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Full Length Research Paper

An evaluation of the bacterial diversity at Tshipise, Mphephu and Sagole hot water springs, Limpopo Province, South Africa

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Tshipise, Mphephu and Sagole are thermal hot water springs in the Limpopo Province of South Africa with temperatures of 58, 43 and 45°C; and pH of 8.85, 8.08 and 9.70, respectively. The bacterial diversity of the hot water springs was determined by pyrosequencing of the two 16S rRNA hypervariable regions V1-3 and V4-7. Analyses of the community DNA revealed that bacterial populations as detectable by the V1-3 or V4-7 region, respectively were dominated by the Bacteriodetes and Proteobacteria for Mphephu, and Proteobacteria and Cyanobacteria for both Tshipise and Sagole. The major differences in the bacterial diversity between the springs was that no Cyanobacteria were detected for Mphephu and the level of Bacteriodetes detected for both Tshipise and Sagole was much lower compared to the levels detected at Mphephu. The Firmicutes were detected at all the springs but at a much lower abundance compared to the other main phyla detected. Various other phyla were detected at the hot springs at levels below 0.20% of the total sequences obtained. It is interesting that very diverse bacterial genera exist in the three hot water springs studied.

Key words: Hot springs, bacterial diversity, metagenome, South Africa.

INTRODUCTION

Exploitable microbial diversity in the environment is inexhaustive and microorganisms represent the largest reservoir of understudied biodiversity (Sogin et al., 2006; Nichols, 2002). Thermal hot springs in different parts of the world have been studied for their thermophilic microbial diversity and often serve as a source of novel microorganisms for various biotechnological applications (Malkawi and Al-Omari, 2010; Narayan et al., 2008; Abou-Shanab, 2007; Baker et al., 2001).

South Africa is endowed with a number of thermal hot springs (Olivier et al., 2008; Kent, 1949) and these hot springs often differ in their physical and elemental chemical characteristics (Olivier et al., 2010). With the exception of a recent article that was published on the bacterial population at Siloam hot spring in Limpopo Province (Tekere et al., 2011), virtually nothing is known about the variation in the diversity of microbes in other hot springs in South Africa. This article describes the bacterial diversity from the hot water springs of Tshipise, Mphephu and Sagole, in the Limpopo Province of South Africa.

Molecular approaches used to analyse the microbial diversity allow the study of unculturable microorganisms and contribute towards our understanding of the microbial world. The recently developed pyrosequencing technique allows quick and detailed analysis of the microbial diversity in different samples in a single run and without the need for cloning (Liu et al., 2008). Literature suggests that pyrosequencing has a better ability to detect rare

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microorganisms that represent only a minority of a community when compared to approaches such as Sanger sequencing that misses these microbes entirely due to low depth of coverage (Petrosino et al., 2009; Krause et al., 2008).

During this study, pyrosequencing was used to study the bacterial diversity in the South African hot springs of Tshipise, Mphephu and Sagole. The physical and chemical water parameters at Tshipise, Mphephu and Sagole were also simultaneously determined for habitat description purposes. The microbial diversity of Tshipise, Mphephu and Sagole hot water springs has not yet been studied and this report presents the first metagenomic study to describe the bacterial diversity present at these thermal hot water springs in the Limpopo Province of South Africa.

MATERIALS AND METHODS

Description of the study areas

Tshipise, Mphephu and Sagole hot water springs are located in the Limpopo Province of South Africa and are of different surface geology. The sampling sites description details are shown in Table 1.

Sampling

Composite water samples were collected in August, 2010 from Tshipise, Mphephu and Sagole hot water springs for physical, chemical and microbial community studies. Samples were collected at 20 to 50 cm depth around the springs. The following water quality parameters were measured *in situ* using the relevant field meters (Mettler Toledo meters, UK): temperature, pH, electrical conductivity (EC), total dissolved solid (TDS) and dissolved oxygen (DO). The water was collected into sterile 2 L bottles and placed into a cooler box for transportation to the laboratory for bacterial diversity studies. Analyses for physical and chemical parameters of the water were conducted by the Institute for Soil, Climate and Water (Agricultural Research Council), Pretoria using standard methods.

DNA extraction

The water samples were concentrated by both filtration [on cellulose nitrate filters, pore size 1.2 um (Sartorius)] for the clear water, and centrifugation (7500 rpm for 10 min) for water with the bulk of the biofilm, and subsequently resuspended in 20 ml of phosphate buffer saline (PBS) (10 mM). Two (2) ml of the samples (both from filtered water and centrifuged biofilm samples) were centrifuged at 7500 rpm for 10 min to collect the pellet. The pellet was resuspended in 1 ml PBS (10 mM) as an additional wash step. DNA was extracted with the genomic DNA Tissue Mini-Prep Kit (Zymo Research), as per protocol; with an additional DNA wash-step. Cell lysis was followed by purification using modern fast-Spin column purification technology. Five (5) µl of the extracted DNA was run on a 1% agarose gel at 90 V for 30 min to verify the efficiency of the extractions.

Polymerase chain reaction (PCR) amplification

The PCR reaction was performed on the extracted DNA samples using universal degenerate primers 27F.1 and 1492R (De Santis et al., 2007) as shown in Table 2. Each PCR reaction contained 5 µl of 10 × Taq Buffer, 2 mM $MgCl_2$, 1.5 U Super-Therm DNA polymerase (Southern Cross), 0.25 mM deoxynucleosides triphosphates (dNTP's), 0.1 µM of each primer, 1 µl of extracted DNA and nuclease free water (NFW) up to the final reaction volume of 50 µl. The PCR cycle started with an initial denaturation step at 94°C for 10 min. This was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min, and a final extension at 72°C for 5 min that was then followed by cooling to 4°C. Five (5) µl of the samples were run on a 1% agarose gel at 90 V for 30 min in order to verify amplification.

The entire PCR reaction was loaded onto a 1% agarose gel and the correct band size (approximately 1500 bp) was excised. The DNA was recovered from the gel slices by using the GeneJET™ gel extraction kit (Fermentas). The DNA was subsequently reamplified with two sets of primers in order to amplify two variable regions of the 16S rRNA gene (V1-3 and V4-7). These primers contained the appropriate adaptor and barcode sequences that were necessary for running the samples on the GS-FLX-Titanium (Roche) as set out in Table 2. Each PCR reaction was done as previously described except that the annealing temperature was either 50 or 56°C dependent on the variable region amplified as shown in Table 2.

The entire PCR product was loaded onto a 1% agarose gel and the correct band size (500 to 600 bp) was excised from the gel and subsequently purified as before. The DNA concentrations were quantified by using a Nanodrop spectrophotometer. The samples were pooled at equal concentrations of the filtration and biofilm samples, and V1-3 samples were equal to V4-7 samples. The pooled samples were sequenced on the GS-FLX-Titanium series (Roche) by Inqaba Biotechnology, South Africa. The resulting data were classified using the Ribosomal Database Project (RDP) Naive Bayesian rRNA Classifier Version 2.2, March 2010, RDP training set 6, based on nomenclatural taxonomy and Bergey's manual with an 80% confidence threshold on the RDP-database. Only sequences that were 50 bp or more were included in the analyses. Data used for the construction of the rarefaction curves were generated by using the RDP pyrosequencing pipeline on the RDP database. The x; y scatter plot charts were then constructed in Microsoft Office Excel 2007 using the different distance levels.

