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Microbiological Analysis of Aweturiver and the Proteolytic and Lipolytic Activities of the Microbial Isolates

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Awetu is the River which passes through Jimma town of south western Ethiopia, and one of the major water resources for irrigation and domestic activities for the area. Improper utility of contaminated water create unlimited health concern for the society that utilizing the water for farm and domestic activities. This study was important to evaluate the microbial load, protolytic and lipolytic activities of the isolated microorganisms. The results showed great number of bacterial contaminant in the river. Mainly, Aerobic mesophilic, Enterobacteriaceae, *Staphylococcus* and aerobic spore formers bacteria range between 10^3-10^6 CFU 100 mL^{-1} while yeast and mold ranged between <30 to 10^3. In a total of 30 samples were analyzed for microbial load determination using conventional culture method. The results of the this study indicate that the human and cattle feces, domestic activities, as well as solid and liquid waste disposal around the river were the main sources of contamination potential of water. Therefore, continuous and accurate assessment of the quality of water had a paramount importance. In addition, the presence of fecal contaminants in the three sites along the river segment indicates the problem of the water quality. Possible remedial actions are needed as recommendations in this study.

Key words: Aerobic mesophilic, Enterobacteriaceae, lipolytic, protolytic, *Staphylococcus*, aerobic spore formers

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INTRODUCTION

The biological contamination in surface/drinking water is a major problem of public health (Suthar *et al*., 2009) in developing world. Increasing population density, and scarcity and pollution of surface waters pose a serious health problem form river water in Ethiopia (Reimann *et al*., 2003). Fecal coli forms are the predominant microorganisms that cause a disease on human beings through consumption of contaminated water (Ashbolt, 2004) . For example, *Escherichia coli*, *Clostridium perfringens* and enterococci are predicted organism and fecal indicators of contaminated water (Lamka *et al*., 1980). Awetu River is used for irrigation by low economic status people of living around the river to grow different fruit and vegetables. Influent from different sources were appended to the river as solid or liquid waste and other chemicals which is released from the town are additional pollutants that affect the microbial activities and also the river ecology.

Microbial analysis of water is a preceding activity to use water for irrigation and consumption by human being. Raw vegetables and fruit become contaminated as a result of irrigation with feacally contaminated water (Seymour and Appleton, 2001). Reports from, Saha *et al*. (2009) and WHO (2009) showed that 90% of children death in developing countries was due to water sanitation and hygienic condition. In addition, Proteinaceous and lipid are organic compound that most abundant components in sewage and removal of these compounds are critical for the effective sewage treatment and function (Zinebi *et al*., 1994). Even if lipolytic bacteria extensively studied in food and dairy industries (Blaise and Armstrong, 1973) relatively few studies have been done from aquatic habitats and unpolluted well waters from rural areas "contaminated" with proteolytic and lipolytic psychophallic bacteria. This study was focused on the isolation, identification and evaluation protolytic and lipolytic activities of the microorganisms used for irrigation in relation to health aspects.

MATERIALS AND METHODS

Study area and period: The study was conducted at Jimma town (Fig.1), from September, 2011 to October, 2012 .Jimma town was located 353 km southwest of Addis Ababa. The town is found at 7°41'N latitude, 36°50'E longitude. It also lies to the west of the Great Rift Valley at an altitude of between 1300 and 2100 m and located between 7°15 to 8°45'N and 35°30 to and 37°30'E. The climatic condition of the town were "Woyna Daga" which is considered best for agriculture as well as human settlement. The maximum and minimum temperature of the town is 30 and 14°C, respectively. The annual rain fall ranges from 1138-1690 mm (Alemu *et al*., 2011).

Sample collection: A total thirty sample were collected from three site of the river with ten from each site and samples were taken below 1-5 cm the surface of water, and transported to laboratory for microbiological analysis. For each sample serial dilutions was made and from appropriate dilution, an aliquots of 0.1 mL was transferred on to their respective pre-solidified media and incubated at 30-33°C for 18-96 h.

Microbiological analysis

Determination microbial spectrum: From the water content, Total Aerobic Mesophilic Counts (TAMC), Counts of Staphylococci', Count of Enterobacteriacea, Bacillus, Counts of yeast and molds and Counts of Coliforms were determined according to the criteria specified in Bacteriological analytical manual, and by standard plate count methods on PCA (Plat Count Agar) (Oxoid) for TAMC at 32°C during 72 h, Mannitol Salt agar (Oxoid) for staphylococci count, Chloramphenicol Bromophenol Blue Agar for yeast and mold and VRBGA(Violet Red Bile Glucose Agar)(Oxoid) for Enterobacteriacea and VRBA (Violet Red Bile Agar) (Oxoid) for total coliform count (TCC) during 24 hrs at 32°C for TCC, and 44°C for FCC.

