Oases of Microbial Life in the Highest Elevation Fumaroles on Earth

By

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ABSTRACT

Investigating Earth for life in extreme environments provides insight into what life might exist elsewhere in the universe. Many celestial bodies have dry and cold landscapes. On Earth the Andes Mountains that form the eastern border of the Atacama Desert in South America are marked by a very dry and cold environment. A previous study in the region, of a volcanic mountain with a summit above 6000 meters above sea level (m.a.s.l.), discovered a fumarole (geothermal vent) that supports an oasis of life in an otherwise barren landscape. This study re-sampled the same fumarole located at 5825 m.a.s.l. and a second fumarole near the summit at 6050 m.a.s.l., which is now the highest elevation fumarole ever investigated for biota. Additionally, three non-fumarole sites were sampled; two at 5825 m.a.s.l. and one at 6050 m.a.s.l. Biogeochemical parameters (enzyme activity, percent water, and percent carbon and nitrogen) were measured in addition to the creation of high-throughput DNA sequencing libraries of 16S and 18S rRNA operational taxonomic unit (OTU) identifiers. Sequence analysis and statistical tests were done to determine the effects of fumarolic activity, elevation, and biogeochemical parameters on community alpha and beta diversity. Fumarole samples had much higher alpha diversity than all non-fumarole samples, with over three times more bacterial and eukaryotic OTUs observed. Furthermore, beta diversity analyses revealed fumarolic soils closely clustered together, to the exclusion of non-fumarolic soils for all three domains of life. This pattern was shown to be statistically significant using Adonis, while elevation and biogeochemical properties were not a significant driver of community composition. These findings indicate that fumarolic activity allows the proliferation of complex microbial communities, even in environments that are seemingly devoid of life due to dry and cold conditions.
Introduction

Are we alone in the universe? The question has long captivated humanity. Philosophers and astronomers of antiquity gazed at the stars and imagined what life might exist beyond our own; Millennia have passed with one generation after another pondering this uncertainty; in the present era, the age of space exploration, the fundamental question continues to allure and inspire. The discovery of life outside of planet Earth would be a momentous and civilization-altering event. For present era scientists the search for life elsewhere is a considerable challenge. In the United States, the National Aeronautics and Space Administration (NASA) has provided a framework to consolidate varying efforts of this search into a concrete roadmap (Des Marais et al. 2008).

The roadmap provides several goals that serve as a guide for investigators. For the present project the fourth and fifth goals of the roadmap are the main points of emphasis. The fourth goal is to seek an understanding of how life and the environment on Earth have co-evolved over time. This goal may be accomplished by studying how life has evolved, and is currently evolving, in varying environmental conditions. The fifth goal of the roadmap is to understand the evolutionary mechanisms and environmental limits of life, including the genetic and biochemical mechanisms that both limit and drive evolution in extreme environments.

While the technical limits of our capability for both manned and un-manned exploration might be readily apparent, the current knowledge and comprehension of where to look for life and what life might exist is still very much undecided. The study of Earth analogues for potential extraterrestrial environments suitable for life is of practical necessity.

Geothermal activity provides important factors necessary for life here on Earth. The deep ocean floor contains an ecosystem found well below the last streaks of light and was believed to be a sparse, relatively barren landscape devoid of biotic diversity. The investigation of deep sea
hydrothermal vents was met with great intrigue and anticipation (Lonsdale 1977, Weiss et al. 1977, Corliss et al. 1979). After the discovery of abundant and unique life forms in the hydrothermal vents along the Galápagos Rift, a hypothesis on the origins of life has developed. It is based upon the abiotic synthesis of organic compounds by result of a reactive chemical environment featuring mixing of high temperature alkaline vent waters with cool acidic ocean waters (Corliss et al. 1981, Baross and Hoffman 1985, Pace 1997, Watchtershauser 2000, Cody et al. 2000, Huber et al. 2003). Deep sea vents host a stunning diversity of life with intricate food webs and important environmental impacts on the ocean as an ecosystem (Jannasch 1985). They serve as archipelagos of rich biotic diversity surrounded by desolate expanses, which are dominated by a few main organisms (Desbruyeres et al. 1994).

