ANADOLU ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ –C
Yaşam Bilimleri ve Biyoteknoloji

ANADOLU UNIVERSITY JOURNAL OF SCIENCE AND TECHNOLOGY –C
Life Sciences and Biotechnology
Cilt/Vol.: 2-Sayı/No: 1 : 31-39 (2012)

ARAŞTIRMA MAKALESİ /**RESEARCH ARTICLE**

MICROBIAL POPULATION OF HOT SPRING WATERS IN ESKİŞEHİR/TURKEY

Nalan YILMAZ SARIÖZLÜ [1], Rasime DEMİREL[1], Merih KIVANÇ[1]

ABSTRACT

In order to investigate and find out the bacterial community of hot spring waters in Eskişehir, Turkey, 7 hot spring water samples were collected from 7 different hot springs. All samples were inoculated using four different media (nutrient agar, water yeast extract agar, trypticase soy agar, starch casein agar). After incubation at 50 ºC for 14 days, all bacterial colonies were counted and purified. Gram reaction, catalase and oxidase properties of all isolates were determined and investigated by BIOLOG, VITEK and automated ribotyping system (RiboPrinter). The resistance of these bacteria was examined against ampiciline, gentamisine, trimethoprime-sulphamethoxazole and tetracycline. As a result, heat resistant pathogenic microorganisms in addition to human normal flora were determined in hot spring waters (43-50 ºC) in investigated area. Ten different species belong to 6 genera were identified as *Alysiella filiformis, Bordetella bronchiseptica, B. pertussis, Molexalla caprae, M. caviae, M. cuniculi, M. phenylpyruvica, Roseomonas fauriae, Delftia acidovorans* and *Pseudomonas taetrolens.*

Keywords: Hot spring, Eskişehir, BIOLOG, VITEK, Ribotype.

ESKİŞEHİR/TÜRKİYE'DEKİ ILICA SULARININ MİKROBİYAL POPULASYONU

ÖZ

Eskişehir/Türkiye'deki ılıca sularındaki bakteriyel topluluğun ortaya konulması ve incelenmesi için 7 ılıca su örneği 7 farklı ılıcadan toplanmıştır. Tüm örnekler dört farklı besi ortamına (nutrient agar, su-maya ekstraktı agar, triptik soy agar, nişasta-kazein agar) inoküle edilmiştir. 50 °C'de 14 günlük inkübasyondan sonra tüm bakteri kolonilerinin sayımı yapılıp saflaştırılmıştır. Tüm izolatların gram reaksiyonları, katalaz ve oksidaz özellikleri belirlenmiş ve BIOLOG, VITEK ve otomatik ribotiplendirme sisteminde (RiboPrinter) incelenmişlerdir. Bu bakterilerin ampisilin, gentamisin, trimetoprim-sülfametoksazol ve tetrasiklin antibiyotiklerine karşı dirençliliği incelenmiştir. Sonuç olarak normal insan florasına ilaveten ısıya dirençli patojenik mikroorganizmalar incelenen alandaki ılıca sularında (43-50 °C) belirlenmiştir. Altı genusa ait 10 farklı tür *Alysiella filiformis, Bordetella bronchiseptica, B. pertussis, Molexalla caprae, M. caviae, M. cuniculi, M. phenylpyruvica, Roseomonas fauriae, Delftia acidovorans* ve *Pseudomonas taetrolens* olarak tanımlanmıştır.

Anahtar Kelimeler: Ilıca, Eskişehir, BIOLOG, VITEK, Ribotip.