Microbial identification: After enumeration of aerobic mesophilic bacteria, about ten to fifteen colonies were picked rando mLy from countable plates and inoculated to tubes containing 5 mL nutrient broth and incubated at 30-32°C for 24 h. The cultures were purified by repeated plating in order to characterize for colony morphology and differentiated into various bacterial group to the genus level by using standard microbiological techniques (cell morphology, Gram reaction, and biochemical test).

Proteolytic activities test: According to the bacteriological analytical manual for food (FDA.1976) Milk agar (MA) was used for isolation and counting of proteolytic bacteria. From broth culture by sterilize surface of milk agar plates and incubated at 34-37°C for 48 h. The presence of clear zone around the colonies against an opaque with back ground in plates of media indicated that these organisms were proteolytic (Rosso and Azam, 1987).

Lipolytic activities test: Nutrient agar containing 1% olive oil (OA) was used for isolation and counting the lipolytic bacteria. Loop full of broth culture streaked on pre solidified surface of nutrient agar olive oil plate and incubated at 32°C for 24-48 h. Plates of medium were flooded with concentrated copper sulfate solution and bluish green colonies surrounded by precipitate predict was considered as lipolytic bacteria (Odeyemi *et al*., 2011).

RESULTS AND DISCUSSION

Microbial load along the three site of river: The town residents have no detail concept about health problem of using contaminated surface water for different purpose, other than drinking. But, the river is highly exposed to fecal contaminants like, Enterobacteriaceae, *Clostridium* and coliform bacteria. Most isolate bacteria genera are gram negative, rode in shape, Catalase, fermentative, proteolytic and lipolytic positive. The most predominate genera in term of microflora and enzyme activities were *Aeromonas*, while the least one in all cases was chromo bacterium species.

The contamination extent of Awetu River at "3" site, were evaluated by taking a afresh sample and culturing on six different media. After counting the cultured microbial result was describe by mean and range, which was tabulated in (Table 1). The microbial load (aerobic mesophilic counts) samples ranged from log 3.61 to log 7.06 CFU mL^{-1} , those of aerobic spore formers from log 3.47 to log 3.89 CFU mL^{-1}, *Staphylococcus* from log 3.47 to log 5.08 CFU mL^{-1}, Coliforms from log 3.47 to log 6.35 CFU mL^{-1} and Enterobacteriacea from log 3.58 to log 6.02 CFU mL^{-1}. The result indicated the low microbial load when compared with the study done by Hoque *et al*. (2009) on bacterial load and chemical pollution level of the river Buriganga, Dhaka, Bangladesh.

A total of 284 bacteria were isolated from the water sample and were grouped to various genera and bacterial groups. The yeast and mold counts ranged between <30 to 3.5 log 10. The microbial load the samples were dominated by Gram-negative bacteria in which aerobic mesophylic were the dominant groups followed by Entrobacteriacea and Coliform spp. Although, these genera are abundant in water environment, appropriate hygienic practice and utility conditions could reduce their load. The detection of Enterobacteriace and Coliforms in all samples signals the need for further strengthening of the management system in the utility of the water for different purposes.

Classification of the isolated bacterial group: Based on the criteria proposed by Cowan and Steel (1965) such as motility, gram reaction, Cytochrome oxidase, Catalasetest,

Table 1: Mean microbial counts log (CFU mL^{-1}) at different sampling sites

			Aerobic mesophilic count		Aerobic spore form bacteria		*Staphylococcus*		Mold		Yeast		Coliform		Enterobacteriaceae	
N	Station	Sample	X	R	X	R	X	R	X	R	X	R	X	R	X	R
1	ASFA	10	4.1	3.61-4.58	3.51	3.47-3.74	3.48	3.47-4.96	<30	<30	<30	<30	3.48	3.47-3.76	3.91	3.58-4.12
2	APP	10	4.3	3.65-4.60	3.59	3.5-3.900	4.04	3.47-5.20	3.48	3.47-3.49	<30	<30	3.90	3.58-5.28	4.02	3.58-4.28
3	ADB	10	6.8	4.04-7.06	3.66	3.47-3.89	4.96	3.47-5.08	3.96	3.57-4.75	3.48	3.5-3.5	5.89	3.5-6.35	5.59	4.06-6.02
	Sum		15.1		10.76		12.48		7.44		3.48		13.27	13.27	13.52	
	Total %		18.9		13.40		15.73		9.8		4.6		16.70	16.7	17.00	