RESULTS

The determined physical and chemical characteristics of the hot spring water at Tshipise, Mphephu and Sagole thermal springs are shown in Tables 3 and 4**.** The three hot springs presented here range from hot to scalding in temperature (Olivier et al., 2008; Kent, 1949). The temperatures of the hot springs were 58, 45 and 43°C for Tshipise, Sagole and Mphephu, respectively. Tshipise is classified as a scalding hot spring (Kent, 1949) and has the highest temperature of these three springs. The hot springs are all alkaline with pH of 8.85, 9.70 and 8.08 for Tshipise, Sagole and Mphephu, respectively. The TDS was highest at Tshipise (460.50 ppm), followed by Sagole (203.76 ppm) and lastly 199.36 ppm at Mphephu. High TDS is a reflection of high dissolved chemical ion concentration. Of the three spring waters, Tshipise has the highest concentrations of sodium, chloride and sulphate ions while Mphephu is rich in calcium and magnesium.

The trace element concentrations determined at the three hot springs are shown in Table 4. The most

Table 1. Sampling site description details.

Sample site coordinate	Spring name	Note	Description of surface geology (Olivier et al., 2011)
22°54.355' S 22°54.355' E	Mphephu	Developed as a tourist resort and the eye of the hot spring is accessible to both the community and wildlife. The spring, at the foot of a mountain, receives a lot of vegetation litter.	Sandstone and quartzite. Reverse fault between Waterberg Group quartzite and Dominion Reef lava.
22°36.521' S 30°10.345'E	Tshipise	Developed as a popular tourist resort. The hot spring is at a foot of a hill. Algae and multicoloured phototropic Cyanobacteria biofilms are evident in the water and on the sediments and rock surfaces.	Basalt, minor andesite. Cream coloured sandstone, Dolerite sills and dykes. Intersection of two post-Permian faults in upper Karoo.
22°31.825' S 30°40.883'E	Sagole	Previously developed as a tourist resort now dysfunctional as a resort. The spring is accessible as a source of water for both the community and wildlife. The spring is surrounded by big trees, thus receives a lot of vegetation litter. The soils are sandy with no visible bedrock. Green algal biofilms are evident on the water surface and on the sediments.	Mudstone, shale, subordinates micaceous sandstone. Shale, carbonaceous shale, siltstone, micaceous sandstone.

Table 2. PCR primers used and annealing temperatures.

Primer name	Reference for primer used	Forward (F)/ Reverse (R)	Primer sequence	Variable region/ annealing temperature
27F.1	DeSantis et al. (2007)	F	5'AGRGTTTGATCMTGGCTCAG 3'	Entire 16S/58°C
1492r	DeSantis et al. (2007)	R	5'GGTTACCTTGTTACGACTT 3'	
A1.2 (Mphephu)	DeSantis et al. (2007) (27F.1)	F	5'**CGTATCGCCTCCCTCGCGCCATCAG**agacgcactcAGRGTTTGATCMTGGCTCAG3'*	V1-V3/50°C
A1.3 (Sagole)			5'**CGTATCGCCTCCCTCGCGCCATCAG**atcagacacgAGRGTTTGATCMTGGCTCAG3'*	
A1.6 (Tshipise)			5'**CGTATCGCCTCCCTCGCGCCATCAG**tatagtagtgAGRGTTTGATCMTGGCTCAG3'*	
B1	Coenye et al. (1999) (pD)	R	5'**CTATGCGCCTTGCCAGCCCGCTCAG**GTATTACCGCGGCTGCTG3'*	
A2.2 (Mphephu)	Dowd et al. (2008) (530F)	F	5'**CGTATCGCCTCCCTCGCGCCATCAG**cgtgtctctaGTGCCAGCMGCNGCGG3'*	V4-V7/56°C
A2.3 (Sagole)			5'**CGTATCGCCTCCCTCGCGCCATCAG**ctcgcgtgtcGTGCCAGCMGCNGCGG3'*	
A2.6 (Tshipise)			5'**CGTATCGCCTCCCTCGCGCCATCAG**cgagagatacGTGCCAGCMGCNGCGG3'*	
B2	Sundquist et al. (2007) (1073R)	R	5'**CTATGCGCCTTGCCAGCCCGCTCAG**ACGAGCTGACGACARCCATG3'*	

*The capital letters in bold indicate the adapter sequences of the primer that is required for 454 pyrosequencing. The lowercase letters indicate the barcode sequences. The capital letters that are not bold indicate the primers that are template specific.

abundant trace elements were bromine, boron, strontium and iodine. In comparison to the other springs, Tshipise has significantly more boron, bromine, iodine, nickel, selenium and strontium whereas the water at Sagole has higher levels of arsenic and Mphephu has more vanadium.

Table 5 shows the summary of the bacterial DNA sequence reads data obtained. Longer sequence read

Table 3. Physical and chemical characteristics of the water at Tshipise, Mphephu and Sagole hot water springs.

Parameter	Hot spring		
	Mphephu	Tshipise	Sagole
Temperature (°C)	43	58	45
DO (%)	65.3	34.7	9.9
pH	8.08	8.85	9.70
TDS (ppm)	199.36	460.56	203.76
Conduct. (mS/m)	44.00	81.00	39.00
Sodium (mg/l)	44.37	156.31	65.15
Potassium (mg/l)	1.14	4.25	1.10
Calcium (mg/l)	13.73	5.58	1.31
Magnesium (mg/l)	11.25	0.17	0.07
Fluoride (mg/l)	3.16	5.63	1.01
Nitrate (mg/l)	2.12	0.61	0.00
Chloride (mg/l)	39.38	168.97	47.85
Sulphate (mg/l)	9.26	53.17	18.20
Phosphate (mg/l)	0.00	0.00	0.00
Carbonate (mg/l)	0.00	6.00	18.00
Bicarbonate (mg/l)	151.28	126.88	102.48

Table 4. Trace element composition of the water at Tshipise, Mphephu and Sagole water springs.