Additionally, investigation into terrestrial geothermal vents in the high latitude, harsh environment of Antarctica has yielded important information. Early investigation of biota among the vents on the continent furthered evidence of the uniqueness of this microenvironment in providing conditions to support diversity in otherwise unfertile terrain (Broady et al. 1987, Bargagli et al. 1996). More recent research has reinforced these findings (Soo et al. 2009, Herbold et al. 2014a, Herbold, et al. 2014b). Other geothermal locations as diverse as the Galápagos Islands, Hawaii, Wyoming, California, New Mexico, and Russia have lent support to vents being hotspots for microbial biodiversity (Mayhew et al. 2007, Tin et al. 2011, Bizzoco and Kelly 2013).

Another extreme environment where geothermal activity occurs is on high elevation volcanoes. Prior to the present study very little formal inquiry had been conducted on this ecosystem. The only previously known study of a fumarole above 5000 m.a.sl. was documented in Costello et al. (2009). Sampling one fumarole on the Volcán Socompa at an elevation of 5820
m.a.s.l. they found an oasis of life amid the barren landscape. Additional exploration of the neighboring Vulcán Llullaillaco did not find any fumaroles, but similarly low diversity communities were found when compared to the non-fumaroles on Socompa (Lynch et al., 2012).

Further investigation of this unique system would help to answer lingering questions from the previous research. Having sampled only one fumarole it was difficult to portray the findings as anything other than unique. Did the sampled fumarole provide indication of what biota would be found in other high elevation fumaroles? Would other fumaroles host similar levels of biodiversity? What about similarity in the community composition? Additionally, sampling across the mountain itself would provide insight into other microenvironments such as high elevation ice deposits. My belief is other fumaroles will not only contain comparably enhanced biodiversity, but microbial community composition will also be similar across geographically separated fumaroles as well. Furthermore, the exploration of this system provides an analog for environments outside of Earth that are dry, cold, exposed to higher levels of radiation, lacking in soil nutrients, and featuring a severe diurnal temperature fluctuation (Costello et al. 2009, Lynch et al. 2012).

Beyond the act of discovery, what will the insight gained from this research contribute to the broader world of science? For ecology the interplay between geothermal vents and high elevation is a fascinating comparison for models of biodiversity. For microbiology the identification of extremophiles found in the low diversity non-fumarole sites provides opportunity to investigate the adaptation of life to Earth’s extremes. Finally, for astrobiology another potential analogue for celestial bodies can give a glimpse into the potential for life elsewhere.
Methods

Sampling locations

Sampling occurred at six different sites on Socompa Volcano at various elevations and microenvironments. The fumarole located at 5820 m.a.s.l., referenced in Costello et al. (2009), was resampled. Two non-fumarole samples at the same elevation were sampled at distances from the fumarole of 20m to the north and 25m to the south. One additional location at this elevation was sampled from the soil between penitentes of an icefield 200 m from the fumarole. Two locations near the summit were selected at ~6049 m.a.s.l. A fumarole site with a visible mat-like carpet of moss was sampled at 6049 m.a.s.l.; the second fumarole sample was 4m below the first and within the vent area, but with no vegetation present. The non-fumarole site a distance of 100 meters to the south of the summit fumarole was sampled three times in a triangle pattern, each sample separated by a distance of 5m from the other two samples. The sampling procedure at all sampling locations was the same, at least three replicate samples of 4 mm in depth were obtained from each site, unfortunately not all samples yielded usable PCR product.

Soil biogeochemical parameters such as levels of organic matter, organic nitrogen, dissolved organic carbon, dissolved nitrogen, pH, water content, and various enzyme assays were measured before I carried out DNA sequence analyses. Biogeochemical analyses are described elsewhere (Weintraub et al. 2007, King et al. 2008, Lynch et al. 2012).

