptidase deformylase	10.67%	9
A9BFU0	50S ribosomal protein, L20	32.23%	9
A9BH72	30S ribosomal protein, S15	51.65%	9
A9BH95	50S ribosomal protein, L14	42.62%	9
A9BIT0	Uncharacterised protein	45.79%	9

Accession	**Description**	**Coverage**	**Peptides**
A9BFR4	Peptidase M55 D-aminopeptidase	7.35%	9
A9BKJ7	NADH dehydrogenase (ubiquinone) 24kDa subunit	48.05%	9
A9BHX2	S-adenosylmethionine synthase	12.22%	9
A9BF41	ATPase AAA-2 domain protein	2.65%	9
A9BF99	Ferroxidase	44.79%	9
A9BFV7	Short-chain dehydrogenase/reductase, SDR	21.51%	9
A9BJ57	D-isomer-specific 2-hydroxyaced dehydrogenase, NAD-binding	11.29%	9
A9BEX9	Uncharacterised protein	1.57%	9
A9BGK8	Amidohydrolase 12.47%	12.47%	9
A9BIU0	(2Fe-2S)-binding domain protein	41.40%	9
A9BJ15	Probable endonuclease 4	3.48%	9
A9BH54	Cobalamin B-12-binding domain protein	51.13%	8
A9BF78	Uncharacterised protein	61.86%	8
A9BHA3	50S ribosomal protein, L23	48.98%	8
A9BFT2	Aspartyl/glutamyl (Asn/Gln) amidotransferase, subunitC	66.00%	8
A9BJK4	Thioesterase-like protein	54.61%	8
A9BJG9	SUF system FeS assembly protein, Nifu family	42.76%	8
A9BJZ6	Imidazoleglycerol-phosphate dehydrogenase	38.76%	8
A9BG02	50S ribosomal protein, L18	27.87%	8
A9BIR2	Uncharacterised protein	40.88%	8
A9BFG0	Cell wall hydrolase/autolysin	16.04%	8
A9BFN4	Putative phosphohistidine phosphatase, SixA	24.22%	8
A9BGK9	Alcohol dehydrogenase GroES domain protein	14.57%	8
A9BH01	Nitroreductase	17.28%	8
A9BHL3	Uncharacterised protein	49.00%	8
A9BHL8	2C-methyl-D-erythrithiol 2,4-cyclodiphosphate synthase	42.59%	8
A9BHV9	Hypoxanthine phosphoribosyltransferase	58.24%	8
A9BER6	Phosphoglycerate mutase	12.44%	8
A9BFM3	Mannose-6-phosphate isomerase, type I	11.07%	8
A9BFQ3	GCN5-relate N-acetyltransferase	29.56%	8
A9BH57	Carboxyl transferase	4.05%	8
A9BK09	Formiminotransferase-cyclodeaminase	5.37%	8
A9BHX0	RNA-binding protein, Hfq	58.75%	7
A9BFY9	50S ribosomal protein, L17	31.25%	7
A9BHA1	30S ribosomal protein, S19	54.64%	7
A9BJ43	Glycine cleavage system H protein	74.14%	7
A9BHB0	30S ribosomal protein, S12	28..23%	7
A9BK14	3-hydroxyacyl-(acyl-carrier-protein) dehydratase, FabZ	38.13%	7
A9BEW1	Uncharacterised protein	38.41%	7
A9BH65	50S ribosomal protein, L9	30.20%	7
A9BI35	Rubrerythrin	19.53%	7
A9BF11	Flagellar basal-body rod protein, FlgG	20.69%	7
A9BF68	Carbon storage regulator, CsrA	50.00%	7
A9BFR5	Methionine sulfoxide reductase, MsrA	6.36%	7
A9BH98	50S ribosomal protein, L16	31.21%	7

Accession	**Description**	**Coverage**	**Peptides**
A9BHP3	3-dehydroquinate dehydratase	25.53%	7
A9BIG1	Transketolase domain protein	2.85%	7
A9BH88	Thiamine pyrophosphate domain protein, TPP-binding	6.15%	7
A9BHF7	Phosphate propanoyltransferase	16.84%	7
A9BIS2	Trascriptional regulator Bad/IM/Rrf2 family	19.87%	7
A9BJT9	DegT/DnrJ/EryC/StrS aminotransferase	3.87%	7
A9BFB2	Stage V sporulating protein S	80.00%	6
A9BEQ8	Stage V sporulating protein S	49.49%	6
A9BF69	Flagellar assembly factor, FliW	33.12%	6
A9BH43	Acetamidase/Formamidase	3.09%	6
A9BHB8	50S ribosomal protein, L2	23.60%	6
A9BHJ9	Homoserine dehydrogenase	8.82%	6
A9BHX3	30S ribosomal protein, S20	31.00%	6
A9BK00	Histidine biosynthesis bifunctional protein, HisIE	19.72%	6
A9BEQ1	Phosphoglucosamine mutase	7.95%	6
A9BER3	Transcriptional regulator Bad/M/Rfr2 family	22.00%	6
A9BFB4	Uncharacterised protein	9.62%	6
A9BFZ2	30S ribosomal protein, S11	29.46%	6
A9BFZ9	50S ribosomal protein, L15	13.91%	6
A9BGQ7	RbsD of FucU transport	33.57%	6
A9BGR8	Tetratricopeptide TPR2, repeat protein	9.40%	6
A9BGV5	Ribosomal silencing factor, RsfS	39.13%	6
A9BFB3	Uncharacterised protein	12.26%	6
A9BGJ1	Uncharacterised protein	7.10%	6
A9BHG2	Uncharacterised protein	1.95%	6
A9BHU1	Phosphodiesterase, MJ0936 family	16.46%	6
A9BG00	50S ribosomal protein, L30	37.70%	5
A9BI57	Arginine-tRNA ligase	2.73%	5
A9BJZ0	Acyl carrier protein	32.94%	5
A9BES3	Uncharacterised protein	35.00%	5
A9BH97	50S ribosomal protein, L29	46.97%	5
A9BHL5	Uncharacterised protein	40.62%	5
A9BGV2	50S ribosomal protein, L32	18.33%	5
A9BHY0	CheW protein	20.57%	5
A9BI89	Flagellar hook-basal body complex protein, FliE	37.37%	5
A9BI93	Ribosomal factor A	27.35%	5
A9BJ07	50S ribosomal protein, L31	35.21%	5
A9BEZ1	Aminotransferase, class I and II	2.49%	5
A9BG28	Adenine phosphoribosyltransferase	16.76%	5
A9BG66	Acetylornithine deacetylase or succinyldiaminopimelate desuccinylase	7.62%	5
A9BHP1	Acylphosphatase	29.21%	5
A9BHU7	Asparigine-tRNA ligase	3.46%	5
A9BI20	Cobrinic acid a,c-diamide synthase	11.90%	5
A9BIA7	3-isopropylmalate dehydratase, small subunit	10.18%	5
A9BJQ0	D-ribose pyranase	17.56%	5
A9BHB5	30S ribosomal protein, S9	34.07%	4

Accession	Description	Coverage	Peptides
A9BH94	50S ribosomal protein, L24	20.75%	4
A9BJL1	Uncharacterised protein	22.58%	4
A9BFA4	Beta-phosphoglucomutase	19.63%	4
A9BGG6	Uncharacterised protein	17.73%	4
A9BH64	30S ribosomal protein, S18	15.79%	4
A9BI17	Sigma 54 modulating protein/ribosomal protein, S30EA	22.11%	4
A9BJ53	Chorismate mutase	42.37%	4
A9BF91	Class II aldolase/adducing family protein	12.15%	4
A9BHB9	Uncharacterised protein	22.45%	4
A9BHE8	Uncharacterised protein	19.78%	4
A9BHX6	Uncharacterised protein	28.19%	4
A9BHZ0	Type IV pilus assembly, PilZ	12.07%	4
A9BJB3	Uncharacterised protein	17.24%	4
A9BJW0	Uncharacterised protein	33.33%	4
A9BJX6	Holo (acyl-carrier-protein) synthase	21.01%	4
A9BF62	MoaD family protein	13.19%	4
A9BG95	Beta-lactamase domain protein	2.76%	4
A9BGA6	Ycfa family protein	52.70%	4
A9BGD5	Uncharacterised protein	25.90%	4
A9BGK1	Isochorismutase hydrolase	5.97%	4
A9BHD4	Uncharacterised protein	12.36%	4
A9BHK0	PfkB domain protein	3.13%	4
A9BHW0	Purine nucleoside phosphorylase	4.00%	4
A9BF66	CheW protein	13.64%	3
A9BFZ5	Translation initiating factor IF1	46.05%	3
A9BEZ8	30S ribosomal protein, S16	6.31%	3
A9BG44	MaoC domain protein, dehydratase	14.07%	3
A9BJT8	Transcriptional regulator, MarR family	10.70%	3
A9BJX2	6-phosphofructokinase	12.43%	3
A9BFC0	Transcriptional elongating factor, GreA	5.66%	3
A9BFD0	Peptidase S58, DmpA	6.27%	3
A9BFG1	Chemotaxis response regulator protein-glutamate methylesterase	3.10%	3
A9BHG1	Endoribonuclease, YbeY	23.08%	3
A9BIX9	ABC transporter-related	3.55%	3
A9BJY6	Uncharacterised protein	33.33%	3
A9BEZ9	UFP0109 protein, Pmob-0321	13.16%	2
A9BF72	Uncharacterised protein	7.27%	2
A9BFR3	Transcriptional regulator, LacI family	6.06%	2
A9BGD1	Glutaredoxin 2	11.58%	2
A9BGD3	Uncharacterised protein	18.18%	2
A9BHC6	Phosphodiesterase, MJ0936 family	7.74%	2
A9BG01	30S ribosomal protein, S5	7.91%	1
A9BID4	Trk-A-N domain protein	11.11%	1
A9BIF0	Uncharacterised protein	15.38%	1
A9BGG7	Uncharacterised protein	5.56%	1
A9BK08	Uncharacterised protein	16.39%	1

Accession	Description	Coverage	Peptides
A9BIX2	Prevent-host-death family protein	44.05%	6
A9BJU4	FoA family protein	19.08%	5
A9BGN9	Glutamate dehydrogenase	9.28%	5
A9BHX4	tRNA (guanine-N-(7)) methyltransferase	3.18%	2
A9BIT6	Aspartate/ornithine carbamoyltransferase, carbamoyl-P-binding domain	3.57%	7
A9BGL0	8-amino-7-oxononanoate synthase	2.54%	8
A9BIL4	4Fe-4S-ferredoxin iron-sulfur-binding domain protein	11.69%	8
A9BJ79	30S ribosomal protein, S2	3.47%	7

Table 7.10: Protein identification of *Kosmotoga olearia*. Virus band?