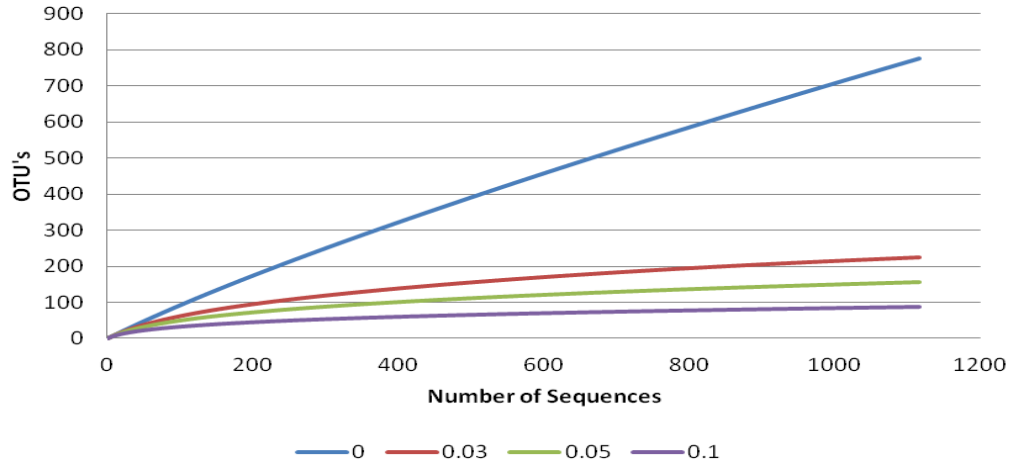
Element	Tshipise	Sagole	Mphephu
	Conc. (µg/l)	Conc. (µg/l)	Conc. (µg/l)
Antimony	0.02	0.31	0.02
Arsenic	0.14	2.88	0.43
Barium	13.63	5.14	51.90
Boron	200.60	56.48	45.92
Bromine	366.30	102.00	102.90
Cadmium	0.02	0.01	0
Chromium	0.70	0.49	1.20
Cobalt	0.10	0.01	0
Copper	0	0	0
Iodine	115.20	6.41	3.25
Lead	0.08	0.12	0.16
Manganese	0	0.20	0
Mercury	0.33	0	0.23
Molybdenum	1.41	1.06	0.91
Nickel	37.19	0	0
Platinum	0.01	0.07	0.01
Selenium	2.35	0.20	0
Strontium	213,30	52.18	40.14
Titanium	3.03	4.01	3.09
Tungsten	4.19	1.54	0.48
Uranium	0	0	0.45
Vanadium	1.81	0.42	10.64
Zinc	2.48	2.05	1.95

length makes alignment to sequence databases easy and classification more complete. The V4-7 had longer average sequence read length for Tshipise and Sagole but less average sequence read length for Mphephu. The 16S rRNA pyrosequencing results indicate that 512 and 120 sequences could be detected at Tshipise for V1-3 and V4-7, respectively; 1117 and 721 sequences detected at Mphephu for V1-3 and V4-7, respectively; and 1046 and 794 sequences could be detected at Sagole for V1-3 and V4-7, respectively. It can be seen that the sequences obtained for Tshipise were much less than those for Mphephu and Sagole and more so the number of sequences obtained for the V4-7 was much less for the V1-3 for the same hot spring. Rarefaction curves were used to indicate that despite the variable number of sequences, whether all the diversity in the samples was detected or not. Rarefaction curves are useful in comparing various samples, and although the operational taxonomic units (OTUs) are approximate values, it gives a good indication of diversity within a sample as the various percentages of distance levels tend to differentiate at different taxonomic levels. It is believed that a distance level at 3% is able to differentiate at species level, where a distance level of 5% is able to differentiate at genus level and a distance level of 10% is able to differentiate at family/class level (Schloss and Handelsman, 2004). Figures 1 to 3 show the rarefaction curves for the V1-3 and V1-4 variable regions for the three hot springs. In cases where the full diversity has been covered, the slope of the lines steeps up and then flattens such that even if more sequences were to be obtained the number of OTU's would not increase. However, if the slope of the lines stay steep it means that more sequences are required to obtain the full diversity within the sample (Schloss and Handelsman, 2004). It can be seen that in as much as a lot of diversity was covered from the sequences obtained, more sequences would be needed if the full diversity in the samples is to be obtained. In the present study, this can be clearly demonstrated from Figure3b where less than 120 sequences were obtained, the slope of the lines are much steeper than the other graphs where more than a thousand sequences were obtained. The rarefaction curves for all the samples are also not flat enough and as such more sequences would have to be obtained in order to reveal the complete diversity in these hot spring samples.

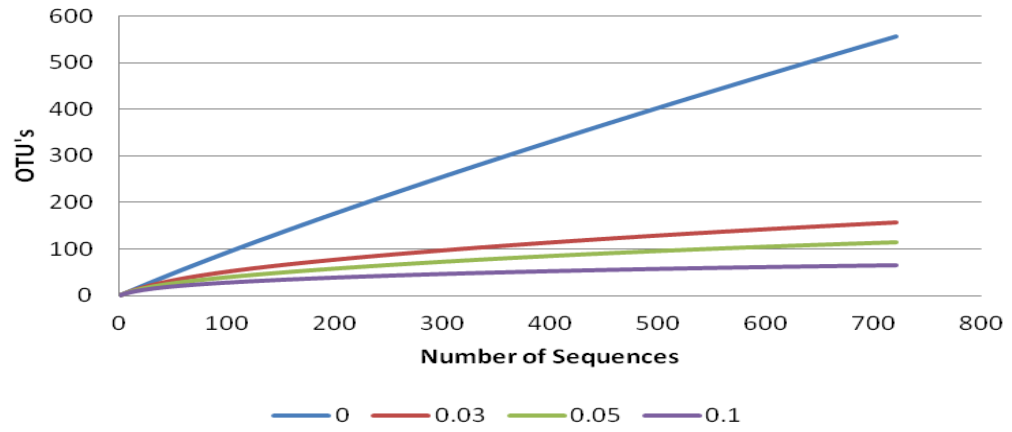
The sequences of the bacterial community DNA targeting the two DNA hyper-variable regions; V1-3 and V4-7, for the three hot springs samples were compared to the 16 rDNA database from the RDP database. The different bacterial phyla detected from the analyses of bacterial community DNA in the hot springs water are shown in Figure 4. It was found that the phyla Bacteriodetes 54.65% (V4-7), Proteobacteria 34.54% (V4-7) and Firmicutes 0.28% (V4-7), were the most detectable phyla at Mphephu. At Tshipise gene sequences belonging to phyla (based on the highest detected by either V1-3 and V4-7), Proteobacteria were 56.67% (V4-7), Cyanobacteria 20% (V4-7), Bacteriodetes 6.67% (V4-7), Fusobacteria 0.83%, Firmicutes 0.83 % (V4-7), Deinococcus-Thermus 0.78%, Chlorobi 0.59%

Table 5. Summary of pyrosequencing data from Tshipise, Mphephu and Sagole hot springs.

Sequence detail	Tshipise		Mphephu		Sagole	
	V1-3	V4-7	V1-3	V4-7	V1-3	V4-7
Number of sequences	512	120	1117	721	1046	794
Total length of sequences	121287	42212	318167	255767	279690	297043
Average length of sequences	234	352	385	355	267	374



(a)



(b)

Figure 1. Rarefaction curves for the total bacterial communities at Mphephu for the variable regions (a) V1-3 and (b) V4-7 at 0% (blue), 3% (red), 5% (green), and 10% (purple) difference levels.