DNA sequencing

Total DNA was extracted with Power Soil DNA Isolation Kit (MOBIO Laboratories, Carlsbad, CA, USA). The 16S small-subunit rRNA in bacteria and archaea and 18S small-subunit rRNA in eukarya were used for OTU identification due to the conserved nature of the sequences through inclusion in the ribosome, which make them a permanent structure in all forms of life (Woese 1987). Additionally, the similarity of the beginning and ending sequences
make them well suited for primer targets. Amplification of the bacterial 16S rRNA was achieved through the use of oligonucleotide primers 515f/806r and eukaryotic 18S rRNA through oligonucleotide primers Euk_1391f/EukBr (Earth Microbiome Project, accessible at http://www.earthmicrobiome.org/emp-standard-protocols/). All forward and reverse primers were modified to include a unique 12 nucleotide barcode. PCR reaction mixtures contained 0.5 μL of forward primer, 0.5 μL of reverse primer, 1 μL of template and 12.5 μL of MM Gotaq Hot start Colorless Master Mix (Promega Corporation, Madison, WI, USA). The reaction volume was adjusted to a total of 25 μL with ultrapure DNase/RNase free water. Thermal cycles consisted of an initial denaturation of 94°C for 3 min, followed by 35 cycles of 94°C for 45 sec; 57°C for 60 sec; and 72°C for 90 sec; with a final elongation step of 72°C for 10 min. To prepare amplicons for sequencing, amplicon purification and normalization was done with Invitrogen SequalPrep Normalization Kit (Invitrogen Inc., CA, USA). Amplicons were combined into a single pool and sequenced using the Illumina MiSeq platform (BioFrontiers Institute, Boulder, CO) using pair-end 2x150 bp chemistry.

Bioinformatics
Data analyses were conducted utilizing QIIME 1.9 (Caporaso et al. 2010a). The 16S rDNA identifier community was limited to all samples with a sequence length greater than 1000 base pairs and the 18S rDNA identifier community was limited to all samples with a sequence length greater than 1400 base pairs. Both datasets were subjected to the QIIME 454 data default pipeline unless noted otherwise. Both communities were subjected to OTU selection at 97% identity level through UCLUST (Edgar et al. 2010) and all singleton OTUs were removed. Taxonomic assignment for 16S followed UCLUST assignments, while 18S utilized the Silva database (Quast et al. 2013) and the BLAST assigner (Altschul et al. 1990). Alignment for the 16S was through PyNAST (Caporaso et al. 2010b), while the 18S utilized SINA (Pruesse et al. 2007).
FastTree (Price et al. 2010) was employed to build the phylogenetic tree. In addition to the Socompa data, existing sequencing data from neighboring volcanic mountain Llullaillaco were also used for comparison. Alpha rarefaction determined the richness of the taxa in each sample with observed species (OTUs) estimator. Beta diversity was calculated through Weighted and Unweighted UniFrac (Lozupone and Knight 2005), and visualized in Principal Coordinate Analysis (PCoA) plots through Emperor (Vazquez-Baeza et al. 2013). The Principal Coordinate Analysis (PCoA) ordinations were constructed based on the QIIME tables of relative abundance of OTUs and weighted and unweighted UniFrac distance matrices for overall communities. Statistical significance of biodiversity between sites was determined with ADONIS from the R programming language Vegan package.

Rank abundance plots for both communities were created with Microsoft Excel.


The Silva database files are available for download: [http://www.arb-silva.de/download/arb-files/](http://www.arb-silva.de/download/arb-files/)

**Data Description**

For purposes of assessment and comparison each sample was assigned a group (five in total) based on a site’s biogeological properties. The two samples from the fumarole at 5820 m.a.s.l. are labeled as “Vent.” The samples from non-fumarole sites 20m north and 25m south of Vent are labeled as “Distance from Vent” or “Dist_Vent.” The final site of same elevation, the icefield, contains three samples high-, mid- and low-icefield and is labeled “Icefield.” Near the summit, at 6049 m.a.s.l., the two samples from the fumarole site with the bryophytic mat are labeled as “Mat”; and the two samples from the non-fumarole site at 6049 m.a.s.l. are labeled as “Summit.”
Results