Accession	Description	Coverage	Peptides
C5CG15	Iron-containing alcohol dehydrogenase	43.78%	25
C5CI57	Aldehyde oxidase and xanthine dehydrogenase, molybdopterin-binding	7.96%	17
B5M6L7	60kDa chaperonin	15.77%	16
C5CFT8	Mandelate racemase/muconate lactonising protein	7.87%	5
C5CD62	RNA-binding protein, Hfq	7.59%	3
C5CTF5	Pyruvate phosphate dikinase	0.00%	27

Table 7.11: Total protein identification of *Thermosipho africanus*

Accession	Description	Coverage	Peptides
B7IFK4	Alcohol dehydrogenase	90.84%	196
B7IDG8	Gap glyceraldehyde-3-phosphate dehydrogenase, type I	88.26%	158
B7IFM2	Pyruvate phosphate dikinase	76.84%	146
B7IG24	Glutamate dehydrogenase	79.63%	125
B7IFN4	Enolase	88.81%	101
B7IFA6	60kDa chaperonin	76.62%	78
B7IFZ4	Pyruvate synthase, subunit PorA	84.18%	70
B7IGN4	Fe-hydrogenase, subunit beta	59.46%	59
B7IDH4	Acetate kinase	79.06%	58
B7IFH8	Aminotransferase, class I	68.86%	57
B7IDJ1	Translation elongating factor G	56.06%	56
B7IDB8	NADP-reducing hydrogenase, aubunit c	42.86%	53
B7IF24	Probable glycine dehydrogenase (decarboxylating) subunit 2	61.25%	51
B7UDZ6	3-oxoacyl (acyl-carrier-protein) synthase 2	72.55%	48
B7ID58	8-amino-7-oxononanoate synthase	69.05%	48
B7IH48	Indolepyruvate ferredoxin oxidoreductase, alpha subunit	40.50%	48
B7ICY5	Propionyl-CoA carboxylase, beta subunit	67.25%	47
B7ID00	Chaperone protein DnaK	59.36%	46
B7IHE6	Serine hydroxymethyltransferase	63.68%	46
B7IE23	Polyribonucleotide nucleotidyltransferase	49.50%	46
B7IDH4	Fba fructose-1,6-bisphosphate aldolase, class II	53.53%	45

Accession	Description	Coverage	Peptides
B7IGN5	Hydrogenase 1	55.78%	44
B7IFW8	Leucine-tRNA ligase	31.27%	44
B7IHU4	Elongation factor Tu	79.50%	43
B7IF23	Probable glycine dehydrogenase (decarboxylating) subunit 1	59.45%	43
B7ICV4	Uncharacterised protein	86.36%	42
B7IGS2	Hydroxylamine reductase	39.72%	41
B7IHX5	Trigger factor	56.55%	39
B7ICT0	Asparigine-tRNA ligase	57.77%	38
B7IH26	Phenylalanine-tRNA ligase, beta subunit	29.54%	38
B7IHF2	2-oxogluterate oxidoreductase, alpha subunit	63.92%	38
B7IFW4	Serine-tRNA ligase	49.77%	37
B7IDM9	Threonine-tRNA ligase	39.47%	37
B7IFI0	Solute hydrogenase, 42kDa subunit	61.32%	36
B7IFW5	Methylthioribose-1-phosphate isomerase	60.12%	36
B7IDT4	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	62.94%	35
B7ICT6	DNA polymerase III, subunit beta	77.11%	35
B7IEA0	Valine-tRNA ligase	21.53%	35
B7IGB0	Rubrerythrin	86.31%	33
B7IF30	Urocanate hydratase	52.82%	33
B7IHS6	Chemotaxis protein, CheA	33.53%	33
B7IFZ3	Pyruvate oxidoreductase, beta subunit	53.23%	32
B7IF29	Succinyl-diaminopimelate desuccinylase	67.57%	32
B7IDP1	Lipopolysaccharide biosynthesis protein	47.36%	31
B7IFK9	Oligoendopeptidase F	29.90%	31
B7IFH6	Phosphate acetyltransferase	66.35%	30
B7IE62	M18 family aminopeptidase	51.46%	30
B7ICR3	DNA-directed RNA polymerase, subunit beta	18.86%	30
B7IH86	Elongating factor Ts	36.73%	30
B7IFI8	Protein UshA	37.91%	30
B7ICR2	DNA-directed RNA polymerase, subunit beta	14.19%	30
B7IEZ8	Methylmalonyl-CoA mutase, N-domain subunit	50.27%	29
B7IGE5	Maltose ABC transporter, periplasmic maltose-binding protein	56.01%	29
B7IGH4	Uncharacterised protein	1.19%	29
B7IFH0	2-oxoglutarate oxidoreductase, gamma subunit	77.09%	28
B7IHJ4	Ornithine carbamoyltransferase, catabolic	19.02%	28
B7IHT7	Pyruvate kinase	52.34%	27
B7IFQ2	Alcohol dehydrogenase, zinc-binding	65.22%	27
B7IDU0	Methenyltetrahydrofolate cyclohydrolase	73.91%	27
B7IF66	Conserved domain protein	96.67%	27
B7IHU3	Elongating factor G	32.27%	27
B7IFC9	Galactose-1-phosphate uridylyltransferase	54.52%	26
B7IH88	CTP synthase	46.96%	26
B7IFB8	Glutamate-tRNA ligase	28.33%	26
B7ID59	L-threonine-3-dehydrogenase	67.64%	25
B7IEY3	Glucose -6-phosphate isomerase	46.77%	25

Accession	Description	Coverage	Peptides
B7ICX0	Spermidine synthase	59.87%	15
B7IGT1	Isoleucine-tRNA ligase	15.38%	25
B7IDE9	Ferritin	44.19%	25
B7IFR9	Endoglucanase M	51.80%	24
B7IHC1	Phosphoglucomutase/phosphomannomutase, family protein	29.20%	24
B7IFZ6	Pyruvate/ketoisovalerate oxidoreductase, common subunit gamma	49.21%	23
B7IGK6	Uncharacterised protein	64.00%	23
B7IFZ5	Pyruvate synthase, subunit PorD	74.55%	23
B7IF63	Phosphate acetyltransferase	69.39%	23
B7IH15	Uncharacterised protein	8.86%	23
B7IH23	NADH-dependent butanol dehydrogenase, a	49.87%	23
B7IFL2	K^+ channel beta subunit	46.25%	23
B7ICR1	Amidohydrolase family protein	42.74%	22
B7IEP7	Transketolase	41.61%	22
B7IEY0	Pyrimidine mucleoside phosphorylase	45.39%	22
B7IHR2	Glutamyl-tRNA (Gln) amidotransferase, subunit A	35.31%	22
B7IG29	Formate tetrahydrofolate ligase	37.93%	22
B7IH22	6-phosphofructokinase	43.54%	22
B7IHP8	Conserved oxidoreductase	42.94%	22
B7IF02	Pyruvate carboxylase, subunit B	8.78%	22
B7IH70	Mrp Mrp protein	62.96%	21
B7IE06	6-phosphofructokinase	35.11%	21
B7IG44	ATP synthase, subunit beta	28.09%	21
B7IGV2	Crispr-associated ramp protein	23.22%	21
B7IFD0	Glyogen synthase	36.08%	21
B7IET8	Chemotaxis response regulator protein, glutamate methyltransferase	45.22%	20
B7ID87	Lysine-tRNA ligase	29.28%	20
B7IFB1	Tld/PmbA family protein	25.98%	20
B7IDA9	Adenylsuccinate lyase	30.59%	20
B7IFB6	Proline-tRNA ligase	29.77%	20
B7IHT3	30S ribosomal protein, S2	47.49%	20
B7IGA5	30S ribosomal protein, S6	78.99%	19
B7IGA9	Glycyl-tRNA synthetase, beta subunit	29.04%	19
B7IEU5	Udk phosphoribulokinase family protein	10.93%	19
B7IGE0	ABC-transporter, ATP-binding protein	33.97%	19
B7IFQ3	L-lysine 2,3-aminomutase	32.15%	19
B7IH58	Uncharacterised protein	66.67%	18
B7ID75	HD domain protein	37.45%	18
B7IDG7	Phosphoglycerate kinase	42.54%	18
B7IDF1	Desulfoferredoxin	75.40%	17
B7IG18	UPF 0173 metal-dependent hydrolase	56.05%	17
B7IDS9	Glutamine-dependent NAD^+ synthetase	15.45%	17
B7IGN1	Ferredoxin 2	14.79%	17
B7IGQ7	Zinc protease	34.40%	17
B7IEH1	2-oxoacid ferredoxin oxidoreductase, alpha subunit	34.35%	16

Accession	**Description**	**Coverage**	**Peptides**
B7ICU9	OsmC/Dhr family protein	66.25%	16
B7IEZ6	Glutamine amidotransferase, subunit PdxT	64.36%	16
B7IEZ7	Pyridoxal biosynthesis lyase, PdxS	46.23%	16
B7IFG4	Endoglucanase	39.94%	16
B7ID31	Aspartate aminotransferase	28.28%	16
B7IFB7	Cysteine-tRNA ligase	36.67%	16
B7IFH1	2-oxoglutarate oxidoreductase, beta subunit	37.60%	16
B7IH82	Conserved protein with A-weak D-galactarate dehydrogenase/altronate hydrolase domain	24.85%	16
B7ICX3	Phosphomannomutase, putative	14.71%	16
B7IFA9	Uncharacterised protein	11.01%	16
B7IHQ0	Threonine synthase	23.79%	16
B7IGG8	Chaperone ClpB	4.31%	16
B7IFN6	50S ribosomal protein, L25	35.48%	15
B7ICG4	50S ribosomal protein, L7/L12	43.75%	15
B7IHS8	Putative chemotaxis protein, CheY-like protein	88.33%	15
B7IFI1	D-3-phosphoglycerate dehydrogenase	56.11%	15
B7IFH7	Probable butyrate kinase	30.20%	15
B7IFG5	Endoglucanase M	22.69%	15
B7IHJ5	Arginine deiminase	28.50%	15
B7ICV9	Uncharacterised protein	26.94%	15
B7ID90	Uncharacterised protein	29.81%	15
B7IGA8	Glycyl-tRNA synthetase, alpha subunit	12.01%	15
B7IHW0	30S ribosomal protein, S8	59.70%	14
B7IHX6	ATP-dependent Clp protease, proteolytic subunit	53.23%	14
B7IEV0	Keto/oxoacid ferredoxin oxidoreductase, gamma subunit, putative	54.50%	14
B7IFW1	DAK2 domain protein	4.28%	14
B7IGA1	Transcription terminating/antiterminating, NusG	37.85%	14
B7ID64	Uncharacterised protein	30.36%	14
B7IDI3	Alanine-tRNA ligase	7.22%	14
B7IFA7	10kDa chaperonin	96.67%	13
B7IGD6	Ribonucleoside diphosphate reductase	7.76%	13
B7IHV2	30S ribosomal protein, S3	50.24%	13
B7IHU2	30S ribosomal protein, S7	53.55%	13
B7IHM6	Uncharacterised protein	19.27%	13
B7IE76	23S rRNA (uracil-5)-methyltransferase, RumA	20.68%	13
B7IHZ0	Csh crispr-associated protein, Csh2 family	17.88%	13
B7IHN8	Glyoxylate reductase	23.03%	13
B7IHR3	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase, subunit B	17.65%	13
B7IHS0	Oligoendopeptidase F	20.03%	13
B7IDE6	Probable peroxiredoxin	33.64%	13
B7IHJ0	Uncharacterised protein	17.36%	13
B7ICP4	Hydrolase, ama/HipO/hyci family	29.12%	13
B7IDE8	Nitric oxide reductase	9.28%	13
B7IF83	L-allothreonine aldolase	14.29%	13
B7IE42	Uncharacterised protein	23.89%	12