(V1-3) and Planctomycetes 0.20% (V1-3). At Sagole, the most detectable phyla were Proteobacteria 32.89% (V1-3), Cyanobacteria 23.93% (V4–7), Fusobacteria 5.79%, (V4-7), Bacteriodetes 3.35% (V1-3), Firmicutes 1.72 (V1-3), Chloroflexi 1.51% and Deinococcus-Thermus 0.63%. The presence of a number of unclassified bacteria is extremely interesting. It can be seen that 49.02 and 47.42% of the sequences as determined by V1-3 and V4–7 for Tshipise and Sagole, respectively could not be classified. A comparison of the percentage of sequences unclassified for Tshipise and Mphephu shows that more sequences could be classified for the V4-7 region than the V1-3. For example, where a 49.02 and 47.42% of the sequences could not be classified at phylum level by the V1-3 for Tshipise and Mphephu, only 15 and 28.34% of the sequences were unclassified for the V4-7 variable region for these hot springs. The V4-7 therefore offered a more resolved classification for Tshipise and Sagole.

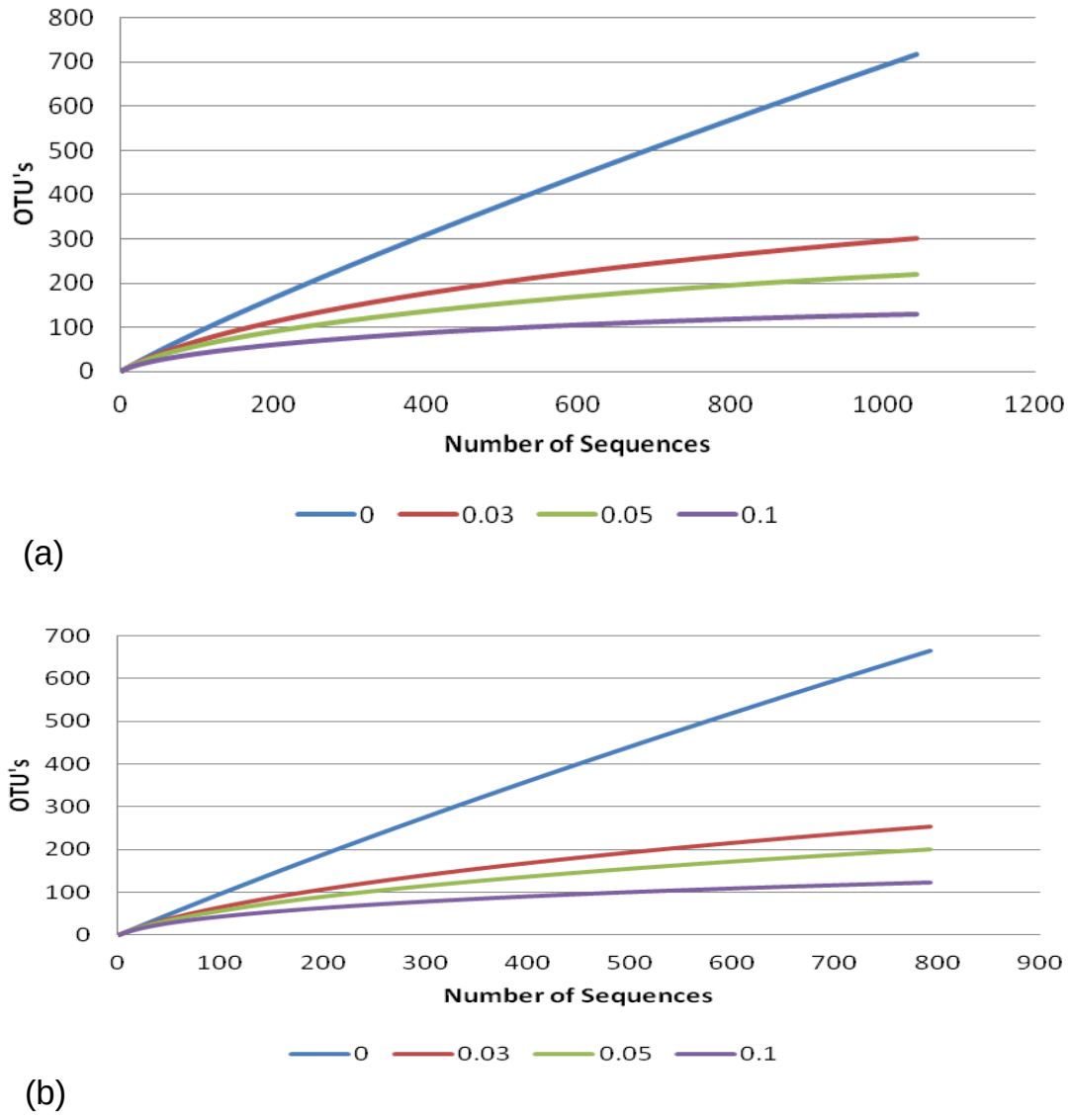
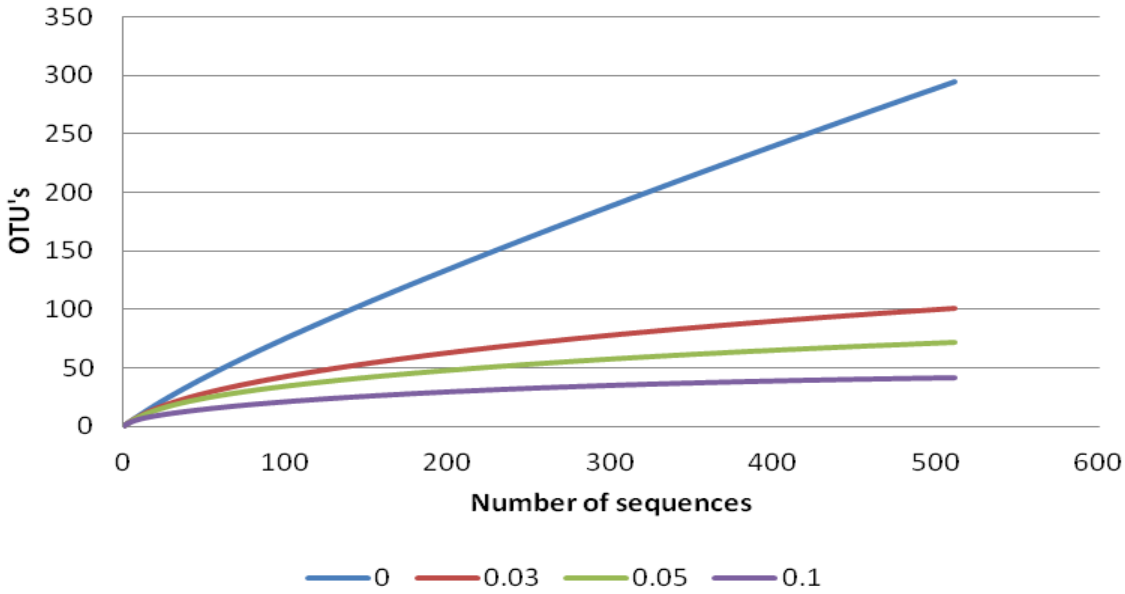