OTU richness of the 16S and 18S communities at both fumarolic sites was notably higher compared to the non-fumarolic sites on Socompa and Llullaillaco (fig 1, 2). On Socompa the two fumarole sites (fig 3, 4) exhibit distinct similarities in their 16S communities, with the top two OTUs being Proteobacteria (Vent- 24%, Mat- 23%), followed by Acidobacteria (Vent-18%, Mat- 14%). They also share relative similarities in percentages of Chloroflexi (Vent- 9%, Mat-12%), Planctomycetes: Vent- 9%, Mat- 6%, and Bacteroidetes: Vent- 2%, Mat- 3%. The main differences occur in the relative abundances of Actinobacteria: Vent- 14%, Mat- 6%, Cyanobacteria: Vent- 2%, Mat- 14%, and Verrucomicrobia: Vent- 4%, Mat- 1.5%. The three non-fumarole sites (fig 5, 6, 7) also share similarities with each other with the highest relative abundance OTU being Actinobacteria: Icefield- 42%, Dist_Vent- 57%, Summit- 48%. Other relative abundance similarities occur with Acidobacteria: Icefield- 2%, Dist_Vent- 3%, Summit-2%. Major differences are seen with the Icefield containing 4% of Cyanobacteria, which is not present in notable quantities in the others. The Dist_Vent is different from the other two by containing only 3% of Proteobacteria, while the other two are at 15% (Icefield) and 18% (Summit). Additionally, the Dist_Vent has 14% of Verrucomicrobia, which is not present in notable quantities in the others. Furthermore, the weighted PCoA plots provide evidence of significant clustering between the two fumaroles to the exclusion of the non-fumarole sites: Adonis, R²= 0.483, p < 0.001(fig 8).

On Socompa the two fumarole sites display a distinct similarity in their 18S communities (fig 9, 10). The top OTU shared by both is the moss Lyellia: Vent- 12%, Mat- 32%. Additionally, there are similarities in the alga Trebouxiphycaceae: Vent- 9%, Mat- 13% and Fungi Mortierellales: Vent- 8%, Mat- 6%. Differences occur in regards to Cerozoa: Vent- 13%, Mat- 5% and the alga Chlorophyceae: Vent- no notable amounts, Mat- 16%. The three non-fumarole
sites (fig 11, 12, 13) do not contain as much homogeneity in their 18S communities as they do in their 16S communities. All three share an abundance of Fungi and indeed the top OTU in all three is a Basidiomycota, however in the Icefield it is Rhodotorula (31%), while in the other two it is Cryptococcus (Dist_Vent- 34%, Summit- 59%). The differences continue with the second most abundant OTU unique to each site: Icefield -Chlorophyceae (23%), Dist_Vent- Amoebozoa (16%), and Summit- Teleostei (10%). Furthermore, the weighted PCoA plots provide evidence of significant clustering between the two fumaroles to the exclusion of the non-fumarole sites: Adonis, \[ R^2 = 0.483, p < 0.001 \] (fig 14).

The Biogeochemical data (Table 1) obtained for the three sites at 5820 m.a.s.l. Socompa shows the soil at each site to have a consistent acidic pH between 4.8 and 5. This is also seen in the sites above 6000 m.a.s.l. on both Socompa and Llullaillaco. Very low levels of organic nitrogen and minute levels of dissolved carbon or nitrogen are shared by all sites. Soil moisture is relatively high in the icefield at 5820 m.a.s.l. as compared to the vent at the same elevation, and when compared with Llullaillaco, the percentage of water in the soil is significantly higher, as is the amount of organic matter and nitrogen found in the vent. Of significance is the elevated amount of organic matter found at the Vent sampling site. The Enzyme activity at each site is very limited and when considering the standard error can be considered insignificant. The one exception is the enzyme Phosphatase which registers some recordable activity around the vent.

**Discussion**

On the wind-swept, weather beaten, unsheltered and exposed surfaces of these volcanic mountains two different community structures exist. In the exposed soils away from the fumaroles there is similarity in community dominance: Bacteria dominated by Actinobacteria and Proteobacteria and Eukaryotes mostly fungi, particularly yeasts. Interestingly, these
basidiomycetous yeast are closely related to *Cryptococcus* species that dominate extreme soils in the Dry Valleys of Antarctica (Vimercati *et al.* 2016) and are very distantly related to Ascomycetous yeast such as Brewer’s Yeast. Recently a *Cryptococcus* isolate from Volcán Llullaillaco was shown to be able to grow exponentially during extreme freeze-thaw cycles (-10°C at night, +30°C during the day) as would be experienced in non-fumarolic soils on high elevation volcanoes (Vimercati *et al.* 2016). It is expected the *Cryptococcus* found on Volcán Socompa in my study would follow similar growth cycles during rare snow melt events and then go dormant and wait (perhaps for years) for the next time water becomes available at this hyperarid location. This adaptation, and the mechanism that allows it do so, is of interest for potential life in extraterrestrial habitats that feature similar physical environments.