[1,] Anadolu University, Faculty of Science, Department of Biology, 26470 Eskişehir, TURKEY.
E-mail: nalany@anadolu.edu.tr

Recieved: 26 October 2010; **Revised:** 25 November 2010; **Accepted:** 21 April 2011

1. INTRODUCTION

Turkey is one of the 7 countries in the world in terms of thermal source richness with almost 1300 thermal springs throughout Anatolia (Anonymous, 2009). Thermal springs of Eskişehir are among the famous ones, most of the thermal springs are near Hamamyolu Street in the center of Eskişehir. The waters (38-45 ºC) contain bicarbonate, sodium, and calcium and suitable for both drinking and bathing cures of rheumatism, neuralgia, post-operational problems, digestive problems, kidney stones and gynecologic complaints, and for the metabolism (Anonymous, 2009). In addition, these spring waters are also consumed as drinking water for health.

Generally, hot springs differ in their physicochemical characteristics, such as water temperature (WT), pH, oxidation–reduction potential (ORP, Eh), electrical conductivity (EC), dissolved oxygen, and in their composition of main anions and cations. Previously, interest in these hydrothermal systems has been restricted to chemical and geological investigations. Both microbiologists and geologists have showed great interest in diversity, structure, and function of the resident microbial communities and most research on the microbiology of the hot springs has concentrated on cultivating and isolating extreme strains (Belkova et al., 2007). Geothermal hot spring streams provide favorable conditions for the development of microbial mat, which may contain physiologically and phylogenetically different groups of prokaryotes, such as chemotropic sulfur bacteria, cyanobacteria, and anoxigenic phototrophic bacteria, depending on the temperature, pH, sulfide (30-60 µM) concentration, and some other environmental conditions (Hiraishi et al., 1999, Wahlund et al., 1991).

Bacterial abundance and biomass in marine, freshwater, and soil ecosystems have been quantified parallel to the enumeration of active bacterial cells. However, bacterial enumerations in the hot-spring water are scarce and restricted to total bacterial abundance (TBA). Furthermore, most of the reported data concern low-density bacterial populations. Nevertheless, the effect of microorganisms on geochemical processes occurring in hot springs is evident. Accordingly, measuring the TBA, especially the fraction of active bacteria in the microbial community of the hot springs, is particularly important.

The purpose of this study is to determine and characterization of bacterial populations in hot spring water from the Eskişehir, Turkey with classical and molecular based methods. For this aim, the majority of isolates are detected and typed by biochemical tests on BIOLOGY and VITEK systems. In addition to these systems, Ribotyping was used to characterize the isolates.

2. MATERIALS AND METHODS

2.1 Samples, Cultures, Media and Growth Conditions

A total of 7 different thermal water samples were collected from thermal springs around the Eskişehir/Turkey. After sampling, temperature and pH values of all samples were measured (Table 1). Samples were transported in dark conditions for analysis within 6 h of sampling. Membrane filtration method was used for the bacterial isolation and 26 isolates were obtained. Different media; nutrient agar (NA), water yeast extract agar (WA), trypticase soy agar (TSA), starch casein agar (CSA) were used to obtain the bacterial population. After incubation at 50 ºC for 14 days, all bacterial colonies were counted and transferred to NA medium for purifying (Table 2). For identification and investigation, sub-cultures were performed on NA for 24 h at 50 ºC.

2.2 Biochemical Tests

Gram staining reaction, oxidase and catalase tests of these isolates were determined with basic microbiological methods (Wistreich, 1997). The tolerances against to different temperatures of these bacterial isolates were investigated by inoculation on NA and incubation at 40 and 60 ºC for 48 h (Table 2). In addition, according to their gram staining reaction, morphology, temperature tolerances, catalase and oxidase properties, some isolates were select and the resistance of these bacteria was examined against ampiciline, tetracycline, gentamisine, trimethoprime-sulphamethoxazole (Table 3).

Two different identification systems, BIOLOG (GP or GN) (OOA 002, 2001; OOA 003, 2001) and VITEK (BAC or GNI) (Laboratory Manager Vitek Manual, 2004), were used to identification of selected isolates according to manufacturing manual. In addition, carbon source metabolic fingerprints of these isolates were determined by these systems. Results of these systems were exported for analysis in NTS files and imported into NTSYSpc 2.1. Clustering analysis was performed by the unweighted pair group method with arithmetic averages (UPGMA) method based on the SM coefficient.