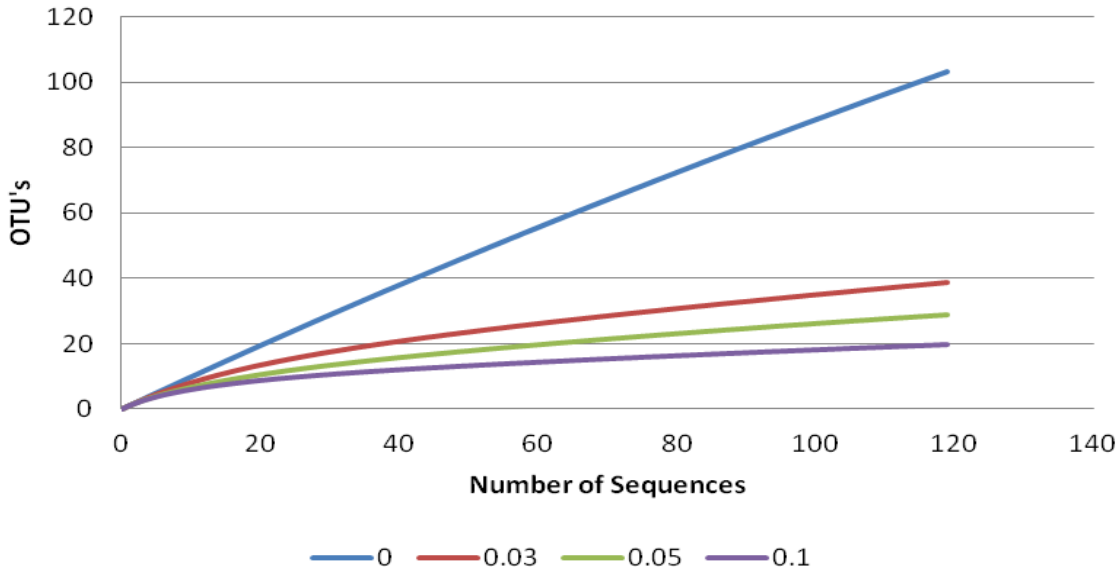
Figure 2. Rarefaction curves for the total bacterial communities at Sagole for the variable regions (a) V1-3 and (b) V4-7 at 0% (blue), 3% (red), 5% (green), and 10% (purple) difference levels.

A total of 9 classes were identified for Mphephu with class Sphingobacteria being the most abundant class [42.86% (V4-7) and 34.47% (V1-3)]. The Proteobacteria classes occurred in the following order of decreasing abundance; β-Proteobacteria (21.39 and 15.95%), γ-Proteobacteria (11.65 and 8.15%), **α-** Proteobacteria (3.05 and 8.08%), **δ-** Proteobacteria (0.14 and 0.36%). A total of 24 classes were detected at Sagole of which 15 of the classes were detected by both the V1-3 and V4-7. The 3 most dominant classes of bacteria detected at Sagole were the β-Proteobacteria, γ-Proteobacteria and the Cyanobacteria. The percentage sequence abundance classified as Cyanobacteria at Sagole with the V4-7 was more by almost 4 times to that detected by the V1-3 region. Ten (10) and 6 bacterial classes were detected at Tshipise with the V1-3 and V4-7 hyper-variable region sequences, respectively. The β-Proteobacteria, γ-Proteobacteria, Cyanobacteria and Flavobacteria classes were the most detected at Tshipise. The bacterial classes detected at Tshipise, Mphephu and Sagole hot springs are shown in Figure 5.

Relative occurrence and phylogenetic diversity of bacterial families at Tshipise, Mphephu and Sagole hot springs are shown in Figure 6. At Mphephu, the bacterial families Cytophagaeceae and Chitinophagaceae were detected with the V4-7 hyper-variable region sequences but were not detected at the other two springs. At Sagole, Fusobacteriaceae and Chromatiaceae were the most detected bacterial families together with other bacterial families with comparable abundance namely: Neisseriaceae, Rhodocyclaceae, Comamonadaceae and Planctomycetaceae. Though the family Chromatiaceae



(a)



(b)

Figure 3. Rarefaction curves for the total bacterial communities at Tshipise for the variable regions (a) V1-3 and (b) V4-7 at 0% (blue), 3% (red), 5% (green), and 10% (purple) difference levels.

was detected at all three springs, it was detected more for Tshipise with the V4-7 hyper-variable region as compared to the other springs.

The bacterial families detected at the hot springs showed some clear distinction with some overlaps as well. Figures 7a and b show the occurrence and overlaps in the bacterial families at the hot springs. From Figure 7a and b, the overlap in bacterial family diversity for the three hot springs is very similar showing that both the V1-3 and the V4-7 variable regions are able to ensure detection of the dominant families that are present in all three hot springs samples. However, from the comparison of the areas that cover only two samples at a time, there is very little overlap between the two variable regions. This could be due to various reasons which include that one variable region is better at distinguishing the various families and that some of the sequences were too small to be classified up to family level. This could