The microbial communities of the fumaroles display a greater richness and increased evenness than the microbial communities found in the surrounding soils. Indeed, all three Domains of life are found: Bacteria, Eukaryotes, and in smaller abundances Archaea, specifically Crenarchaeota. The increased level of organic matter at the fumaroles sites is indicative of the greater support for life the fumaroles provide. The presence of small amounts of phosphatase enzyme activity is another indication of increased microbial diversity and functioning biogeochemical cycles at the fumarole sites. The richness of the fumarole sites is in keeping with previous studies of geothermal vents (Costello *et al.* 2009, Bizzoco and Kelly 2013, Herbold, *et al.* 2014b) and indicates the important life supporting capabilities of geothermal events in a variety of extreme environments. None of the non-fumarolic sites proved capable of supporting anything more than a sparse community of a few hardy extremophiles; reinforcing the forbidding nature of the surrounding environment. The fact the wet soils of the icefield did not support high microbial diversity suggests water availability per se is not the only factor limiting
life at extreme high elevation sites in the Atacama Desert. Besides water (in the form of water vapor), fumaroles provide simple carbon sources (CO$_2$, CO, and CH$_4$, Costello et al. 2009) that can be utilized by microbes to build biomass and increase soil carbon levels. Fumaroles are also warming the soils, thus alleviating potential temperature limitations to life at high elevations. Future work on Volcán Socompa will be needed to unravel the true limitations to life at this and other high elevation sites in the Atacama region.

Additionally, Socompa is the only mountain yet discovered in the Atacama region to host vegetation at elevations above 5100 m.a.s.l. Bryophytic mats of moss cling to the mountainsides 900m above any other vegetation. Their ability to survive such alien territory is explained by the presence of fumaroles; without such a hospitable microenvironment the moss would not survived and grown. The resources provided by the vents host not only abiotic benefits, but also a microbial soil community supportive for plant growth (e.g. through nitrogen fixation and other biogeochemical processes). This is consistent with findings of similarly extreme environments at lower elevations in Antarctica (Broady et al., 1987).

**Conclusion**

The astrobiological implications of these findings present another analog for celestial locations that are dry, cold, highly irradiated, lacking in soil nutrients, and featuring a severe diurnal temperature fluctuation. The microenvironments of fumaroles and the barren non-fumaroles present the potential of how life may adapt to similarly harsh environments. The OTUs (“species”) that dominate these sites provide a glimpse into what life might be possible elsewhere, if not in exact structure and form then in the functional, metabolic, and environmental adaptations exhibited in these communities. A further and more complete investigation would be interesting to determine if other locations comparable to Socompa and Llullaillaco feature
similar communities and functional niches that draw out a template for life in the extremes, for life elsewhere beyond our own planet Earth. Future considerations could investigate the functional niches that are provided by this ecosystem and the adaptations of the organisms that function there. Additionally, currently unexplored locations of geothermal activity in the form of fumaroles and other structures could be investigated for biotic activity and compared to existing biogeographic, biogeochemical, and genomic research. One such location is located 50 km south of Llullaillaco, the Volcán Listerria, which contains high elevation sulfurous fumaroles in contrast to the non-sulfurous fumaroles found on Socompa.

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First, I would like to thank each member of my committee; my advisor Dr. Steve Schmidt for his insight and guidance; the departmental honors representative Dr. Pieter Johnson for his perspective and support; and Dr. Brian Hynek for his time and interest. Furthermore, I would like to thank fellow Schmidt lab members Jack Darcy, Lara Vimercati, and Eli Gendron for their assistance and encouragement. Finally, I offer thanks to the both the CU Honors program and the Undergraduate Research Opportunities Program (UROP) for helping to make this original research possible.