Table 1. Sampling points, temperature, pH and total colony counts on different media and isolates number of thermal water samples.

Sample No	Sampling point	Temperature (ºC)	pH	Media/Total Colony Counts	Isolate Number*
1	A	50	7.8	NA: 470 cfu/ml	S6
				WA: 3 cfu/ml	S10
2	B	50	7.5	NA: 340 cfu/ml	S23
				WA: 1 cfu/ml	
3	C	43	7.9	No Growing	
4	D	48	7.3	NA: 173 cfu/ml	S22, S4, S5
				TSA: 1 cfu/ml	
				CSA: 21 cfu/ml	S11
5	E	43	7.4	NA: 3 cfu/ml	S19, S20, S21
				TSA: 2 cfu/ml	S18
6	F	43	7.4	NA: 36 cfu/ml	S16, S15, S14
				CSA: 90 cfu/ml	S17
				TSA: 24 cfu/ml	S13
				WA: 92 cfu/ml	S12
7	G	45	7.5	NA: 571 cfu/ml	S7, S8, S9
				CSA: 127 cfu/ml	S24, S25, S26
				TSA: 24 cfu/ml	S1, S2, S3

***S=Strain**

2.3 Ribotyping and Ribotype Analysis

As a molecular method based on the analysis of the restriction fragment length polymorphisms (RFLPs) of rRNA genes (Cordevant et al., 2003), Ribotyping were used for identification and/or characterization by analyzing ribosomal DNA banding pattern of isolates. For this aim, all isolates were characterized by use of the RiboPrinter system (Qualicon, Wilmington, Del., USA), as described previously (Ito et al., 2003), with respect to the procedures and conditions recommended by the manufacturer. EcoRI and PVUII were used as the restriction enzymes. At the end of the process, densitometry scan and molecular weight of the restriction fragments were obtained for each samples analyzed.

Restriction fragments of ribotypes were exported for analysis in NTS files and imported into NTSYSpc 2.1. Clustering analysis was performed by the unweighted pair group method with arithmetic averages (UPGMA) method based on the SM coefficient for band matching. Bands for analysis with the SM coefficient were allocated manually, according to densitometry curves and the accompanying hard copy photograph.

3. RESULTS AND DISCUSSION

Thermal water samples in this study have a temperature value between 43-50 ºC and pH value between 7.3 and 7.9 (Table 1). While these temperatures are suitable for growing of mesophilic and thermophilic microorganisms, pH values of investigated samples are supporting to microbial growing. Optimum pH value for many microorganisms is between 6 and 8 already many natural environments have pH value between 5 and 9 (Madigan and Martinko, 2006). We counted to colony forming unit between 36 and 571 cfu/ml at different media (Table 1). The highest mean numbers of microorganisms were obtained from sample G with 571 cfu/ml at NA and 127 cfu/ml at CSA. We determined 470 cfu/ml at NA from sample A, 340 cfu/ml at NA from sample B, 173 cfu/ml at NA from sample D, 92 cfu/ml at WA and 90 cfu/ml at CSA from sample F. Only sample E had colony forming unit out of 30-300 but this sample were took to isolation process because of this microorganisms could be pathogenic microorganisms. By the sampling process, we couldn't found colony forming unit at sample C (Table 2).

Table 2. Properties of cell morphology, gram staining reaction, catalase and oxidase, temperature tolerances of isolates.