Accession	Description	Coverage	Peptides
B7IGN3	Fe-hydrogenase, gamma subunit	37.58%	12
B7IH61	Response regulator	68.85%	12
B7IDK5	CBS domain par protein	39.00%	12
B7IDD4	Methionine-tRNA ligase	5.02%	12
B7IEV2	S-adenosylmethionine synthase	6.33%	12
B7IFM5	Ribose-phosphate pyrophosphokinase	36.39%	12
B7IGT4	Maltodextrin glycosyltransferase	12.16%	12
B7IHH3	Glutamate-1-semialdehyde, 2,1-aminomutase	20.33%	12
B7IEL9	Hydroxydechloroatrizine ethylaminohydrolase	5.53%	12
B7IF06	Metallo-beta-lactamase family protein	39.76%	12
B7IF25	Alcohol dehydrogenase, iron-containing	36.21%	12
B7IG63	Pleiotrophic regulating protein	21.32%	12
B7ICP3	Diaminopimelate decarboxylase	13.28%	12
B7IG13	Tryptophan-tRNA ligase	12.39%	12
B7IH60	Regulatoric protein,putative	22.10%	12
B7IHS7	Chemotaxis protein, CheW	21.85%	11
B7IFN7	Tyrosine-tRNA ligase	9.05%	11
B7ICV3	Thioredoxin reductase	19.94%	11
B7IE00	Malonyl-CoA acyl carrier protein, transacyclase	27.95%	11
B7IE30	Aspartate-tRNA ligase	6.54%	11
B7IDT6	Transcriptional terminating/antiterminating protein, NusA	20.35%	11
B7ID06	Acetyl-CoA acetyltransferase	34.25%	11
B7IE24	Processsing protease, putative	9.67%	11
B7IER9	Carbohydrate kinase family protein	19.92%	11
B7IEX4	3-oxoacyl-(acyl-carrier-protein) reductase	19.84%	11
B7IFB2	Amino acid racemase	20.39%	11
B7IGH3	ATP-dependent nuclease, subunit A, putative	1.06%	11
B7IGQ5	Glutamine-fructose-6-phosphate aminotransferase (isomerising)	13.14%	11
B7HH1	Delta-aminolevulinic acid dehydrogenase	19.18%	11
B7IED6	Uncharacterised protein	1.57%	11
B7IGA2	ATP synthase, subunit alpha	4.14%	11
B7IGT2	Transcriptional regulatoric protein, ResD	73.95%	10
B7IG48	Ribosomal subunit interface protein	40.91%	10
B7ICS3	Endoribonuclease L-PSP, putative	50.81%	10
B7IDQ8	Uncharacterised protein	23.88%	10
B7IDC1	Glutamate forminimotransferase	21.26%	10
B7IE29	Probable transaldolase	31.48%	10
B7ICR8	Flagellin	5.17%	10
B7IF21	Uncharacterised protein	11.86%	10
B7IGE4	Negative regulator of genetic competanse, Clp/MecB	7.35%	10
B7IGU1	Trehalose/maltose-binding protein	13.29%	10
B7IEC2	Beta-galactosidase	13.15%	10
B7IGJ1	Phosphoribosylamine-glycine ligase	11.84%	10
B7IHE5	Alpha-galactosidase	9.06%	10
B7IE59	Exopolyphosphatase family protein	22.56%	9
B7IF37	6-phosphofructokinase	22.39%	9

Accession	**Description**	**Coverage**	**Peptides**
B7IDI6	Ggdef domain protein	23.86%	9
B7IFW6	Alkaline phosphatase 3	7.34%	9
B7IH5	Phosphate propanoyltransferase	37.24%	9
B7IE87	Signal recognition particle receptor, FtsY	37.41%	9
B7IF92	Iron (III) ABC transporter, periplasmic-binding	35.27%	9
B7IG23	Acyl carrier protein	46.05%	9
B7ICT1	Thermostable carboxypeptidase 1	11.86%	9
B7IFH5	Probable butyrate kinase	9.37%	9
B7IHM4	Uncharacterised protein	28.15%	9
B7IDB2	Radical SAM domain protein	11.62%	9
B7IF05	Methyl-accepting chemotaxis protein, McpA	33.33%	9
B7IF31	2-oxogluterate synthse, subunit KorA	26.17%	9
B7IFL3	Aminopeptidase 2	19.85%	9
B7IFY6	Orotidine-5’-phosphate decarboxylase	33.50%	9
B7IGR5	Uncharacterised protein	9.22%	9
B7IHU6	50S ribosomal protein, L3	19.51%	9
B7IDV7	CheY-phosphatase, CheC	36.10%	9
B7IE66	Uncharacterised protein	8.77%	9
B7IFH3	Conserved domain protein	15.07%	9
B7IGI4	Electron transfer flavoprotein, subunit beta	8.08%	9
B7IH52	Purine nucleoside phosphorylase	11.49%	9
B7IHX9	Chemotaxis protein methyltransferase	4.81%	9
B7IEX8	DNA-binding response regulator	22.18%	9
B7IG17	MutL protein	12.97%	9
B7IDZ7	(3R)-hydroxymyristoyl-(acyl-carrier-protein) dehydratase (3R-hydroxymyristoyl ACP dehydrase)	48.53%	8
B7IHC0	CoA-binding protein	52.42%	8
B7IDK8	3-methyl-2-oxobutanoate hydroxymethyltransferase	38.49%	8
B7IDR1	2’-3’-cyclic nucleotide 2’-phosphodiesterase	3.67%	8
B7IFI7	Stage V sporulating protein S	78.16%	8
B7ICP5	Phenylalanine-tRNA ligase, alpha subunit	12.31%	8
B7ID95	Oligopeptide ABC transporter, periplasmc oligopeptide-binding protein	2.07%	8
B7IF03	Translation-initiating factor, IF2	6.18%	8
B7IGH6	DNA-binding protein, HU1	43.48%	8
B7IGP4	Uridylate kinase	21.03%	8
B7ICY2	Methylmalonyl-CoA mutase, subunit alpha	75.00%	8
B7IF53	Carboxymuconolactone decarboxylase	17.36%	8
B7IGL2	Aspartate aminotransferase	20.91%	8
B7IH47	Indolepyruvate ferredoxin oxidoreductase, beta subunit	33.82%	8
B7ICZ9	Heat-shock protein, Hsp20 family	27.46%	8
B7IF42	Bifunctional protein, FolD	19.56%	8
B7IGG9	Bactoferritin comigratory protein	30.00%	8
B7IGI3	Acyl-CoA dehydrogenase, short-chain specific	14.25%	8
B7IH43	Uncharacterised protein	13.68%	8
B7IEW1	Transcriptional regulator, XylR-related	21.83%	8
B7IF64	D-aminopeptidase superfamily	17.34%	8
B7IF78	PHP domain protein	7.51%	8

Accession	**Description**	**Coverage**	**Peptides**
B7IGP2	CheY-P phosphatase CheX	32.26%	8
B7IC!9	Sucrose-phosphate synthase	4.27%	8
B7IEV1	2-oxoglutarate synthase, subunit KorB	10.95%	8
B7OFI3	Ornithine carbamoyltransferase	31.31%	8
B7IHV5	30S ribosomal protein, S17	73.74%	7
B7ICR9	Glycosyl transferase, group 2 family protein	1.27%	7
B7IEX3	1-phosphofructokinase	15.31%	7
B7IFQ1	Uncharacterised protein	16.18%	7
B7IG66	Lipoprotein, putative	13.33%	7
B7ID72	Geranylgeranyl diphosphate synthase	16.27%	7
B7IDM7	Metal-dependent phosphohydrolase	21.55%	7
B7IHW3	30S ribosomal protein, S5	36.36%	7
B7IHX3	DNA-directed RNA polymerase, subunit alpha	11.52%	7
B7ICN8	Stage V sporulating protein S	68.89%	7
B7IEQ3	50S ribosomal protein, L19	19.13%	7
B7IEV9	UPF 0273 protein, THA-115	23.93%	7
B7IFB3	Uncharacterised protein	14.05%	7
B7IFG8	UPF 0296 protein, THA-332	51.06%	7
B7IFJ7	Conserved domain protein	58.82%	7
B7IH89	Cin-like protein	13.56%	7
B7IHG9	Porphobilinogen deaminase	6.07%	7
B7IHW7	Adenylate kinase	18.69%	7
B7IDC0	Fe-hydrogenase, gamma subunit	17.18%	7
B7IDD0	Anti-sigma factor antagonist	38.39%	7
B7IFD7	D-isomer-specific-2-hydroxyacid dehydrogenase, NAD-binding, putative	2.51%	7
B7IG99	50S ribosomal protein, L1	26.29%	7
B7IGE3	Isoaspartyl dipeptidase	7.67%	7
B7IGU0	Glycosyl transferase, group 1	13.37%	7
B7IHW9	Translation initiating factor, IF1	23.08%	7
B7IHX1	30S ribosomal protein, S11	37.21%	7
B7IDE3	Pyrimidine base biosynthesis protein	6.29%	7
B7E34	GTPase Der	4.56%	7
B7IEF4	Uncharacterised protein	8.64%	7
B7IGF8	Rubrerythrin subfamily	31.65%	7
B7IGQ6	Peptidase M16 inactive domain family	2.44%	7
B7IHH0	Uroprophyrinogen III synthase/methyltransferase	8.23%	7
B7IHK2	Glucose-1-phosphate, thymidylyltransferase	10.58%	7
B7IDB9	NADP-reducing hydrogenate, subunit b	34.68%	6
B7IDI0	Ribosome recycling factor	31.35%	6
B7ICY6	Methylmalonyl-CoA epimerase	53.62%	6
B7IGH0	Uncharacterised protein	37.08%	6
B7IHU5	30S ribosomal protein, S10	27.45%	6
B7IEU3	Adenosylhomocysteinase	14.00%	6
B7IHJ3	Carbamate kinase	17.57%	6
B7IHP9	Uncharacterised protein	41.18%	6
B7IDU4	Phosphoglucosamine mutase	2.56%	6

Accession	Description	Coverage	Peptides
B7IDX9	Cdd cytidine deaminase	37.21%	6
B7IDY9	Sensor histidine kinase	6.45%	6
B7IGP1	MTA/SAH nucleosidase	27.19%	6
B7IER4	Phosphorylase	1.56%	6
B7IHF7	Nicotinate-nuclotidedimethylbenzimidazole phosphoribosyltransferase	5.23%	6
B7IDU7	50S ribosomal protein, L9	15.89%	6
B7IGW6	Glucose-1-phosphate, thymidylyltransferase	3.79%	6
B7IHU9	50S ribosomal protein, L2	12.41%	6
B7IE58	Purine nucleoside phosphorylase	6.77%	6
B7IE74	Phosphate-specific transport system accessory protein, PhoU	6.01%	6
B7IE81	Peptide release factor 2	26.29%	6
B7IFA1	SUF system FeS assembly protein, NifU family	12.12%	6
B7IFZ0	Purine-binding chemotaxis protein	19.87%	6
B7IGV4	Crispr-associated ramp protein	3.00%	6
B7IHI6	5-methyltetrahydrofolate S-homocysteine methyltransferase	1.68%	6
B7IHY2	Glutamine synthetase	5.01%	6
B7IEQ1	Uncharacterised protein	6.90%	5
B7IDC9	5-phosphate isomerase B	8.97%	5
B7IHX0	30S ribosomal protein, S13	31.15%	5
B7IFW2	Alkaline phosphatase	33.03%	5
B7IHR1	Uncharacterised protein	8.63%	5
B7IEB8	Quinolinate synthetase complex A, subunit 1	26.07%	5
B7IEH2	Ferredoxin oxidoreductase, beta subunit	15.47%	5
B7IFP7	Ribosomal protein, L1 methyltransferase	7.69%	5
B7IHV6	50S ribosomal protein, L14	46.72%	5
B7ICN2	50S ribosomal protein, L27	19.32%	5
B7ID86	Transcriptional elongating factor GreA	6.41%	5
B7IF84	Uncharacterised protein	10.96%	5
B7IGA0	50S ribosomal protein, L11	37.59%	5
B7ICT2	Acetyltransferase-related protein	17.73%	5
B7IDT9	Enzyme of the cupin superfamily	12.36%	5
B7IDY4	Probable dual-specific RNA methyltransferase, RlmN	8.80%	5
B7IEQ8	30S ribosomal protein, S16	36.17%	5
B7IID5	Glycoprotease family	9.72%	5
B7ICK4	UPF 0597 THA 1286	4.73%	5
B7ICS7	Uncharacterised protein	6.39%	5
B7IDE0	Alpha-galactosidase, putative	3.26%	5
B7IDG3	TRK system potassium uptake protein, TrkA	21.92%	5
B7IDX1	Response regulator receiver	11.98%	5
B7IDZ8	Putative enoyl-(acyl-carrier-protein) reductase II	11.61%	5
B7IEB9	Nicotinate-nucleotide pyrophosphorylase	5.17%	5
B7ICF6	Pyrazinoamidase/nicotinamidase-related protein	33.33%	5
B7IFG9	Uncharacterised protein	3.44%	5
B7IGZ8	Conserved protein with A-weak D-galactarate dehydrogenase/altronate hydrolase domain	3.80%	5