$\bar{x}$: Mean value of count, R: Range of count

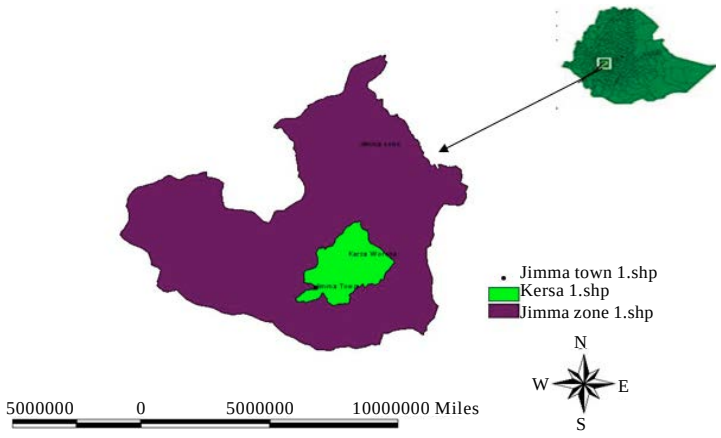

Fig. 1: Map of study area

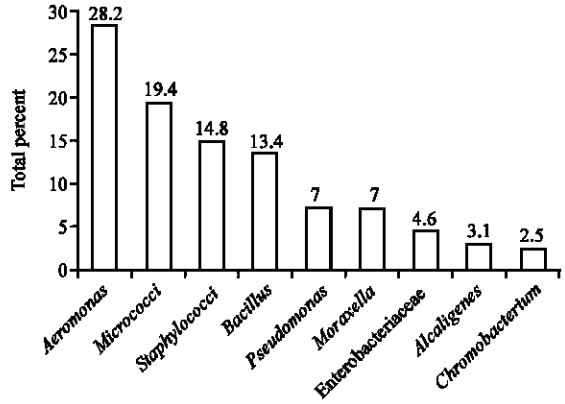

Fig. 2: Percentage of bacteria genera isolated from the three sites of Awetu River to determine the microbial load and spp.

oxidation-fermentation, shape and endospore, 284 isolate were grouped into nine genera of bacteria. These were: *Aeromonas* 80(28.2%), *Micrococci* 55(19.4%) *Staphylococci* 42(14.8%), *Bacillus* 38(13.4%) *Pseudomonas* 20(7%), *Moraxella* 20(7%), Enterobacteriaceae 13(4.6%), *Alcaligenes* 9(3.1%) and *Chromobacterium* 7(2.5%) was identified as (Fig. 2).

In case of micrococcus no uniform varied was seen at three sites, regard on *Alcaligenes* and *Chromobacterium* there was no appeared in both cases at Lower River site. This study was in line with(Blaise and Armstrong, 1973) i.e., the result obtained in this study show that the *Aeromonas* genera was predominant in both case, which was reported in Ottawa River, where *Pseudomonas* was abundance in that case.

Enzyme activities of isolated bacteria: The Contamination level of Awetu River and enzyme activities of bacteria along three sites were evaluated and comparable study was done their (i.e., ASFA, APP and ADB). The results of proteolytic activities, 59(26.6%), 65(30%), 75(33.8%) and lipolytic activities 71(32.7%) 88(39.6%) and 81(37.3) of the isolate from the three site were obtained (Table 2) and (Fig. 3a, b).

Proteolytic and lipolytic bacteria are the dominant in polluted river. They are useful microorganisms in degrading the solid and liquid waste added to the river in different ways. It includes bacteria genera such as, *Bacillus*, *Pseudomonas*, *Aeromonas* etc. Related, results were recorded by Blaise and Armstrong (1973) that 85% of unpolluted well waters from rural areas across Canada were "contaminated" with proteolytic and lipolytic psychrophilic bacteria, many of these members of the genus *Pseudomonas*. In this study both isolates bacillus, and aerobic spore bacteria are highly proteolytic. The