Isolate Number*	**Morphology**	**Gram Staining Reaction**	**Catalase**	**Oxidase**	**Temperature Tolerances**	
					40 ºC	**60 ºC**
S1	Bacil	Gr(+)	-	+	Positive	Poor
S2	Coccobacil	Gr(-)	-	+	Positive	Poor
S3	Bacil	Gr(-)	-	+	Positive	Negative
S4	Coccus	Gr(-)	-	+	Negative	Negative
S5	Coccus	Gr(-)	+	+	Positive	Negative
S6	Coccobacil	Gr(-)	+	+	Negative	Negative
S7	Coccobacil	Gr(-)	+	+	Positive	Positive
S8	Coccobacil	Gr(-)	-	+	Positive	Negative
S9	Coccobacil	Gr(-)	-	+	Positive	Negative
S10	Coccobacil	Gr(-)	+	+	Positive	Negative
S11	Bacil	Gr(+)	+	+	Positive	Positive
S12	Coccobacil	Gr(-)	+	+	Positive	Negative
S13	Coccobacil	Gr(-)	+	+	Positive	Poor
S14	Coccobacil	Gr(-)	-	+	Positive	Negative
S15	Coccobacil	Gr(-)	+	+	Positive	Negative
S16	Coccobacil	Gr(-)	-	+	Positive	Positive
S17	Coccus	Gr(-)	+	+	Positive	Positive
S18	Coccobacil	Gr(-)	+	+	Positive	Negative
S19	Coccobacil	Gr(-)	-	+	Negative	Negative
S20	Coccobacil	Gr(-)	-	+	Negative	Negative
S21	Coccobacil	Gr(-)	-	+	Negative	Negative
S22	Coccus	Gr(-)	+	+	Positive	Negative
S23	Coccobacil	Gr(-)	-	+	Negative	Negative
S24	Coccus	Gr(-)	+	+	Positive	Positive
S25	Coccus	Gr(-)	+	+	Positive	Positive
S26	Coccus	Gr(-)	+	+	Positive	Positive

Table 3. Resistance of some isolates against to ampiciline, tetracycline, gentamisine, trimethoprime-sulphamethoxazole.

Isolate Number*	Inhibition Zones of Tested Antibiotics (mm)			
	Ampiciline	**Tetracycline**	**Gentamisine**	**Trimethoprime-sulphamethoxazole**
S1	51.0	29.0	15.5	59.0
S5	0.0	30.0	30.0	52.0
S7	30.0	43.0	20.0	52.0
S8	51.0	25.0	16.0	55.5
S10	27.0	39.5	29.0	43.0
S11	0.0	35.5	30.5	51.0
S12	0.0	34.0	33.5	53.0
S13	0.0	35.0	32.0	53.5
S14	0.0	16.0	30.5	55.0
S15	0.0	16.5	30.5	54.0
S16	0.0	17.5	29.0	51.5
S17	0.0	27.5	30.5	51.0
S18	0.0	16.0	30.5	45.0
S22	0.0	37.5	34.5	54.5
S24	51.0	54.0	43.0	65.0

End of the isolation process, we obtained 26 isolates from samples from 6 samples. While twenty four isolates (92%) were determined as Gram negative, two isolates (8%) were determined as Gram positive cell properties. The sixteen isolates (61.5%) have cocobacil cell shape, seven isolates (26.9%) have coccus cell shape and three isolates (11.6%) have bacil cell shape (Table 2). While all of the isolates showed positive oxidase reaction, 13 isolates showed positive catalase reaction. In temperature tolerances studies, twenty isolates grew very well at 40 ºC and six isolates grew very well at 60 ºC (Table 2). While isolate S6 belong to sampling point A did not grow at 40 °C and 60 °C, S10 grew at 40 °C. Isolate S23 belong to sampling point B did not grow both at 40 and 60 °C; it only grew at 50 °C that is isolation temperature. As S23, S4 belong to sampling point D only grew at isolation temperature. While S5 and S22 grew only at 40 °C, S11 grew both at 40 and 60 °C. Only isolate S18 belong to sampling point E grew at 40 °C, the other isolates did not grow both at 40 and 60 °C. While S16 and S17 isolates belong to sampling point F grew both at 40 and 60 °C, the other isolates grew only at 40 °C. While there are isolates belong to sampling point G which grew at 40 and 60 °C such as S7, S24, S25 and S26, the other isolates of grew only 40 °C (Table 2). According to these results, bacterial community with different properties such as thermo tolerant, thermopile and extreme ther-

mopile obtained from hot spring environments in sampling area. Baker et al. (2001) were found different type bacteria from Indonesian hot springs. These bacteria types includes new extremely thermopilic strains belong to *Bacillus* species and extreme thermopilic species together non-thermopilic pathogenic species such as *Flavobacterium* spp.