Accession	**Description**	**Coverage**	**Peptides**
B7IHW8	Methionine aminopeptidase	9.41%	5
B7ID42	Cysteine synthase A	9.47%	4
B7IHV8	50S ribosomal protein, L5	22.65%	4
B7ICM9	30S ribosomal protein, S9	18.94%	4
B7ID41	N5-carboxyaminoimidazole ribonucleotide mutase	28.67%	4
B7IEG3	Glucosylase	9.52%	4
B7IEL8	Ribosomal RNA, small subunit methyltransferase A	6.53%	4
B7IEV0	M23 peptidase domain protein	6.54%	4
B7IFQ0	Beta-alanyl-CoA: ammonia lyase 2	43.31%	4
B7IHG3	Precorrin-2 methyltransferase	22.22%	4
B7ICN4	50S ribosomal protein, L21	22.64%	4
B7ID45	N-acetyl-beta-hexosamidinase	3.17%	4
B7IE32	Cytiylate kinase	5.86%	4
B7IEQ4	tRNA (guanine-N-(1)-) methyltransferase	6.38%	4
B7IF87	Radical SAM domain protein	5.72%	4
B7IH65	Cationic outer membrane protein	6.79%	4
B7IHU7	50S ribosomal protein, L4	15.84%	4
B7IHW5	50S ribosomal protein, L15	22.45%	4
B7IDV9	Uncharacterised protein	9.60%	4
B7IDX6	DNA ligase	2.11%	4
B7IF44	Thymidylate synthase, ThyX	5.70%	4
B7IFM4	Bifunctional protein, GlmU	5.10%	4
B7IG16	Uncharacterised protein	20.00%	4
B7IG35	Xaa-Pro dipeptidase	2.53%	4
B7IH08	Brached-chain aminoacid aminotransferase 1	12.60%	4
B7IHV0	30S ribosomal protein, S19	21.05%	4
B7IHV9	30S ribosomal protein, S15, type Z	18.03%	4
B7IGC5	Glycerol kinase	2.25%	3
B7IF90	Thiamine pyrophosphokinase	11.71%	3
B7IE57	Ribulose-phosphate 3-epimerase	12.21%	3
B7IEN0	Glycine cleavage system H protein	31.97%	3
B7IEW0	Uncharacterised protein	15.28%	3
B7IGA3	30S ribosomal protein, S18	22.97%	3
B7IGI0	Putative periplasmic metal-binding protein	15.97%	3
B7ICT9	Uncharacterised protein	7.93%	3
B7ID16	Response regulator	2.59%	3
B7IDQ7	Redox-sensing transcriptional repressor, Rex	5.80%	3
B7IF43	Uncharacterised protein	23.28%	3
B7IFG6	DNA-directed RNA polymerase, subunit omega	16.00%	3
B7IFV2	Glucose-1-phosphate adenylyltransferase	14.56%	3
B7IG97	Acetyltransferase, gnat family	13.64%	3
B7IGI7	Uncharacterised protein	13.97%	3
B7IGX9	UDP-glucose-6-dehydrogenase	3.59%	3
B7IHT4	50S ribosomal protein, L20	29.66%	3
B7IF04	Uncharacterised protein	14.71%	2
B7IF94	NagD protein	13.48%	2

B7IFW6	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase, subunit C	48.39%	2
B7IG20	Adenylsuccinate synthetase	4.24%	2
B7IHU1	30S ribosomal protein, S12	15.32%	2
B7ICR5	50S ribosomal protein, L10	12.22%	2
B7ICV2	Uncharacterised protein	16.25%	2
B7IFA2	Cysteine desulfurase	2.91%	2
B7IFD3	Uncharacterised protein	8.09%	2
B7IGU9	Acetyltransferase	15.60%	2
B7IHV4	50S ribosomal protein, L29	45.45%	2
B7IDG5	Response regulating protein	8.00%	2
B7IE92	Hpt hypoxanthine phosphoribosyltransferase	6.47%	2
B7IER0	ABC-type thiamine transport system, periplasmic component	3.38%	2
B7IES9	Uncharacterised protein	16.67%	2
B7IFH4	Uncharacterised protein	7.94%	2
B7IFU9	Uncharacterised protein	12.94%	2
B7IHV3	50S ribosomal protein, L16	9.15%	2
B7IHW1	50S ribosomal protein, L6	9.78%	2
B7IEU4	Cytoplasmic axial filament protein	1.94%	1
B7IHW4	50S ribosomal protein, L30	24.59%	1
B7ID89	S4 domain protein	12.66%	1
B7IDG1	6-carboxy-5,6,7,8-tetrahydropterin synthase	15.00%	1
B7IDV3	Uncharacterised protein	8.16%	1
B7IEZ9	Chain-P, L-fuculose-1-phosphate-aldolase	6.13%	1
B7IFE3	Major facilitator superfamily, MFS1	2.61%	1
B7IHV1	50S ribosomal protein, L22	9.46%	1
B7IG31	Chaperonin HslO	14.63%	5
B7IDZ2	Ion protease	2.71%	16
B7IFW7	Basic membrane protein, putative	7.51%	8
B7IF19	DegT/DnrJ/EryC/StrS aminotransferase	12.73%	13
B7IHL2	Fructose-1,6-bisphosphatase	11.50%	10
B7IE45	Glutamine synthetase, type I	2.63%	9
B7IH59	Elongating factor P	4.86%	5
B7ID40	Phosphoribosylaminoimidazole/succinocarboxyamide synthase	7.89%	8
B7IDN8	tRNA modifying GTPase, MnmE	4.31%	6
B7IF91	1-deoxy-D-xylulose-5-phosphate reductoisomerase	6.23%	4
B7IFU1	ATP-dependent protease, subunit HsIV	11.93%	3
B7IF77	ClpC ATPase	6.35%	15
B7IHX2	30S ribosomal protein, S4	8.61%	5
B7IDP4	NADH-ubiquinone oxidoreductase, 49kDa subunit	5.99%	6
B7IDA8	Transcriptional regulator, ArsR family	7.00%	3
B7IFY9	S1 RNA-binding domain protein	2.07%	11
B7IFI9	ABC transporter, ATP-binding protein	2.96%	8
B7IDD3	DNA gyrase, subunit A	1.74%	12

Table 7.12: Total protein identification of *Petrotoga mobilis*

Accession	**Description**	**Coverage**	**Peptides**
A9BEZ5	Pyruvate phosphate dikinase	55.98%	93
A9BGX9	Alpha-glucan phosphorylase	36.14%	46
A9BF22	Leucine-tRNA ligase	30.43%	44
A9BHA7	Elongating factor Tu	64.66%	42
A9BII4	Phosphoglycerate kinase, Triose phosphate isomerase	44.73%	40
A9BJ45	Peptidase S9 prolyl oligopeptidase, active site domain protein	45.95%	40
A9BI29	Isoleucine-tRNA ligase	28.29%	40
A9BGY0	Ribonucleoside diphosphate reductase	30.83%	37
A9BIW3	Phosphoglucomutase/phosphomannomutase alpha/beta alpha domain	49.91%	36
A9BII5	Glyceraldehyde-3-phosphate dehydrogenase, type I	50.90%	34
A9BFA2	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	58.56%	34
A9BHK4	60kDa chaperonin	55.19%	33
A9BHF0	Threonine-tRNA ligase	23.75%	33
A9BHI4	NADH flavin oxidoreductase/NADH oxidase	23.24%	33
A9BF47	Alpha amylase catalytic region	13.51%	33
A9BFV1	Hydrogenase, Fe-only	41.70%	32
A9BHA8	Elongating factor G	39.28%	32
A9BJ22	Carboxypeptidase Taq	28.26%	32
A9BFM9	Alpha amylase catalytic subdomain	25.00%	31
A9BGA8	Proline-tRNA ligase	33.85%	31
A9BHJ5	Hydrogenase, Fe-only	28.12%	31
A9BJE0	Alanine-tRNA ligase	21.35%	31
A9BG71	Alpha-glucosidase	25.14%	31
A9BJU0	Nucleotide sugar dehydrogenase	58.16%	30
A9BF90	Glycoside hydrolase, family 4	52.27%	30
A9BI24	Aspartate-tRNA ligase	34.22%	30
A9BHL0	Peptidase U62 modulator of DNA gyrase	36.81%	29
A9BET9	Ornithine carbamoyltransferase, catabolic	47.11%	29
A9BIG1	Tramsketolase domain protein	46.91%	28
A9BIV8	Formate-tetrahydrofolate ligase	48.01%	28
A9BIT2	Pyridoxal-5'-phosphate-dependent protein, beta subunit	45.90%	28
A9BFA3	Kojibiose phosphorylase	19.92%	28
A9BH06	Glutamate synthase (NADPH) homotetrameric	17.00%	28
A9BHU9	Uncharacterised protein	28.74%	27
A9BFV2	NADH dehydrogenase (quinone)	34.23%	27
A9BJK9	Small GTP-binding protein	30.87%	26
A9BJ56	Response regulator receiver protein	53.93%	25
A9BIB0	Trigger factor	38.91%	25
A9BIS7	Enolase	51.85%	24
A9BFC1	Lysine-tRNA ligase	28.29%	24
A9BI32	ATPase AAA-2 domain protein	5.81%	24
A9BHU7	Asparigine-tRNA ligase	38.71%	24