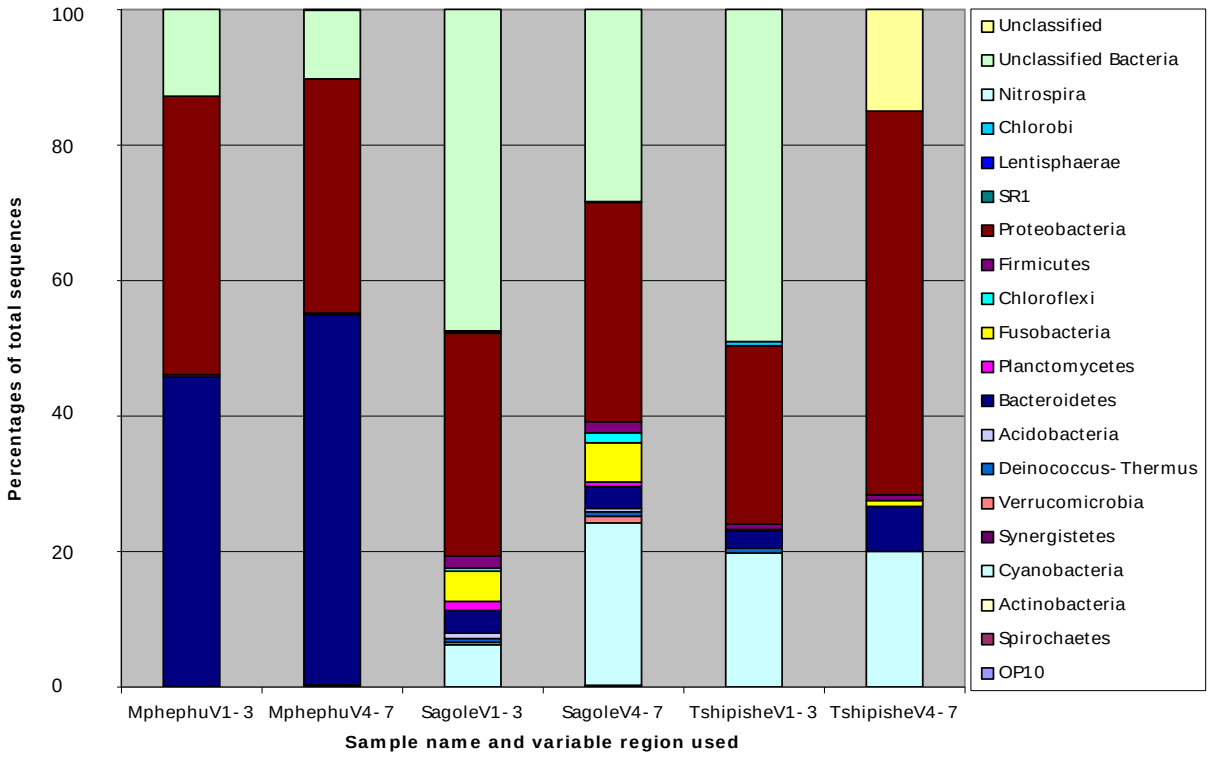


Figure 4. Relative abundance and phylogenetic diversity of bacteria phyla at Mphephu, Sagole and Tshipise hot springs.

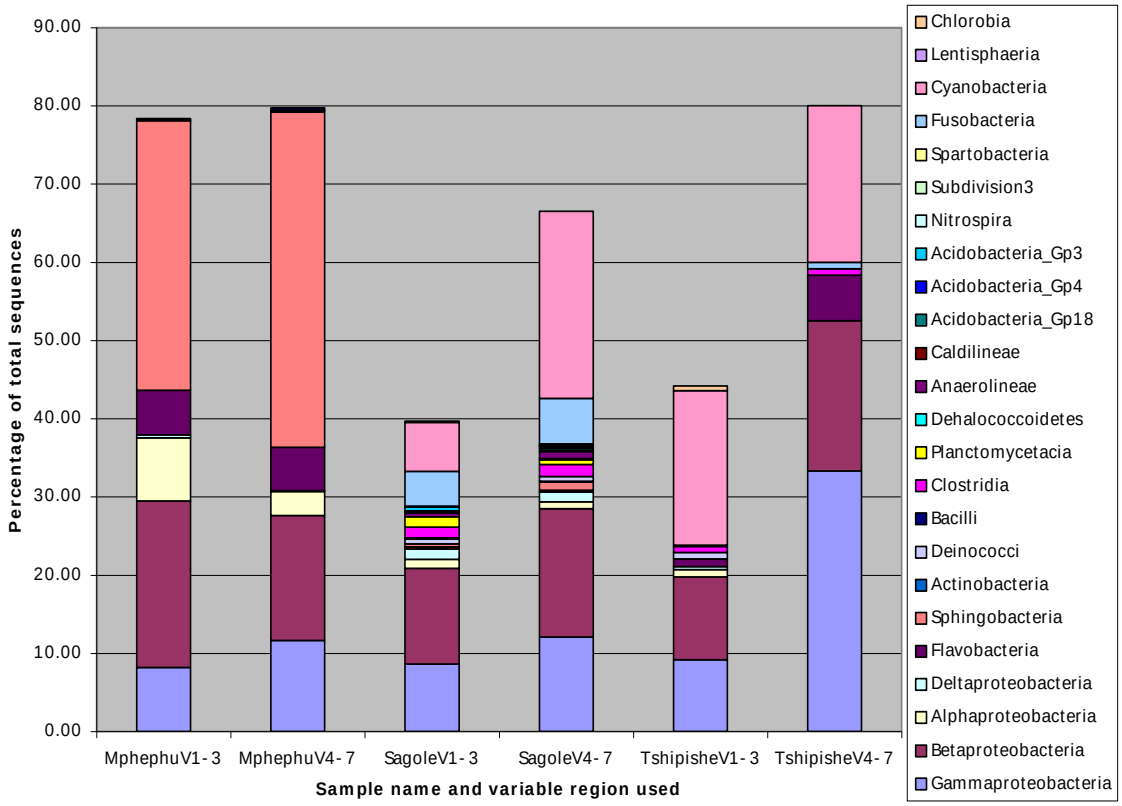


Figure 5. Relative occurrence and phylogenetic diversity of bacteria classes at Tshipise, Mphephu and Sagole hot springs.