Due to be found of probable pathogen bacteria in hot springs, we applied a resistance test of our some hot spring isolates. According to these cellular and biochemical properties, 15 isolates were selected for antibiotic resistance test. For this test, we used to ampiciline, tetracycline, gentamisine, trimethoprime-sulphamethoxazole antibiotics. Ten isolates (S5, S11, S12, S13, S14, S15, S16, S17, S18 and S22) showed resistance to ampiciline. The other antibiotics showed antimicrobial activity against all of the isolates at the different levels (Table 3). These ten isolates have coccus and coccobacil cell forms and Gram negative properties except S11. According to these results, current some commercial antibiotics could be use against probable pathogen bacteria in hot spring for protect public health.

In addition to these morphological and biochemical studies, we used to three different methods for diagnose of isolates impending VITEK, BIOLOG and Ribotyping. S1 and S11 isolates were identified by VITEK system as *Bacillus sphericus* with 95% similarity. The other isolates were identified with BIOLOG system based on basic biochemical principles. In addition to identification results, we obtained information about using capabilities of different carbon sources of these isolates with BIOLOG system. According to these results, we investigated to similarity relationship between all isolates (Figure 1). The highest coefficient is belonging to S5 and S16 with 80%. Also S13 is too near to this group with 65.6%. These three isolates (S5, S16 and S13) have already identified as *Alysiella filiformis* in BIOLOG system. *Alysiella filiformis* is a member of *Neisseriaceae* family and similar to *Simonsiella* genus. Even though these bacteria have been considered as a normal flora, they are well understood as part of oral microflora. Despite their low pathogenecitiy, these bacteria have been isolated from erosive lesions of the human oral cavity and from the gastric aspirate of a neonate (Boone et al., 2001)**.** S12 and S17 isolates have 73.6% coefficient value and S14 is similar to these isolates with 63.4% similarity. While S14 and S17 were identified as *Moraxella caviae*, S12 was identified as *Moraxella cuniculi* in this system. *Moraxella caviae* is a member of *Moraxellaceae* family and a species of subgenus *Branhamella* (Enright et al., 1994). Although there are information about reports pathological properties of other species of *Moraxella* genus (Boone et al., 2001), we couldn't be obtained information about pathogenity of *Molexalla caviae*. Likely *Moraxella cuniculi* is a member of *Moraxellaceae* family and we couldn't determine as a human pathogen to this species. Although *Alysiella* and *Molexalla* genuses were considered in the *Neisseriaceae* family, after some DNA-DNA and DNA-rDNA hibridization tecniques, *Moraxella* genus were removed from *Neisseriaceae* family. So *Alysiella filiformis*, *Molexalla caviae* and *Moraxella cuniculi* were showed 57% coefficient according to result of BIOLOG system. In addition to capabilities of using to different carbon sources, these isolates that are member of *Neisseriaceae* and *Moraxellaceae* family showed 89% and 85.8% coefficient in ribotypes results. Also these isolates were cut by only ECORI (Figure 2).