A9BFG8	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase, subunit B	23.48%	24
A9BGL0	8-amino-7-oxononanoate synthase	45.55%	23
A9BJ57	D-isomer-specific 2-hydroxyacid dehydrogenase NAD-binding	64.84%	23
A9BFW9	M18 family aminopeptidase	43.22%	23
A9BIJ7	Valine-tRNA ligase	20.25%	23
A9BJG4	Acetate kinase	36.66%	23
A9BEX3	Serine-tRNA ligase	50.24%	22
A9BIT6	Aspartyl/ornithine carbamoyltransferase, carbamoyl-P-binding domain	39.29%	22
A9BHJ6	NADH dehydrogenase (quinone)	49.01%	22
A9BFW2	O-acetylhomoserine/O-acetylserine sulfhydrylase	31.70%	22
A9BGA5	Methionine-tRNA ligase	13.33%	22
A9BFH1	Phenylalanine-tRNA ligase, beta subunit	20.37%	22
A9BI52	Chaperone protein DnaK	22.33%	21
A9BGH1	1,4-alpha-glucan branching enzyme, GlgB	8.52%	21
A9BHV4	Adenosylsuccinate synthetase	36.90%	21
A9BI65	Phosphoribosylamineimidazole carbxyamide formyltransferase	34.56%	21
A9BH91	Pyruvate/ketoisovalerate oxidoreductase, gamma subunit	62.30%	20
A9BK16	3-oxoacyl (acyl-carrier-protein) synthase 2	42.93%	20
A9BHG2	Uncharacterised protein	22.93%	20
A9BIK7	Adenosyl/homocysteinase	35.54%	20
A9BJ12	Flagellin domain protein	35.31%	20
A9BJG3	Fructose-1,6-bisphosphate aldolase, class II	43.27%	20
A9BGX5	Sucrose-phosphate synthase	23.94%	20
A9BIT4	M20/DapE family protein, YgeY	27.25%	20
A9BG55	Glycine-tRNA ligase, beta subunit	13.35%	20
A9BIW8	Peptidase S41	4.32%	20
A9BJ00	Alpha amylase catabolic region	22.47%	20
A9BG66	Acetylornithine deacetylase or succinyldiaminopimelate desuccinylase	43.00%	19
A9BH73	Polyribonucleotide nucleotidyltransferase	18.99%	19
A9BIK8	Serine hydroxymethyltransferase	32.85%	19
A9BF11	Flagellar basal-body rod protein, FlgG	52.49%	19
A9BIM0	Cysteine synthase A	47.97%	19
A9BHX9	CheA signal transduction histidine kinase	8.62%	19
A9BI31	PAS7PAC sensor signal transduction histidine kinase	3.01%	19
A9BJ39	Urocanate hydratase	1.78%	19
A9BEX9	Uncharacterised protein	13.82%	19
A9BGR2	L-fucose isomerase-like protein	8.28%	19
A9BI96	Peptidase M3A and M3B thimet/oligopeptidase F	16.10%	19
A9BG68	Glycogen synthase	24.64%	19
A9BIA2	Carbamoyl-phosphate synthase, large subunit	1.80%	19
A9BGW5	Phosphoglucomutase/phosphomannomutase alpha/beta alpha domain	63.55%	19
A9BHL6	D-lysine 56-aminomutase, alpha subunit	17.49%	19

Accession	**Description**	**Coverage**	**Peptides**
A9BG29	Glucose-6-phosphate isomerase	46.17%	18
A9BID2	Pyruvate kinase	31.38%	18
A9BKD2	Iron-containing alcohol dehydrogenase	27.84%	18
A9BJ05	Histidine-tRNA ligase	27.08%	18
A9BIC8	Probable dehydrogenase (decarboxylating) subunit 1	18.53%	18
A9BEU2	Glycosyltransferase family 2	33.33%	18
A9BHS5	Uncharacterised protein	18.18%	18
A9BF30	Tryptophanyl-tRNA synthase	15.38%	18
A9BG95	Beta-lactamase domain protein	28.07%	17
A9BH57	Carboxyl transferase	27.61%	17
A9BHF5	Alkyl hydroperoxidase-like protein, AhpD family	34.78%	17
A9BG26	Uncharacterised protein	26.39%	17
A9BGS5	Aminotransferase, class I and II	32.21%	17
A9BHJ8	Threonine synthase	44.25%	17
A9BFU5	Heavy-metal transport/detoxification protein	42.03%	17
A9BI57	Arginine-tRNA ligase	14.75%	17
A9BF41	ATPase AAA-2 domain protein	3.73%	17
A9BJ27	Xylose isomerase	5.25%	17
A9BK01	Histidinol-phosphate aminotransferase	50.00%	16
A9BEV7	Rubrerythrin	55.36%	16
A9BIP1	2,5-didehydrogluconate reductase	58.39%	16
A9BJ58	tRNA (guanine-N-(7)-)-methyltransferase	96.67%	16
A9BGK8	Amidohydrolase	27.27%	16
A9BHL1	Peptidase U62 modulator of DNA gyrase	28.60%	16
A9BH90	Pyruvate ferredoxin/flavodoxin oxidoreductase, delta subunit	48.00%	16
A9BHK0	PkfB domain protein	50.47%	16
A9BFG9	Glutamyl-tRNA (Gln) amidotransferase, subunit A	17.00%	16
A9BHL4	Pyridoxal-5’-phosphate-dependent protein, beta subunit	22.67%	16
A9BJZ1	CTP synthase	13.66%	16
A9BG28	Adenine phosphoribosyltransferase	49.13%	15
A9BES9	Tyrosine-tRNA ligase	22.92%	15
A9BJ20	Adenylsuccinate lyase	23.15%	15
A9BHN3	Carbohydrate kinase, YjeF-related protein	32.45%	15
A9BHX2	S-adenosylmethionine synthase	23.94%	15
A9BJW4	Cobalamin B-12-binding domain protein	10.87%	15
A9BIT3	Threonine synthase	28.27%	15
A9BH53	Methylmalonyl-CoA mutase, large subunit	18.96%	15
A9BGH0	Mannose-1-phosphate guanyltransferase	27.08%	15
A9BI72	Diguanylate cyclase	3.61%	15
A9BJ21	Peptidase S16 Ion domain protein	21.39%	15
A9BTJ8	Transcriptional regulator, MarR family	39.07%	15
A9BHL7	DNA polymerase III, subunit beta	28.11%	15
A9BIC9	Probable glycine dehydrogenase (decarboxylatig) subunit 2	19.13%	15
A9BEV7	Short-chain dehydrogenase /reductase, SDR	33.47%	14
A9BI89	Flagellar hook-basal body, complex protein	57.58%	14

A9BGV4	Glutamine-fructose-6-phosphate aminotransferase (isomerising)	11.11%	14
A9BHP4	Phospho-2-dehydro-3-deoxyheptonate aldolase	21.53%	14
A9BH36	Putative GAP sensor protein	23.17%	14
A9BHM7	RNA-binding S1 domain protein	5.42%	14
A9BJZ9	Imidazole glycerol/phosphate synthase, subunit HisF	36.51%	14
A9BHN8	Iron-containing alcohol dehydrogenase	27.10%	13
A9BJH2	FeS assembly protein, SufB	2.55%	13
A9BGX7	NAD$^+$ synthetase	18.61%	13
A9BFL1	6-phosphogluconate dehydrogenase, decarboxylating	21.40%	13
A9BJT9	DegT/DnrJ/EryC/StrS aminotransferase	23.20%	13
A9BFW0	Acetate kinase	19.20%	13
A9BKD5	GTPase Obg	12.05%	13
A9BEM3	Linocin M18 bacteriocin protein	32.83%	13
A9BIZ4	Glutamine synthetase	29.48%	13
A9BEX7	Flagellar hook-length control protein	5.68%	13
A9BFE4	PfkB protein	15.94%	13
A9BFK9	Carbohydrate kinase, FGGY	17.23%	13
A9BTU2	Sulfate adenylyltransferase	24.22%	13
A9BG84	Peptidase M42 family protein	41.62%	13
A9BHL2	Dihydropicolinate reductase	30.47%	13
A9BJA6	Tryptophanase	13.45%	13
A9BGL2	tRNA uridine-5-carboxymethylaminomethyl-modifying enzyme, MnmG	10.24%	13
A9BGY7	CRISPR-associated protein, Csh2 family	33.44%	13
A9BIT7	Xanthine dehydrogenase	11.78%	13
A9BIU8	Dyhydroorotate dehydrogenase family protein	21.76%	13
A9BJ72	Delta-1-pyrroline-8-carboxylate dehydrogenase	18.60%	13
A9BG70	Peptidase M28	7.64%	13
A9BID1	6-phosphofructokinase	10.15%	13
A9BIU1	Amidohydrolase	19.56%	13
A9BIX9	Bifunctional purine biosynthesis protein, PurH	20.28%	13
A9BFY6	Amidohydrolase	4.98%	13
A9BIK0	Radical SAM domain protein	12.35%	13
A9BHJ9	Homoserine dehydrogenase	20.19%	12
A9BHK1	Aldo/keto reductase	32.80%	12
A9BEZ7	Adenylate kinase	36.74%	12
A9BJK5	Two-component transcriptional factor, winged helix family	28.10%	12
A9BJN8	GTPase-like protein	32.50%	12
A9BK13	Nitropropane dioxygenase	30.60%	12
A9BJU1	Oxidoreductase domain protein	38.10%	12
A9BGN9	Glutamate dehydrogenase	23.43%	12
A9BH88	Thiamine pyrophosphate domain protein, TPP-binding	33.23%	12
A9BIL1	Phosphate butyryltransferase	33.67%	12
A9BI20	Cobrynic acid a,c-diamide synthase	53.53%	12
A9BIC7	Aminomethyltransferase	8.62%	12
A9BIX5	Sua5/YciD/YrcD/YwlC family protein	18.90%	12
A9BER7	GMP synthase (glutamine hydrolysing)	19.13%	12

Accession	Description	Coverage	Peptides
A9BFL6	Basic membrane lipoprotein	1.60%	12
A9BFV0	Glycoside hydrolase, family 38	8.02%	12
A9BGT2	Protein GrpE	9.83%	12
A9BHA2	50S ribosomal protein, L2	35.40%	12
A9BIM5	Peptidase M24	7.02%	12
A9BIU5	Aldehyde oxidase and xanthine dehydrogenase, alb hammerhead	9.88%	12
A9BG49	Chaperone protein, HtpG	5.78%	12
A9BG96	Glucose-6-phosphate-1-dehydrogenase	12.69%	12
A9BIB9	Inosine-5'-monophosphate dehydrogenase	12.69%	12
A9BGG5	DegV family protein	19.18%	12
A9BJB2	Glutamate-tRNA ligase 1	11.13%	12
A9BJ43	Glycine cleavage system H protein	78.45%	11
A9BI17	Sigma 54 modulation protein/ribosomal protein S30EA	45.23%	11
A9BI23	Phosphoribulokinase/uridine kinase	8.50%	11
A9BIR0	D-isomer-specific 2-hydroxyacid dehydrogenase, NAD-binding	30.00%	11
A9BK12	Malonyl-CoA-acyl carrier protein, transacyclase	23.37%	11
A9BI68	Aldo/keto reductase	7.16%	11
A9BJH1	SufBD protein	18.52%	11
A9BHV9	Hypoxanthine phosphoribysyltransferase	63.53%	11
A9BH78	Phosphoesterase RecJ domain protein	26.87%	11
A9BIV9	Bifunctional protein, FolD	47.25%	11
A9BJZ5	Histidinol dehydrogenase	20.80%	11
A9BK09	Formiminotransferase-acyclodeaminase	28.29%	11
A9BEQ5	Transcriptional terminating factor, Rho	9.07%	11
A9BG54	Glycine-tRNA ligase, alpha subunit	14.63%	11
A9BHA5	50S ribosomal protein, L3	29.11%	11
A9BHM5	Phosphate butylyltransferase	39.12%	11
A9BFN0	Polyphosphate kinase	2.11%	11
A9BFX3	ATP synthase, subunit beta	12.21%	11
A9BHJ0	Uncharacterised protein	3.37%	11
A9BJ25	Xylulokinase	8.37%	11
A9BJ54	Translation initiating factor, IF2	8.57%	11
A9BIJ4	Bifunctional protein, GlmU	12.56%	11
A9BJF8	Methylthioribose kinase	15.80%	11
A9BHK3	Uncharacterised protein	62.92%	11
A9BF34	50S ribosomal protein, L7/L12	54.69%	10
A9BI62	tRNA synthetase, class II (D,K and N)	23.20%	10
A9BFM6	Alkyl hydroperoxide reductase/Thiol-specific antioxidant/Mal allergen	45.70%	10
A9BH01	Nitroreductase	32.98%	10
A9BK15	DegV family protein	27.66%	10
A9BFB0	PhoH family protein	18.93%	10
A9BFZ1	30S ribosomal protein, S4	28.91%	10
A9BHS0	Aspartate semialdehyde dehydrogenase	23.22%	10
A9BFM4	Pyruvate flavodoxin/ferredoxin oxidoreductase domain protein	10.00%	10