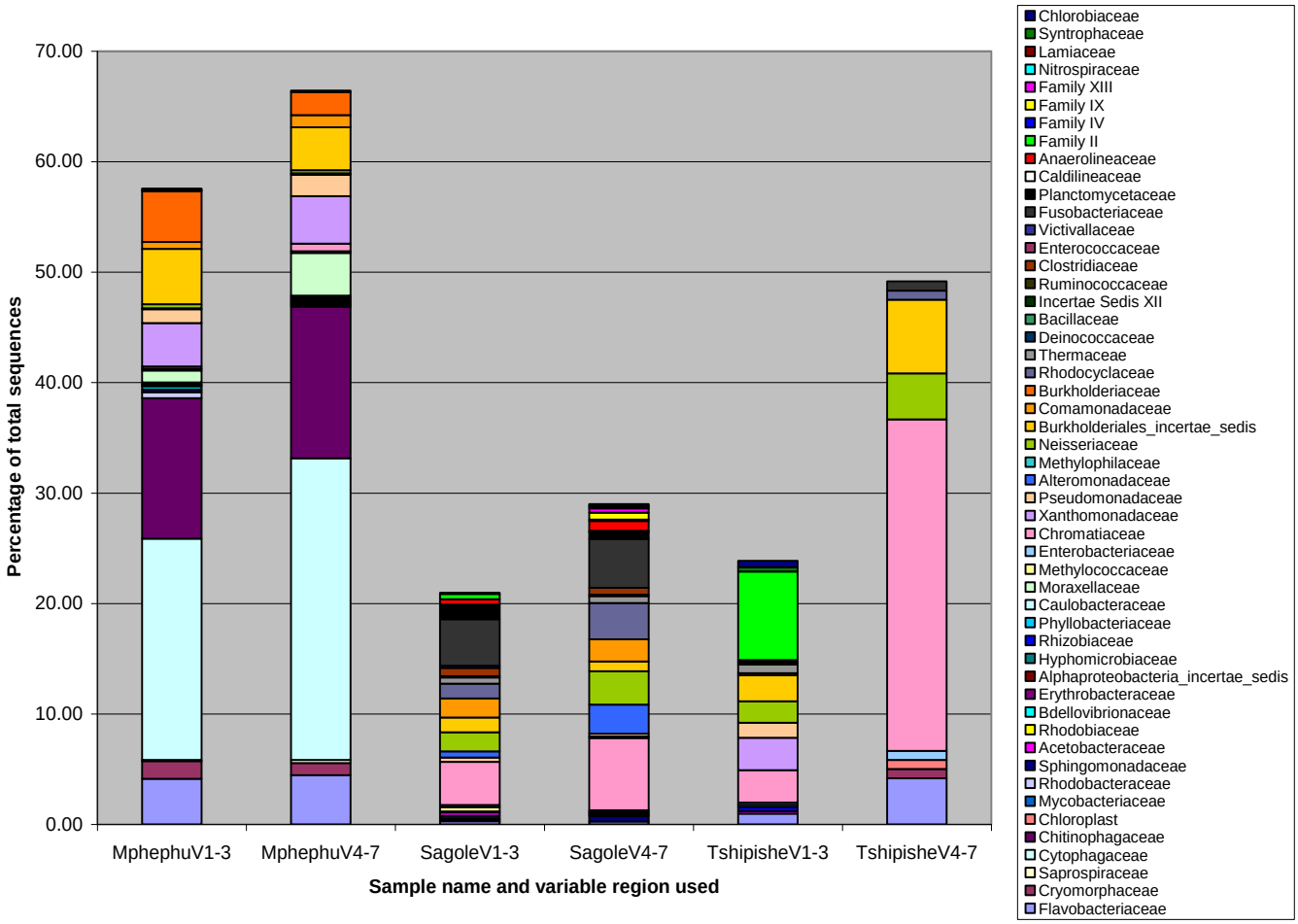


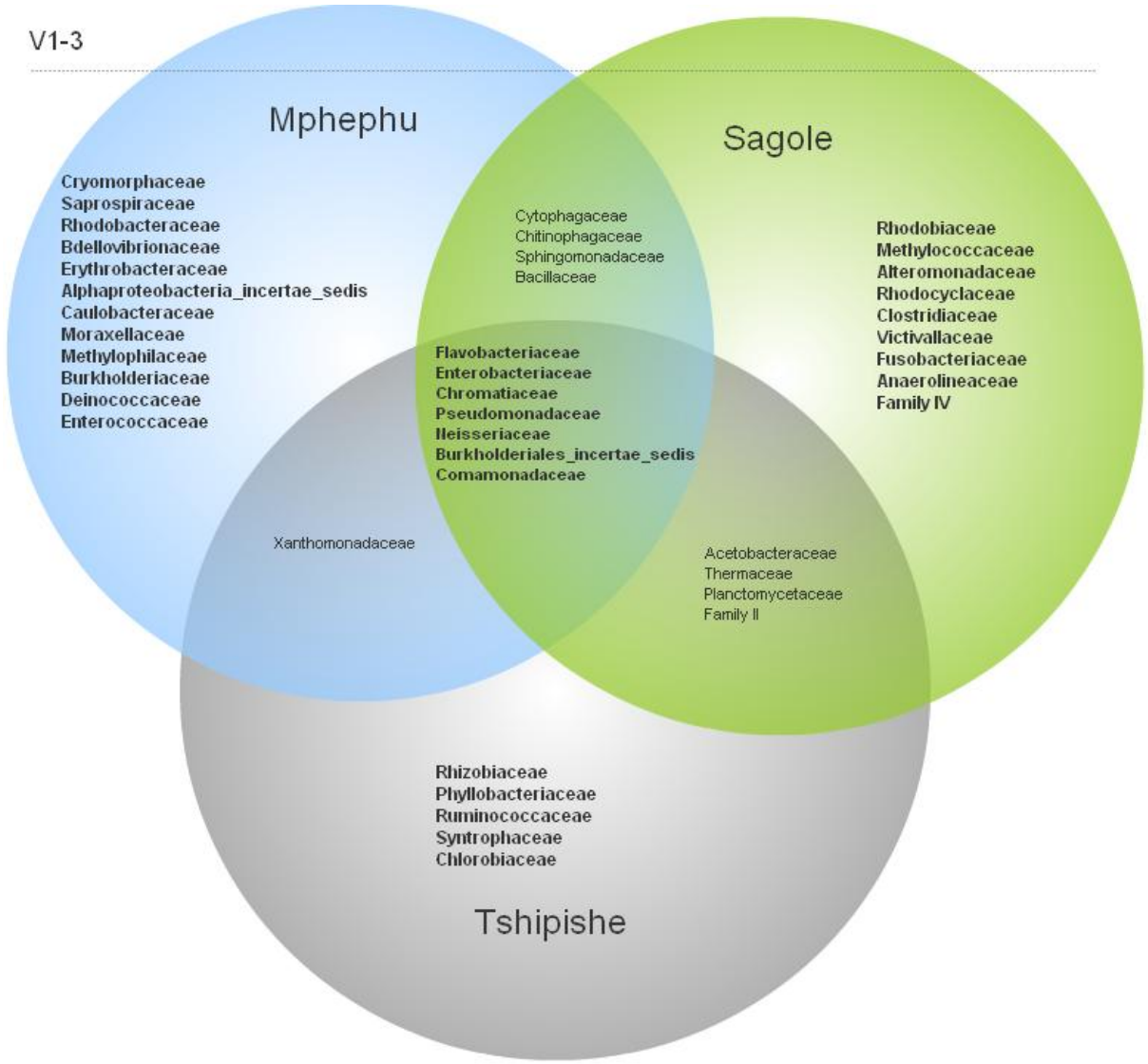
Figure 6. Relative occurrence and phylogenetic diversity of bacteria families at Mphephu, Sagole and Tshipise hot springs.

mean that instead of only focusing on one variable region it might be better to do two or more regions in order to detect as much diversity as possible.

DISCUSSION

Accurate taxonomic assignment of microorganisms has been shown to be dependent on the region of the 16S rRNA gene that is targeted during sequencing (Liu et al., 2008; Petrosino et al., 2009). Different hypervariable regions have been found to have different efficacies with respect to species calls in different genera (Sundquist et al., 2007; Petrosino et al., 2009). The primers used in this study were two separate sets shown in Table 1, which were used to amplify two variable regions (V1-3 and V4-7) in order to determine if the two variable regions would give the same phylotypes, and also determine which region gives a more resolved classification. In this study, the V4-7 and the V1-3 hypervariable regions had similar and overlapping efficacies. The V4-7 region was more effective, enabling a more complete classification of the bacterial phylotypes at Tshipise and Sagole which could not be done by the V1-3 region. The V4-7 hyper viable region is also more efficient at identifying the bacterial families at the highly alkaline Sagole, while the V1-3 region distinguishes thermophilic and halophilic bacterial families at Tshipise. Either hypervariable regions can be used to identify the bacterial families at Mphephu.