S15 was identified as *Roseomonas fauriae* that is one of the six members of *Methylobacteriaceae* family. Species of *Roseomonas* are opportunistic pathogens and have been isolated from human infections including septicemia (Helsel et al., 2006). *Roseomonas fauriae* has been associated with bacteremia and wound infections of humans (Boone et al., 2001). In addition, S15 showed so far relationship to previous six isolates with 52.2% coefficient. *Bordetella bronchiseptica* that was obtained result for isolate S18 is one of the seven species of the family *Alcaligenaceae* of the *Betaproteobacteria*. *B. bronchiseptica* is predominantly animal pathogens and causes respiratory tract infections in various animals such as swine, dogs, cats, rabbits. It has been isolated from warm-blooded animals, including humans (Boone et al., 2001). This isolate showed 50.6% coefficient to members of *Neisseriaceae* family belong to the *Betaproteobacteria* class.

S8 was identified as *Psychrobacter phenylpyruvicus* (formerly *Moraxella phenylpyruvica*), a member of *Moraxellaceae* family (BSOP ID 11, 2007). *Psychrobacter* spp. has been isolated from different habitats such as seawater, skin and gills of fish, in addition to from human samples. Stepanović et al. (2007) reported that *Psychrobacter phenylpyruvicus* associated with surgical wound infection.

While S10 and S22 isolates showed 64%, S7 showed 47.4% coefficient. Also these isolates were identified as *Moraxella caprae, Bordetella pertussis* and *Delftia* (formerly *Comamonas*) *acidovorans* respectively.

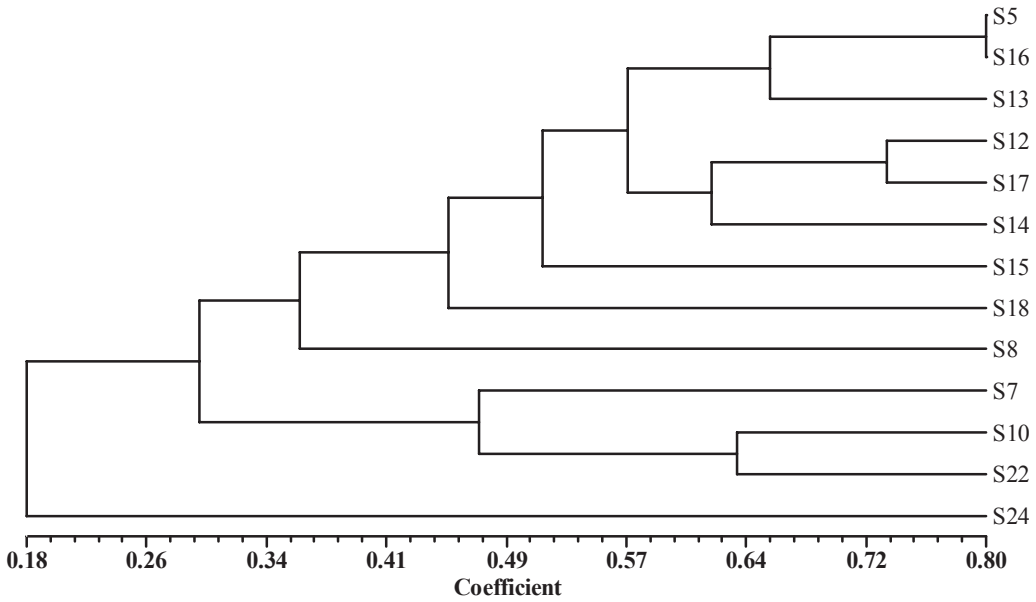

Figure 1. Dendogram based on UPGMA cluster analysis of BIOLOG results. All isolates were compared according to their capabilities of using to 96 different carbon sources (S=Strain).