Accession	Description	Coverage	Peptides
A9BHC5	Methylthioribose-1-phosphate isomerase 2	30.37%	10
A9BIJ2	50S ribosomal protein, L25	32.42%	10
A9BF99	Ferroxidase	22.09%	10
A9BFN4	Putative phosphohistidine phosphatase, SixA	56.52%	10
A9BFR6	Alpha/beta hydrolase fold	25.10%	10
A9BFV5	Oxidoreductase domain protein	11.81%	10
A9BH75	Metal-dependent phosphohydrolase	22.83%	10
A9BHF4	Dihydrolipoamide dehydrogenase	7.10%	10
A9BHR1	ROK family protein	6.96%	10
A9BIA4	N-acetyl-gamma-glutamyl-phosphate reductase	12.75%	10
A9BIK9	Putative transcriptional regulator, GntR family	11.38%	10
A9BG01	30S ribosomal protein, S5	24.86%	10
A9BGE8	dDTP-4-dehydrorhamnose reductase	10.37%	10
A9BGS2	30S ribosomal protein, S1	9.59%	10
A9BI98	Arginie biosyntheisi bifunctional protein, ArgJ	6.13%	10
A9BIB5	Alpha amylase catalytic region	14.32%	10
A9BJN0	Pseudouridine-5’-phosphate glycosidase	22.22%	10
A9BJP4	ADP-ribosylating/crystalline, J1	5.00%	10
A9BES6	UDP-N-acetylglucosamine-1-carboxyurinyltransferase	10.00%	10
A9BGF1	Chemotaxis response regulator protein-glutamine methyltransferase	28.45%	10
A9BGT9	Cysteine-tRNA ligase	11.09%	10
A9BHC4	MCP methyltrnsferase, CheR-type	13.75%	10
A9BI67	Aldo/keto reductase	14.59%	10
A9BIU9	Adenine deaminase	2.63%	10
A9BJ40	Glutamate formiminotransferase	2.68%	10
A9BJP3	Glycerophosphoryl diester phosphodiesterase	27.12%	9
A9VJY4	ATP-dependent Clp protease, proteolytic subunit	25.37%	9
A9BH70	Glutaredoxin-like domain protein	42.60%	9
A9BJ18	Gamma-glutamyl-phosphate reductase	20.19%	9
A9BJ03	DegT/DnrJ/EryC/StrS aminotransferase	15.83%	9
A9BEU0	Carbamate kinase	22.36%	9
A9BFZ6	Methionine aminopeptidase	8.40%	9
A9BGK9	Alcohol dehydrogenase, GroES domain protein	13.43%	9
A9BH48	Peptidase U32	16.02%	9
A9BJZ3	ATP phosphoribosyltransferase, predicted regulatoric subunit	15.71%	9
A9BG83	Cellulase	12.94%	9
A9BGJ1	Uncharacterised protein	12.22%	9
A9BGT8	Glutamate-tRNA ligase 2	8.61%	9
A9BGZ6	Beta-lactamase domain protein	24.77%	9
A9BGZ9	L-lactate dehydrogenase	13.68%	9
A9BHJ7	NADH dehydrogenase (ubiquinone) 24kDa subunit	11.69%	9
A9BI02	DNA polymerase III, subunit gamma and tau	2.36%	9
A9BI78	Uncharacterised protein	27.46%	9
A9BIE3	Phosphate-specific transport system accessory protein, PhoU	23.18%	9
A9BIP9	Elongation factor 4	9.57%	9

Accession	Description	Coverage	Peptides
A9BIS5	Elongating factor Ts	22.14%	9
A9BJN9	Glucose-1-phosphate adenylyltransferase	7.66%	9
A9BEQ3	Orotate phosphoribosyltransferase	24.49%	9
A9BGG7	Putative signal-transduction protein with CBS domain	33.33%	9
A9BAI6	3-isopropylmalate dehydrogenase, large subunit	11.22%	9
A9BID5	Trk-A-C domain protein	19.20%	9
A9BJV0	Alanine racemase domain protein	20.90%	9
A9BFC0	Transcriptional elongating factor, GreA	20.75%	9
A9BH99	30S ribosomal protein, S3	19.05%	9
A9BHF7	Phosphate propanoyltransferase	32.14%	9
A9BI35	Rubrerythrin	19.53%	9
A9BIC4	Non-canonical purine, NTP phosphorylase	20.77%	9
A9BIX4	Type III pantothenate kinase	12.94%	9
A9BJN2	Arginine deaminase	6.90%	9
A9BEQ1	Phosphoglucosamine mutase	20.45%	8
A9BF98	Alkyl hydroxyperoxide reductase/Thiol-specific antioxidant/Mal allergen	38.27%	8
A9BHX6	Uncharacterised protein	8.05%	8
A9BH59	Biotin/lipolyl attachment domain-containing protein	20.51%	8
A9BI54	UDP-N-acetylmuramate alanine ligase	7.69%	8
A9BI88	Flagellar-body rod protein, FlgC	25.71%	8
A9BI95	Riboflavin biosyntheisi protein, RibF	16.55%	8
A9BJ15	Probable endonuclease 4	19.16%	8
A9BFM0	Radical SAM domain protein	12.82%	8
A9BGT0	UDP-N-acetylmuramoyl-tripeptide synthase	12.82%	8
A9BH62	30S ribosomal protein, S6	52.00%	8
A9BJA5	Endoribonuclease L-PSP	42.52%	8
A9BJQ6	Gamma-glutamyltransferase	7.30%	8
A9BGG0	Uncharacterised protein	46.32%	8
A9BIA0	Acetylornithine aminotransferase	5.33%	8
A9BIX1	ABC transporter-related	3.83%	8
A9BIX7	Phosphoribosylamine-glycine ligase	9.47%	8
A9BEN2	Elongation factor P	18.38%	8
A9BFZ9	50S ribosomal protein, L15	37.75%	8
A9BGR9	Uncharacterised protein	9.14%	8
A9BIU3	Molybdopterin dehydrogenase, FAD-binding	17.70%	8
A9BI30	Response regulator receiver protein	47.06%	7
A9BIZ9	Inositol-phosphate phosphatase	13.95%	7
A9BFE9	Polyprenyl synthase	3.12%	7
A9BHA9	30S ribosomal protein, S7	45.16%	7
A9BHK7	Probable transaldolase	37.56%	7
A9BHR8	4-hydroxy-tetrahyderdipicolinate synthase	15.75%	7
A9BHV1	Phosphodiesterase, MJ0936 family	13.92%	7
A9BIA8	Isocitrate dehydrogenase (NAD(+))	17.52%	7
A9BIG7	PPS modulated sigma 54 specific translational regulator, Fis family	9.36%	7
A9BEW1	Uncharacterised protein	38.41%	7
A9BF67	Cupin 2 conserved barrel domain protein	25.41%	7

Accession	Description	Coverage	Peptides
A9BFD0	Peptidase S58, OmpA	12.85%	7
A9BFS9	Beta-lactamase domain protein	17.51%	7
A9BFY8	Conserved hypothetical radical SAM protein	8.55%	7
A9BG82	Peptidase M42 family protein	18.50%	7
A9BH56	Methylmalonyl-CoA epimerase	10.45%	7
A9BJN7	Nucleoside diphosphate kinase	29.05%	7
A9BEN0	Deoxyribose-phosphate aldolase	6.83%	7
A9BEZ1	Aminotransferase, class I and II	20.70%	7
A9BFA4	Beta-phosphoglucomutase	24.50%	7
A9BFL0	D-isomer-specific 2-hydroxyacid, NAD-binding	12.87%	7
A9BFR4	Peptidase M55 D-aminopeptidase	4.41%	7
A9BGJ9	Uncharacterised protein	5.39%	7
A9BGT7	NUDIX hydrolase	13.64%	7
A9BHR0	DegV family protein	8.63%	7
A9BIS6	Uridylate kinase	15.52%	7
A9BJ01	Glucose-1-phosphate thymidylyltransferase	14.57%	7
A9BES4	Csf-like hydrolase	9.03%	7
A9BEW9	Uncharacterised protein	8.16%	7
A9BF43	Uncharacterised protein	3.25%	7
A9BF76	Methenyl tetrahydrofolate cyclohydrolase-like protein	12.44%	7
A9BGH7	NAD-dependent epimerase/hydratase	12.78%	7
A9BGV6	30S ribosomal protein, S2	6.60%	7
A9BA38	Hydroxypyruvate reductase	5.78%	7
A9BHF3	Aldo/keto reductase	9.32%	7
A9BHS3	Thymidylate synthase, ThyX	5.75%	7
A9BID5	Spermidine synthase	21.18%	7
A9BI22	OsmC family protein	21.74%	7
A9BIL0	Probable butyrate kinase	2.49%	7
A9BJH0	Cysteine desulfurase	7.11%	7
A9BJH3	FeS assembly ATPAse, SufC	14.40%	7
A9BJN1	Phosphomethylpyrimidine kinase, type 1	12.29%	7
A9BHL5	Uncharacterised protein	48.44%	6
A9BJC2	Uncharacterised protein	4.68%	6
A9BFT4	Uncharacterised protein	17.33%	6
A9BI34	Desulfoferredoxin, ferrous iron-binding region	18.80%	6
A9BJX7	Cell division protein FtsA	1.44%	6
A9BK08	Uncharacterised protein	16.39%	6
A9BHY1	Response regulator receiver protein	32.77%	6
A9BIZ0	Aldo/keto reductase	19.46%	6
A9BFX5	ATP synthase, subunit alpha	3.37%	6
A9BG04	30S ribosomal protein, S8	21.64%	6
A9BGE6	Polysaccharide biosynthesis protein, CspD	14.86%	6
A9BGS7	UDP-N-acetylmuramoylalanine-D-glutamate ligase	4.18%	6
A9BHV6	Glutamate formimidoyltransferase	12.13%	6
A9BJY2	Uncharacterised protein	19.33%	6
A9BJZ8	1-(5-phosphoribosyl)-5-(-(5-phosphoribosylamino)-methylidenamino)-imidazole-4-carboxyamide isomerase	26.30%	6