Different bacteria can exist in the same ecotype, each exploiting a minute variable niche (Lau et al., 2009). The diversity of bacteria at Tshipise, Mphephu and Sagole hot springs is described here. Sagole had the highest number of phyla (14) when compared to Mphephu and Tshipise. The extent of the bacterial diversity detected is not surprising as the bacteria found in these hot springs occur commonly in the environment and have been described in many different environments studied elsewhere. For example, Bacteriodetes play an important role in the carbon cycle, including a role in anaerobic fermentation and as such it is not surprising that they were detected at high percentage at Mphephu where there is a lot of carbon input from the vegetation litter within the hot spring than at the other hot springs. It is



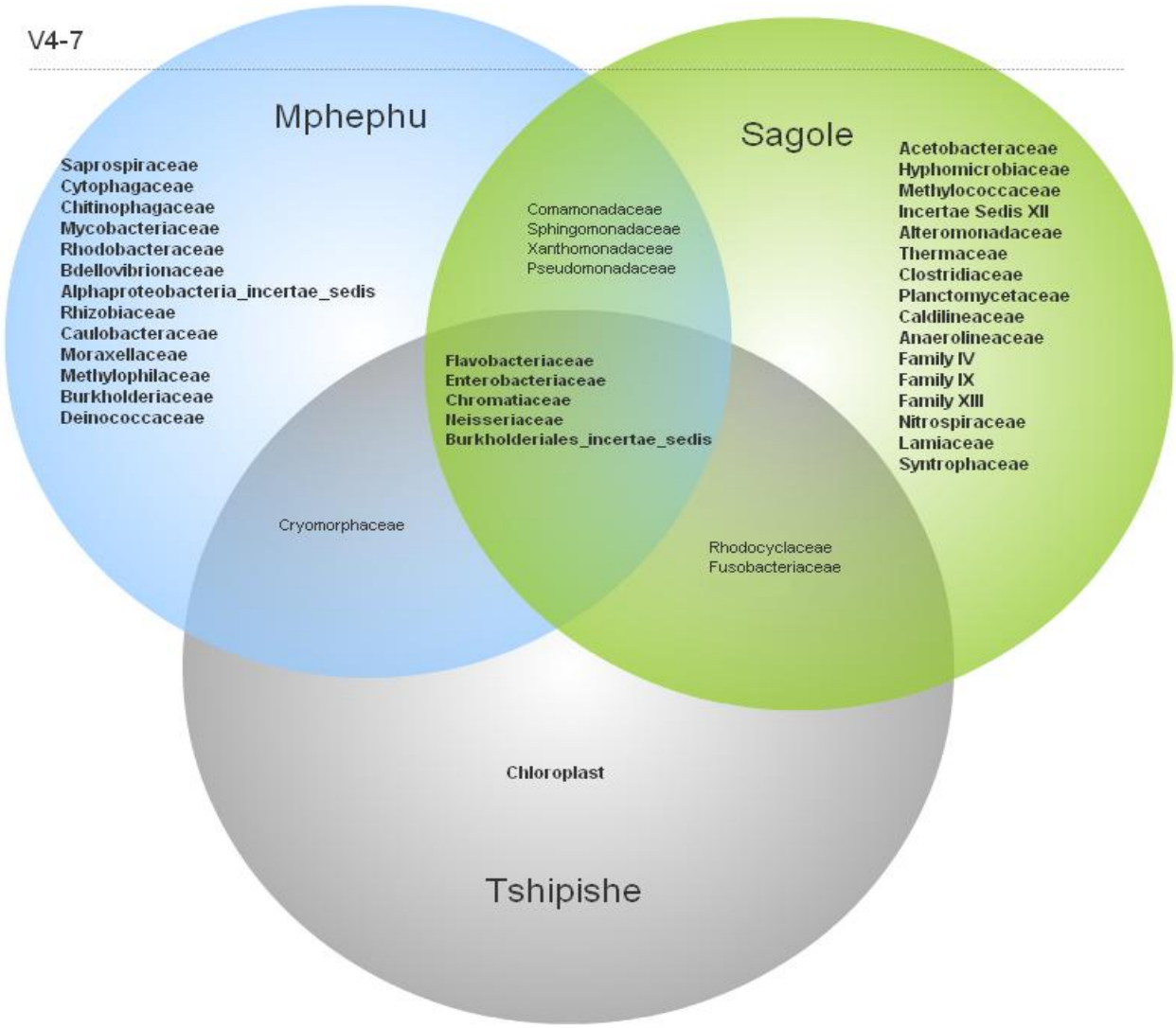
(a)

Figure 7. Bacterial family occurrence comparison for Mphephu, Sagole and Tshipise as determined by the sequencing of the (a) V1-3 and (b) V4-7 regions.

also notable that the Proteobacteria were detected in the thermal water though they were previously suggested to be non-thermophilic (Baker et al., 2001; Lau et al., 2009). However, recent research indicates that Proteobacteria are important microbial groups in hot springs (Lau et al., 2009). It should be noted that no attempt was made in this study to characterise the bacteria with respect to their thermophilic status. The possible contamination of the hot water spring by normal surface water, soil and spores cannot be excluded. Nevertheless, it can be concluded that the bacterial phylotypes detected in these hot springs can possibly proliferate in these thermophilic environments.

Among the phylotypes present at Tshipise and Sagole, was an abundance of the phototropic Cyanobacteria. At Tshipise, these grew as noticeable yellow-green-blackish Cyanobacteria mats on substrata of the spring water flow. The results also show that there were fewer bacteria phylotypes at Tshipise where high temperatures and dissolved mineral salts existed. In such environmental habitats as Tshipise, only bacteria which can tolerate such harsh conditions thrive.

The difference in microbial diversity with location variation is also evident as shown by the distinction in the bacterial families and the occurrence at differing abundance within hot springs. The main bacterial family Chromatiaceae detected at Tshipise is a family of the purple sulphur bacteria, known to oxidize sulfide and produce sulphur under anaerobic conditions (Childs et al., 2008; Tao et al., 2008; Whitaker et al., 2003). The



(b)

Figure 7 Cont:

family Chromaticeae was also dominant at Sagole where anaerobic conditions exist.

Groups important to a particular environment are generally known to be enriched and also to be in correlation to the hydrogeochemistry of the area (Edwards et al., 2006; Meyer-Dombard et al., 2005; Whitaker et al., 2003). The chemical analysis of the thermal spring water at Tshipise indicates high sodium, chloride, bromide, iodine, boron and strontium concentrations and it is yet to be established how these might influence the microbial diversity and function. A number of different trace elements have been described to be essential to the functions of microbial enzymes and growth (Sengör et al., 2005). The occurrence of bacteria in springs with elevated levels of trace metal elements such as strontium, boron, bromide and iodine at Tshipise could be the subject of further studies to investigate the bio-potentials of the microorganisms from this hot spring.

Although the dominant groups are more emphasised; it is worth noting that the microorganisms found in low abundance in rare or extreme environments are important individuals of the community that could constitute an unexplored reservoir of genomic innovation and novelty (Sogin et al., 2006).

Conclusion

This study showed that a considerable diversity of microbial communities can be revealed by metagenomics using 454 pyrosequencing and gives insight into microbial genetic diversity, community composition, distribution and abundance at Mphephu, Tshipise and

Sagole thermal springs in South Africa. Here, we gave a general description of the bacterial diversity in these three South African hot springs. The significantly high number of the sequences that could not be assigned to any phyla could be due to a variety of reasons and could indicate that some of these bacteria have not yet been classified or are not known. Our study offers important insights into the bacterial community diversity in these South African hot springs and can offer the basis for the search for novel species or for future bio-prospecting studies of the microorganisms in these hot springs.

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