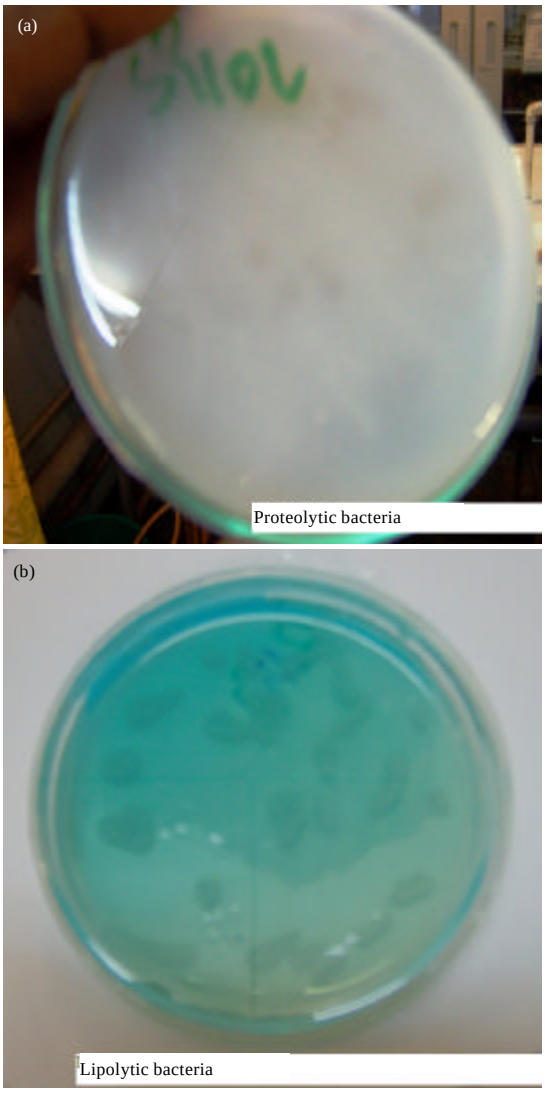

Fig. 3(a-b): Protolytic and lipolytic properties of Bacteria, (a) Protolytic Bacteria and (b) Lipolytic Bacteria

percent of gram reaction of proteolytic bacteria was 49.6% gram positive, 50.4% Negative and for lipolytic 50.2% (+) ve and 49.8(-) ve, respectively was observed (Table 2). The number of population of Proteolytic and lipolytic isolate were low in the river and they may pose a serious health hazard and release by products toxic to other aquatic life, or deplete available.

Therefore, on this study the bacteria genera increase down the river in both cases (i.e., proteolytic and Lipolytic activities) were *Pseudomonas*, Enterobacteriaceae, and *Staphylococcus* also their count in down river (ADB) was almost two-fold of the upper

Table 2: Activities of lipolytic and proteolytic bacteria along (down) the River in the town

Enzyme activities	Sampling site	Bacterial group										
		Aeromananas	*Micro cocci*	*Staphylococci*	*Bacillus*	*Pseudomonas*	*Moraxella*	Enterobacteriacea	*Alcali genes*	*Chromo bacterium*	Total	Total (%)
Proteolytic	ASFA	17	13	8	9	2	3	2	2	3	59	26.6
Lipolytic		25	11	9	5	2	4	3	2	4	65	30.0
Proteolytic	APP	19	21	10	8	2	4	3	4	4	75	33.8
Lipolytic		13	17	15	8	4	2	4	4	4	71	32.7
Proteolytic	ABD	29	8	19	5	11	11	5	-	-	88	39.6
Lipolytic		18	16	16	9	9	9	5	-	-	81	37.3

(ASFA) river, so, it was more or less similar to what was observed by another workers Blaise and Armstrong (1973) in Ottawa River. In addition proteolytic a genus of *Aeromonas* and *Moraxella* also increases down the river, but their lipolytic activates was decreased. On the other hand the genera of *Bacillus* were decreased down the river in this study, which was in contrast with another demonstration (Lamka *et al*., 1980).

CONCLUSION

The Liquid and solid waste management process and the effluent released from town to the river was found to be a contributing factor for the contamination of sampled river water, especially when analyzed for Bacillus ,coliform, and Enterobacteriacea spp. were the predominantly isolated bacteria. Water safety and quality analysis is a preceding activity to use water for different purposes. The use contaminated water for irrigation and lack of awareness on consumption of contaminated fruit and vegetables grown with unsafe water is the major problem for different outbreak in the community. This also would imply that there is a need for improving the waste management process, in order to reduce microbial contamination that contributes the increasing health problem of the society at the study area.

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