Accession	Description	Coverage	Peptides
A9BF31	Flagellar hook-associated 2 domain protein	4.38%	6
A9BF36	50S ribosomal protein, L1	20.76%	6
A9BF66	CheW protein	13.64%	6
A9BF96	Carbohydrate kinase, FGGY	5.36%	6
A9BGK3	Tetratricopeptide TPR-2 repeat protein	5.03%	6
A9BGW3	Uncharacterised protein	19.16%	6
A9BH95	50S ribosomal protein, L14	27.05%	6
A9BHU0	Phage SPO1 DNA polymerase-related protein	17.70%	6
A9BI25	Uncharacterised protein	11.88%	6
A9BIW9	Phosphofructokinase	2.70%	6
A9BJW3	NAD7NADP octopine/nopaline dehydrogenase	2.49%	6
A9BHA4	50S ribosomal protein, L4	19.46%	5
A9BI87	Flagellar basal body rod prote7glnin, FlgB	7.52%	5
A9BIT5	Dihydropyrimidinase	5.32%	5
A9BHY0	CheW protein	33.33%	5
A9BF69	Flagellar assembly factor, FliW	24.84%	5
A9BFT2	Aspartyl/glutamyl-tRNA (Asn/Gln) amidotransferase, subunit 1	41.00%	5
A9BFZ0	DNA-directed RNA polymerase, subunit alpha	4.25%	5
A9BGK1	Isochorismutase hydrolase	11.94%	5
A9BGN7	Homoserine kinase	8.14%	5
A9BIT8	Molybdopterin dehydrogenase, FAD-binding	11.30%	5
A9BJ61	Uncharacterised protein	17.42%	5
A9BFQ9	Beta-galactosidase	3.36%	5
A9BFV4	NADH dehydrogenase (ubiquinone) 24kDa subunit	24.26%	5
A9BH97	50S ribosomal protein, L29	39.39%	5
A9BIA7	3-isopropylmalate dehydrogenase, small subunit	22.75%	5
A9BJD6	Ribosome recycling factor	16.04%	5
A9BJG8	CoA-binding domain protein	21.38%	5
A9BJM8	Aldo/keto reductase	14.84%	5
A9BJU3	Isoaspartyl dipeptidase	8.24%	5
A9BJX2	6-phosphofructokinase	11.54%	5
A9BJZ7	Imidazole glycerol phosphate synthase, subunit HisH	18.37%	5
A9BK00	Histidine biosynthesis bifunctional protein, HisE	13.76%	5
A9BEQ8	Stage V sporulating protein S	30.30%	5
A9BEU9	Ribosomal protein, S12 methylthiotransferase, RimO	2.76%	5
A9BFG0	Cell wall hydrolase/autolysin	7.69%	5
A9BFU1	Adenylyl-sulfate kinase	18.91%	5
A9BFW1	Homoserine O-succinyltransferase	3.88%	5
A9BFZ3	30S ribosomal protein, S13	16.53%	5
A9BG03	50S ribosomal protein, L6	5.38%	5
A9BGZ3	Uncharacterised protein	7.10%	5
A9BH55	ArgK protein	4.52%	5
A9BH65	50S ribosomal protein, L9	22.82%	5
A9BHA3	50S ribosomal protein, L23	24.49%	5
A9BHB5	30S ribosomal protein, S9	23.70%	5
A9BHR2	2-C-methyl-D-erythritol-4-phosphate cytidyltransferase	15.42%	5

Accession	**Desription**	**Coverage**	**Peptides**
A9BHX8	Cin-like protein	2.48%	5
A9BIK4	Maf-like protein, Pmob-1464	7.89%	5
A9BK11	Short-chain dehydrogenase /reductase, SDR	14.92%	5
A9BEN1	Cold-shock DNA-binding domain protein	41.54%	4
A9BFB2	Stage V sporulating protein S	72.22%	4
A9BF38	Transcriptional termination/antitermination protein, NusG	4.24%	4
A9BIE9	D-tyrosyl-tRNA (Tyr) deacyclase	27.33%	4
A9BFL8	HAD superfamily hydrolase, subfamily IA, variant 3	13.57%	4
A9BI45	ATP-dependent protease, subunit HsIV	23.73%	4
A9BJ24	D-isomer-specific 2-hydroxyacid dehydrogenase, NAD-binding	9.15%	4
A9BJZ4	ATP phosphoribosyltransferase	20.48%	4
A9BFG5	CMP/dCMP deaminase, zinc-binding	6.51%	4
A9BFM3	Mannose-6-phosphate isomerase, type I	5.90%	4
A9BG00	50S ribosomal protein, L30	19.67%	4
A9BG06	50S ribosomal protein, L5	12.43%	4
A9BG59	Thymidylate kinase	18.53%	4
A9BGV1	Uncharacterised protein	5.88%	4
A9BHB6	50S ribosomal protein, L13	17.88%	4
A9BHI9	Glycerol transferase, family 2	15.08%	4
A9BIL2	Probable butyrate kinase	11.83%	4
A9BIU6	Aldehyde oxidase and xanthine dehydrogenase, molybdopterin-binding	35.91%	4
A9BJU7	Uncharacterised protein	15.75%	4
A9BER9	5’-nucleotidase, SurE	3.17%	4
A9BEU6	Appr-1p processing domain protein	13.74%	4
A9BF03	50S ribosomal protein, L19	8.40%	4
A9BF12	Flagellar basal body rod protein	4.58%	4
A9BF86	UDP-N-acetylglucosamine-2-epimerase	3.81%	4
A9BGS4	Cytidylate kinase	10.18%	4
A9BGX3	HEAT domain protein	5.34%	4
A9BFZ1	Uncharacterised protein	5.10%	4
A9BI00	Uncharacterised protein	4.48%	4
A9BI38	Metal-dependent phosphohydrolase	9.94%	4
A9BIF4	1-deoxy-D-xylulose-5-phosphate reductoisomerase	7.79%	4
A9BJV3	UDP-N-acetylenolpyrimoylglucosamine reductase	3.27%	4
A9BFB3	Uncharacterised protein	15.09%	4
A9BFY9	50S ribosomal protein, L17	10.94%	3
A9BIZ1	HAD superfamily hydrolase, superfamily IIA	5.42%	3
A9BJG5	Ferripyochelin-binding protein (Fbp)	15.48%	3
A9BFU0	50S ribosomal protein, L20	17.36%	3
A9BFV3	NADH dehydrogenase (quinone)	24.19%	3
A9BHA0	50S ribosomal protein L22	10.74%	3
A9BHQ2	Tryptophan synthase, alpha chain	12.40%	3
A9BIB6	Signal recognition particle receptor, FtsY	3.62%	3
A9BIE2	Two-component transcriptional regulator, winged helix family	4.31%	3

Accession	**Description**	**Coverage**	**Peptides**
A9BIN8	Uncharacterised protein	3.44%	3
A9BFE3	Putative signal transduction protein with CBS domain	3.95%	3
A9BFE8	Uncharacterised protein	5.81%	3
A9BFT8	Translation initiating factor, IF3	4.57%	3
A9BGL7	Uncharacterised protein	4.78%	3
A9BHA1	30S ribosomal protein, S19	8.25%	3
A9BI28	Uncharacterised protein	11.06%	3
A9BIL6	Thiamine pyrophosphate protein, domain protein, TPP-binding	3.98%	3
A9BIX3	Radical SAM domain protein	2.68%	3
A9BEZ8	30S ribosomal protein, S16	19.82%	2
A9BEZ9	UPF 0109 protein, Pmob-0321	13.16%	2
A9BJT7	Flavoprotein, WrbA	7.87%	2
A9BEW2	PEBP family protein	8.97%	2
A9BFH2	Phenylalanine-tRNA ligase, alpha subunit	3.20%	2
A9BHD4	Uncharacterised protein	25.84%	2
A9BHV2	Thiamine pyrophosphate kinase	5.05%	2
A9BIL7	Pyruvate/keto/isovalerate oxidoreductase, gamma subunit	7.78%	2
A9BJ19	Pyrrolo-5-carboxylate reductase	5.47%	2
A9BJG6	PHP domain protein	5.99%	2
A9BJY6	Uncharacterised protein	10.00%	2
A9BIU4	(2Fe-2S)-binding domain protein	9.15%	1
A9BF62	MoaD family protein	13.19%	1
A9BG02	50S ribosomal protein, L18	7.38%	1
A9BG51	MEMO1 family protein, Pmob-1088	4.38%	1
A9BGA6	YcfA family protein	17.57%	1
A9BGL6	Macrophage migration inhibitory factor family protein	8.04%	1
A9BIY8	Uracil phosphoribosyltransferase	6.22%	1
A9BJZ0	Acyl carrier protein	14.12%	1
A9BJX5	DNA ligase	5.24%	12
A9BJZ6	Imidazoleglycerol-phosphate dehydratase	21.35%	4
A9BGV8	Putative esterase	10.14%	8
A9BIR2	Uncharacterised protein	23.90%	4
A9BHW5	Flagellar protein export ATPase, FliL	8.14%	13
A9BG64	UvrD/REP helicase	1.97%	14
A9BJI6	Radical SAM domain protein	1.56%	18
A9BH43	Acetamidase/Formamidase	7.90%	12
A9BG47	D-lysine 56-aminomutase, alpha subunit	5.41%	6
A9BEZ4	Uncharacterised protein	5.40%	9
A9BET8	Uncharacterised protein	23.33%	6
A9BGS6	RNA methyltransferase, Trm family	3.81%	9
A9BJX3	Deoxyribodipyrimidine photo-lyase	2.16%	9
A9BIF3	Metal-dependent phosphohydrolase	2.50%	5
A9BH77	Purine nucleoside phosphorylase	8.89%	5
A9BIM9	Glycoside hydrolase, family 38	1.34%	12
A9BIU0	(2Fe-2S)-binding domain protein	14.65%	3

Table 7.13: Total protein identification of *Kosmotoga olearia*, sterile filtered

Accession	**Description**	**Coverage**	**Peptides**
C5CTF5	Pyruvate phosphate dikinase	24.83%	56
C5CG15	Iron-containing alcohol dehydrogenase	62.44%	47
C5CFP9	Proline-tRNA ligase	3.44%	33
C5CET5	Translation elongation factor G	24.70%	31
C5CFX5	Uncharacterised protein	24.70%	30
C5CHD5	Phosphopentomutase	20.57%	29
C5CHA1	Isoleucine-tRNA ligase	6.73%	29
C5CI62	Aldehyde oxidase and xanthine dehydrogenase, mollybdopterin-binding	3.00%	29
C5CGU7	Probable transcriptional regulatoric protein, Kole-1935	12.30%	28
C5CIG5	Leucine-tRNA ligase	1.52%	28
C5CEP3	Enolase	36.68%	26
C5CHM3	Phosphoglycerate kinase	45.68%	23
C5CD52	Spermidine synthase	20.83%	23
C5CHS3	Ornithine carbamoyltransferase	28.27%	22
C5CHS1	M20/DapE family protein, YgeY	18.80%	20
C5CDU0	Pphosphoribosylkinase/uridine kinase, family protein	6.14%	20
C5CEI1	Iron-containing alcohol dehydrogenase	36.81%	19
B5M6P0	Acetate kinase	45.20%	19
C5CIR4	4Fe-4S ferredoxin iron-sulfur-binding protein	83.05%	19
C5CFT9	Transglutaminase domain protein	17.33%	19
C5CEN6	Probable 2.3-bisphosphoglycerate-independent phosphoglycerate mutase	24.50%	19
C5CGC3	Serine-tRNA ligase	3.54%	19
C5CEY5	3-oxoacyl-CoA transferase, B subunit	6.39%	17
C5CHS6	Dihydroorotate dehydrogenase, family protein	10.53%	17
C5CIU5	Asparigine-tRNA ligase	2.78%	17
C5CEP1	ABC-type nitrate/sulfonate/bicarbonate transport system, periplasmic components-like protein	30.23%	16
C5CFS0	50S ribosomal protein, L25	33.95%	16
C5CGZ4	Galactokinase	8.99%	16
B5M6L7	60kDa chaperonin	24.12%	16
C5CHM2	Glyceraldehyde-3-phosphate dehydrogenase, type I	32.02%	16
C5CHF3	Uncharacterised protein	10.84%	16
C5CDK1	D-lysine 56-aminomutase, alpha subunit	5.75%	16
C5CDZ3	Glutamate dehydrogenase	24.22%	16
C5CEN4	Iron-containing alcohol dehydrogenase	2.96%	16
C5CIR3	D-isomer-specific 2-hydroxyacied dehydrogenase, NAD-binding	2.81%	16
C5CF34	Ribosome recycling factor	23.78%	15
C5CDQ0	Carboxyl transferase	2.51%	15
C5CHM4	Triosephosphate isomerase	42.97%	14
C5CFG7	Formimidoyltetrahydrofolate cyclodeaminase	25.84%	14
C5CHF7	Peptidase U62 modulator of DNA gyrase	4.10%	14
C5CDT5	Pyruvate/ketoisovalerate oxidoreductase, gamma subunit	42.86%	13