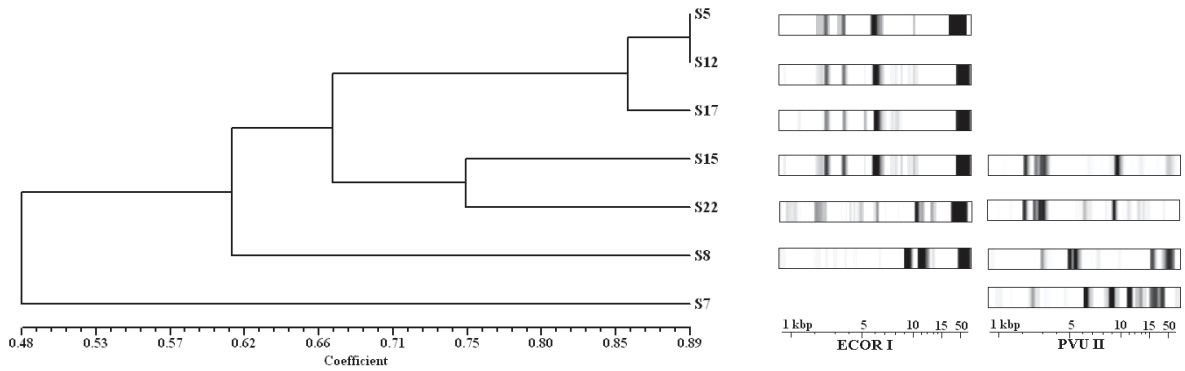

Figure 2. Dendogram based on UPGMA cluster analysis of ribotypes of isolates used in this study (*S=Strain).

Although *Moraxella caprae* is a member of genus *Moraxella* of *Moraxellaceae* family, *Bordetella pertussis* is a species of the family *Alcaligenaceae* and *Delftia acidovorans* is a species of *Comamonadaceae* family. These species showed so different capabilities on using to different carbon sources and they showed 30.8% coefficient to previous species. We couldn't obtain information about pathogenity of *Molexalla caprae* from these species. Although *Delftia acidovorans* has been isolated from clinical specimens and the hospital environments, it has been outshining with have potential for biological control against fungal pathogens (El-Banna, 2007). *Bordetella pertussis* is type species of *Bordetella* genus and this species causes pertussis (whooping cough) in humans.

According to ribotyping results, S15 and S22 that were determined as members of *Methylobacteriaceae* family and *Alcaligenaceae* family respectively showed 75% coefficient and they were cut by both ECOR I and PVU II. Similarly, S8 were cut by both ECOR I and PVU II and this isolate showed 62% coefficient values to previous family members. However, S7 were cut by only PVU II and showed 48% coefficient to other isolates (Figure 2).

S24 was identified as *Pseudomonas taetrolens* that is a member of *Pseudomonadaceae*.

So this isolate showed 18% coefficient with previous isolates. This microorganism had been used for oxidation of isomaltose by Sternberg and Lockwood (1969). But it causes mustiness to different food samples (Tompkin and Shaparis, 1972).

In this stage of our study, we have reached our goal that is determination to cultivable bacterial community of hot springs of Eskişehir /TURKEY. We found that different hot spring water using by public contain pathogen microorganisms for humans in addition to normal flora. Thus, our result showed that this hot springs are not suitability for using and have risk factors for public health. In addition to determination of pathologic risk factors in hot spring, we obtained some microorganisms that have industrial and biotechnological potentials from thermal extreme areas.

4. CONCLUSION

In this study we have described the hot spring bacterial population for aim of the public health and obtained microbial sources for further industrial applications. In addition, profiling of hot spring environments is useful not only for characterization of microbial community structures but also for evaluation of the energy-yielding modes of the microbes present in these type environments. Further work is being done on apply molecular identification techniques such as 16S r RNA analysis for clearly determination bacterial composition and should provide a better understanding of the microbial ecology of these extreme habitats and allow comparison to other thermal regions of the world, furthering the knowledge of the microbial ecology of these environments.

REFERENCES

Anonymous (2009). Thermal Springs. (http://turkeytravelresource.com/view/253/thermal-springs--).

Baker, G.C., Gaffar, S., Cowan, D.A. and Suharto, A.R. (2001). Bacterial community analysis of Indonesian hot springs. FEMS *Microbiology Letters* 200, 103-109.