References


**Figures and Tables**
Figure 1. Alpha rarefaction plot correlating the number of 16S sequences sampled with the number of OTUs observed in each community. The color-sample site combinations are as follows: orange-Mat, yellow-Vent, red-DistVent, blue-Icefield, green-Summit, purple-Llullaillaco.
Figure 2. Alpha rarefaction plot correlating the number of 18S sequences sampled with the number of OTUs observed in each community. The color-sample site combinations are as follows: orange-Mat, yellow-Vent, red-DistVent, blue-Icefield, green-Summit, purple-Llullaillaco.
Figure 3. The percent relative abundance of bacterial/archaeal OTUs found within the Mat sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
Figure 4. The percent relative abundance of bacterial/archaeal OTUs found within the Vent sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
Figure 5. The percent relative abundance of bacterial/arachael OTUs found within the DistVent sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
Figure 6. The percent relative abundance of bacterial/arachael OTUs found within the Summit sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
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Figure 12. The percent relative abundance of eukaryotic OTUs found within the Summit sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
Figure 13. The percent relative abundance of eukaryotic OTUs found within the Icefield sampling site. The blue bar represents the mean of the samples collected from the site and the red bar is the standard error.
Figure 14. The PCoA plot represents the clustering of the 18S communities as a function of a weighted unifrac distance matrix. The color-site combinations are as follows: yellow-Mat, purple-Vent, blue-Icefield green-Summit, and red-DistVent
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<td><strong>TON (µg N/g dry soil)</strong></td>
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<td><strong>DOC (µg g dry soil^-1)</strong></td>
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<td><strong>TDN (µg g dry soil^-1)</strong></td>
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</tr>
<tr>
<td>MBC (µg g dry soil^{-1})</td>
<td>267 (57.3)</td>
<td>72.0 (31.6)</td>
<td></td>
</tr>
<tr>
<td>MBN (µg g dry soil^{-1})</td>
<td>8.5 (8.5)</td>
<td>&lt; d.l.</td>
<td></td>
</tr>
<tr>
<td>PHO* (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>0.5 (0.2)</td>
<td></td>
</tr>
<tr>
<td>BG (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>0.31 (0.22)</td>
<td></td>
</tr>
<tr>
<td>AG (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>&lt; d.l.</td>
<td></td>
</tr>
<tr>
<td>BXLY (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>&lt; d.l.</td>
<td></td>
</tr>
<tr>
<td>NAG (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>&lt; d.l.</td>
<td></td>
</tr>
<tr>
<td>LAP (nmol h^{-1} g^{-1} soil)</td>
<td>ND</td>
<td>&lt; d.l.</td>
<td></td>
</tr>
</tbody>
</table>
Enzyme activities are abbreviated β-glucosidase (BG), N-acetylgalactosaminidase (NAG), and phosphatase (PHO).

Activity of α-glucosidase, β-xylanase, cellobiase, leucine aminopeptidase were all below detection limits.

All values are the means of at least 3 replicates with the standard error of the mean in parentheses.

Below detection limit

OM = organic matter: organic carbon (not dissolved)

TON = total organic nitrogen

DOC = dissolved organic carbon

TDN = total dissolved nitrogen: inorganic + organic

MBD = microbial biomass carbon: amount of C in microbes, somewhat unreliable (note-large errors)

MBN = microbial biomass nitrogen: almost worthless because so close to detection limits

PHO = Acid phosphatase: mineralizes organic P into phosphate by hydrolyzing phosphoric (mono) ester bonds under acidic conditions

BG = β-1,4-Glucosidase: catalyzes the hydrolysis of terminal 1,4-linked β-D-glucose residues from β-D-glucosides, including short-chain cellulose oligomers, especially cellulose

AG = α-1,4-Glucosidase: principally a starch-degrading enzyme; catalyzes the hydrolysis of terminal, nonreducing 1,4-linked α-D-glucose residues, releasing α-D-glucose

BXYL = β-1,4-Xylosidase: degrades xylooligomers (short xylo chains) into xylose. Xylans are β-1,4-linked polymers of xylopyranose—a plant structural polymer less tightly associated with plant cell walls than cellulose

NAG = β-1,4-N-Acetylglucosaminidase: catalyzes the hydrolysis of terminal 1,4-linked N-Acetyl-beta-D-glucosaminide residues in chitooligosaccharides (chitin-derived oligomers)

LAP = Leucine amino peptidase: catalyzes the hydrolysis of leucine and other amino acid residues from the N-terminus of peptides; most reactive towards leucine. Amino Acids amides and methyl esters are also readily hydrolyzed by this enzyme, which has broad specificity.