Accession	Description	Coverage	Peptides
C5CIU7	Aspartate-tRNA ligase	2.20%	13
C5CIR2	Aminotransferase, class V	17.89%	13
C5CGG8	DNA polymerase III, subunit beta	20.44%	13
C5CGM0	Transketolase domain protein	5.84%	13
C5CGT4	Glycogen synthase	8.42%	13
C5CFH6	6-phosphofructokinase	11.47%	13
C5CGI1	Adenylate kinase	18.14%	12
B5M6P1	Fructose-bisphosphate aldolase (Fragment)	22.18%	12
C5CF47	Aminomethyltransferase	6.79%	12
C5CEX3	Peptidase M42 family protein	10.47%	12
C5CFQ0	Purine nucleoside phosphorylase	23.43%	12
C5CHD0	Peptidase M42 family protein	7.35%	12
C5CF11	NUDIX hydrolase	14.51%	11
C5CE23	Ornithine carbamoyltransferase, catabolic	11.18%	11
C5CFT8	Mandelate racemase/muconate lactonising protein	18.95%	11
C5CET1	Two-component transcriptional regulator, winged helix family	19.57%	10
C5CGE0	50S ribosomal protein, L7/L12	18.75%	10
C5CHH3	Dipeptidase	3.24%	10
C5CDT7	Pyruvate flavodoxin/ferredoxin oxidoreductase, domain protein	22.62%	9
C5CH14	Malonyl-CoA –acyl carrier protein, transacyclase	6.45%	9
C5CHC9	Peptidase M42 family protein	24.71%	9
C5CHP6	Cytosine deaminase and related metal-dependent hydrolase-like protein	6.78%	9
C5CEA6	Methylthioribose-1-phosphate isomerase	6.05%	9
C5CFU7	OsmC family protein	19.73%	9
C5CHC8	Peptidase M42 family protein	9.52%	9
C5CGB5	Rubrerythrin	9.18%	9
C5CI92	Elongating factor P	6.49%	9
C5CG6	Elongating factor Tu	6.77%	9
C5CIQ9	Probable butyrate kinase	11.80%	9
C5CE22	Carbamate kinase	24.68%	8
C5CDQ4	Methylmalonyl-CoA mutase, large subunit	2.32%	8
C5CIQ7	Probable butyrate kinase	9.09%	7
C5CD88	Glutaredoxin-like domain protein	5.58%	7
C5CF46	Glycine cleavage system H protein	21.14%	7
C5CDB3	N-acetylmuraminate-9-phosphate synthase	3.66%	7
C5CHD8	Uncharacterised protein	4.38%	7
C5CD96	30S ribosomal protein, S6	20.53%	7
C5CDS1	Dihydrolipoamide dehydrogenase	2.89%	7
C5CHX9	4-hydroxytetrahydrodipicolinate synthase	4.08%	7
C5CIH0	Glucose-6-phosphate isomerase	3.05%	7
C5CDQ1	Methylmalonyl-CoA epimerase	22.96%	6
B5M6L6	Pyruvate kinase	3.60%	6
C5CHE0	3-oxoacyl-(acyl-carrier-protein) reductase	10.08%	6
C5CF51	10kDa chaperonin	32.58%	6
C5CDS8	Response regulator receiver protein	27.88%	5

Accession	Description	Coverage	Peptides
C5CDH1	Histone family protein, DNA-binding protein	30.00%	5
C5CFG9	Metal-dependent phosphorylase	8.24%	5
C5CGH2	Probable transaldolase	5.50%	5
C5CD93	50S ribosomal protein, L9	10.60%	5
C5CDJ7	Dihydropicolinate reductase	4.12%	5
C5CG18	Peptide deformylase	17.68%	5
C5CDJ0	Aminotransferase, class IV	5.61%	4
C5CE51	Uncharacterised protein	7.84%	3
C5CHB7	Uncharacterised protein	21.49%	3
C5CFQ4	Cytidine deaminase	9.09%	3
C5CGN8	NAD/NADP octopine/nopaline dehydrogenase	2.77%	3
C5CIN3	Desulfoferredoxin ferrous iron-binding region	12.71%	2
C5CDZ6	Zn-dependent hydrolase of the beta-lactamase fold-like protein	22.64%	8
C5CHV3	Uncharacterised protein	7.79%	6
C5CGT8	Hydrolase of HD superfamily-like protein	6.12%	5
C5CES3	2’-5’-RNA ligase	5.77%	8
C5CFS9	Valine-tRNA ligase	4.04%	24
C5CH15	Acyl carrier protein	22.37%	4
C5CH17	Uncharacterised protein	10.75%	2
C5CHB0	Ribose-5-phosphate isomerase B	7.28%	9
C5CEU0	Peptidase U62 modulator of DNA gyrase	6.80%	19
C5CIP5	M18 family aminopeptidase	3.85%	11
C5CE76	Alpha amylase catalytic region	0.00%	42
C5CI06	Heavy-metal transport/detoxification protein	17.65%	4
C5CGY3	Alcohol dehydrogenase, GroES domain protein	6.99%	9
C5CIX5	Phosphate propanoyltransferase	4.57%	11
C5CDH8	Alpha amylase catalytic region	3.13%	13
C5CFP2	L-lactate dehydrogenase	3.72%	8
C5CFG8	Deoxyribose-phosphate aldolase	6.53%	9
C5CGE1	50S ribosomal protein, L10	5.59%	4

Table 7.14: Protein identification of *Thermotoga maritima*, TCEL2, grown on Avicel

Accession	Desription	Coverage	Peptides
A5IJI7	Alpha amylase catalytic region	7.69%	22
A5IJR6	60kDa chaperonin	39.22%	19
A5IKR8	Uncharacterised protein	12.36%	15
A5U99	Glyceraldehyde-3-phosphate dehydrogenase	33.63%	12
A5IM81	Elongating factor Tu	22.75%	10
D2C6U2	Extracellular solute-binding protein	15.37%	10
D2C4E5	Type IV pilin-related protein	17.61%	9
A5IM81	Elongating factor Tu	15.50%	7
O05651	FAD synthase, subunit PorA	23.72%	7
B1L9A0	FAD-dependent pyridine nucleotide-disulfide oxidoreductase	13.77%	7
B1LAP9	Probable 2,3-bisphosphoglycerate-independent phosphoglycerate kinase	15.96%	6

Accession	**Description**	**Coverage**	**Peptides**
A5ILE6	Lysine-tRNA ligase	6.57%	6
A5IKV9	Iron-containing alcohol dehydrogenase	6.72%	6
Q56317	Pyruvate synthase, subunit PorB	9.26%	6
A5IM41	Flagellar basal-body rod protein, FlgG	4.60%	5
A5IJ65	Serine hydroxymethyltransferase	3.75%	5
A5IJL6	Ferritin, Dps family protein	19.23%	5
Q9WZF8	Trigger factor	2.35%	5
A5IK30	Alkyl hydroxyperoxidase-like protein, AhpD family	48.65%	5
A5ILS4	S-adenosylmethionine synthase	12.91%	5
A5ILS7	Two-component transcriptional regulator, winged helix family	20.50%	4
A5IIY2	3-oxoacyl-(acyl-carrier-protein) synthase 2	12.86%	4
A5IM86	50S ribosomal protein, L2	4.35%	4
B1LBS5	Aminotransferase, class V	4.17%	4
A5IIR5	Glutaredoxin-like domain protein	19.00%	3
A5IIG8	Uncharacterised protein	28.70%	3
Q9WZS7	Alcohol dehydrogenase (NADP(+))	8.35%	3
P29396	50S ribosomal protein, L7/L12	27.34%	3
O05650	Pyruvate synthase, subunit PorC	16.15%	3
A5IMA4	Adenylate kinase	5.91%	3
A5IML3	Response regulator receiver protein	21.55%	3
D2C7B0	Lysine-2,3-aminomutase, YodO family protein	9.86%	3
D2C/96	Diguanylate cyclase	13.96%	3
A5IM92	30S ribosomal protein, S17	30.84%	3
O08398	Cell division protein, FtsZ	4.27%	3
B9KBU6	Alkyl hydroxiperoxidase-like protein, AhpD family	24.56%	2
A5ILI5	Uncharacterised protein	11.11%	2
A5ILV4	Ribose-phosphate pyrophosphokinase	4.44%	2
A5IM88	50S ribosomal protein, L3	14.98%	2
A5IMT0	ABC transporter-related	3.81%	2
P96108	Ornithine carbamoyltransferase	4.47%	2
A5IIQ1	O-acetylhomoserine/O-acetylserine sulfhydrylase	3.02%	2
Q9WZ72	30S ribosomal protein, S6	10.94%	2
A5IJM0	3-isopropylmalate dehydratase, small subunit	10.24%	2
A5IMK7	Flagellar hook-basal body complex protein, FliE	15.96%	2
B1LBD7	50S ribosomal protein, L25	8.84%	2
Q9WZC6	Putative superoxide reductase	12.21%	1
A5ILJ5	Elongating factor P	6.49%	1
A5IM95	50S ribosomal protein, L5	5.18%	1
A5IMH3	D-isomer-specific 2-hydroxyacid dehydrogenase, NAD-binding	5.52%	1
Q56312	Chemotaxis protein, CheY	9.17%	1
A5IJC5	Acyl carrier protein	16.05%	1
A5IKN6	Phosphofructokinase	3.50%	1
A5IMF0	CoA-binding domain protein	8.40%	1
P46797	Ferredoxin	45.00%	3
A5IMF8	Hydrogenase, large subunit domain protein	3.88%	4
O05651	Pyruvate synthase, subunit PorA	29.34%	11

Accession	**Description**	**Coverage**	**Peptides**
A5IJ26	Flagellin domain protein	3.36%	2
Q4FF54	Flagellin	5.65%	5
P38516	50S ribosomal protein, L4	4.68%	3
Q9X1H0	Cupin 2, conserved barrel domain protein	13.16%	3
Q9XDK7	Flagellar hook-associated protein 2	1.81%	4
Q56316	Pyruvate synthase, subunit PorD	16.16%	8
B1LBI3	30S ribosomal protein, S1	3.13%	5
Q9X0E0	Cellulase	3.32%	3