Belkova, N.L., Tazaki, K., Zakharova, J.R. and Parfenova, V.V. (2007). Activity of bacteria in water of hot springs from Southern and Central Kamchatskaya geothermal provinces, Kamchatka Peninsula, Russia. *Microbiological Research*. 162, 99-107.

Bsop I.D.11 (2007). *Identification of Moraxella Species and Morphologically Similar Organisms*. Issue No: 2, Issue Date: 14.08.07, Issue by: Standards Unit, Evalutions and Standards Laboratory 1-13.

Boone, D.R., Castenholz, R.W., Bergey, D.H., Garrity, G.M. and Staley, J.T. (2001). *Bergey's Manual of Systematic Bacteriology*, Second Edition, Springer, New York.

Cordevant, C., Tang, J.S., Cleland, D. and Lange, M. (2003). Characterization of members of the *Legionellaceae* family by automated ribotyping. *Journal of Clinical Microbiology* 41(1), 34-43.

El-Banna, N.M. (2007). Antifungal activity of *Comamonas acidovorans* isolated from water pond in south Jordan. *African Journal of Biotechnology* 6(19), 2216-2219.

Enright, M.C., Carter, P.E., MacLean, I.A. and McKenzie, H. (1994). Phylogenetic relationships between some members of the genera *Neisseria*, *Acinetobacter, Moraxella* and *Kingella* based on partial 16S ribosomal DNA sequence analysis. *International Journal of Systematic Bacteriology* 44(3), 387-391.

Helsel, L.O., Hollis, D.G., Steigerwalt, A.G. and Levett, P.N. (2006). Reclassification of *Roseomonas fauriae* Rihs *et al.*1998 as a later heterotypic synonym of *Azospirillum brasilense* Tarrand *et al.* 1979. *International Journal of Systematic and Evolutionary Microbiology* 56, 2753-2755.

Hiraishi, A., Umezawa, T. Yamamoto, H., Kato, K. and Maki, Y. (1999). Changes in quinone profiles of hot spring microbial mats with a thermal gradient. *Applied and Environmental Microbiology* 65(1), 198-205.

Ito, Y., Linuma, Y., Baba, H., Sugino, Y., Hasegawa, Y., Shimokata, K., Ichiyama, S., Hasegawa, T. and Ohta, M. (2003). Evaluation of automated ribotyping system for characterization and identification of verocytotoxin-producing *Escherichia coli* isolated in Japan. *Japanese Journal of Infectious Disease* 56(5-6), 200-204.

Laboratory Manager Vitek Manual (2004). TML/MSH Microbiology Department

Policy & Procedure Manual. (http://microbiology.mstinai.on.ca/manual/vitek/mi_vitek.pdf).

Madigan, M.T. and Martinko, J.M. (2006). Brock Biology of Microorganisms, Eleventh Edition, Pearson Prentice Hall.

OOA 002 (2001). Gram positive identification test panel (http://www.biolog.com/).

OOA 003 (2001). Gram negative identification test panel (http://www.biolog.com/).

Stepanović, S., Vuković, D., Bedora-Faure, M., K'ouas, G., Djukić, S., Švabić-Vlahović, M. and Carlier, J.P. (2007). Surgical wound infection associated with *Psychrobacter phenylpyruvicus*-like organism. *Diagnostic Microbiology and Infectious Disease* 57, 217-219.

Sternberg, M. and Lockwood, L.B. (1969). Oxidation of isomaltose by *Pseudomonas taetrolens*. *Journal of Bacteriology* 99(2), 623.

Tompkin, R.B. and Shaparis, A.B. (1972). Potato aroma of lamb carcasses. *Applied Microbiology*. 24(6), 1003-1004.

Wahlund, T.M., Woese, C.R., Castenholz, R.W. and Madigan, M.T. (1991). A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum* sp. nov., *Arch Microbiol*. 156, 81-90.

Wistreich, G.A. (1997). Microbiology Laboratory Fundamental and applications, Prentice Hall, US.