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Studies on Protease Producing Bacterial Biodiversity from Soil Collected from Arba Minch University, Abaya Campus, Southern Ethiopia

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Abstract

Microbes are small microscopic organisms and they are found in every habitat such as soil, water, food, and animal intestines, as well as in more extreme environment under extreme conditions such as rocks, glaciers, hot springs, and deep-sea vents. Hydrolytic enzymes are one of the most important groups of microbial products that have various application in various industries. The aims of the present study was study the biodiversity of protease producing bacteria from soil collected from the study area (Arba Minch University, Abaya campus). Skim milk agar medium was used for isolation and screening of protease producer. Totally 157 bacterial strain were isolated from the study area, 38 of them are protease producer. The diversity of protease producer were studied based on colony morphology, Gram staining, endospore staining and biochemical tests. These organisms were then identified in accordance with the Bergey's Manual of Determinative Bacteriology. Accordingly, 15 *Bacillus sp.*, 9 *Staphylococcus sp.*, 5 *Psuedomonas sp*., 4 *Micrococcus sp.*, 2 *Corynebacterium sp.*, 2 *Lactobacillus* and 1 *Streptococcus sp*. were protease producer identified in the present study. Among four potential protease producing isolates, three strains are belongs to *Bacillus spp*, however, AMUa31 isolate was found to be *Psuedomonas sp*. Isolate AMUa38 and AMUa12 produce relatively higher clear zone with 26.5 and 16mm size respectively. The use of microorganisms to produce industrially important enzymes has a number of technical and economic advantages and in recent years has become the predominant mode of enzyme production. In this regard, members of the genus Bacillus produce a large variety of extracellular proteases which have significant application in various industries.

Keywords

Biodiversity, Protease, Bacteria, Isolation, Abaya Campus

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1. Introduction

Microorganisms are microscopic organisms with great contribution for sustainability of ecosystem. They have a Profound impact in every facet of human life and everything around us because, they can be either beneficial in several ways or become a threat to plant, animal and human health. The beneficial microbes are, fascinating, versatile and capable of growing on a wide range of substrates and carry out extremely useful processes that can't be achieved by other physical and chemical means. The sum total of microbial chemical reactions and biosynthetic pathways generates an extraordinary diverse and broad range of metabolites or compound, simple and complex low molecular weight and polymeric [1].

Moreover, microorganisms are able to degrade many substance to a remarkable spectrum of products that support the growth of living organisms and fulfill numerous human

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requirements. Accordingly, microbes are said to the fundamental element and building blocks of biotechnology. The role of microbes in biotechnology is much and without microbes, biotechnology would not be as advanced as it is, nor would it include Such a broad range of applications. Hence microbial biotechnology could be best explained as the branch of biotechnology which utilizes microorganisms (bacteria, fungi, viruses etc) whole cell, their parts (plasmids, chromosomal DNA, cell surface etc), process (anabolisin and catabolism), and products (enzymes, vitamins, amino acids, Carbohydrates, lipid etc) to benefit mankind [2, 3].

Microbial biotechnology explores and exploits the beneficial microbial wealth For various human requirements. Humans over the ages have been highly Successful in applying processes carried out by microorganisms to solve problem. In agriculture food production, human health, chemical industry and environmental Quality. Microbial biotechnology has made significant advances in recent years with an overwhelming impact on the society the developments are very fast and new dimensions are being added every day that changes all aspects of human life microbial biotechnology is being extensively applied in almost all sectors of the industry currently its share in the global bio-economy is estimated in multibillion dollars with annual rise [4-8].

Enzymes are highly efficient environmental-friendly protein catalysts, synthesized by living systems. They have significant advantages over chemical catalysts, of which the most important is specificity, high catalytic activity, ability to work at moderate as well as extreme temperatures, and the ability to be produced in large qualities. The current demand for better utilization of renewable resources and pressure on industry to operate within environmentally compatible limits stimulated development of new enzyme-catalyze industrial process [9].

Recent developments in industrial biotechnology has offered an alternative approach for the reduction (or in some cases total elimination) of pollution from many industrial sectors without affecting production efficiency and product quality. At present enzymes find increasing application in many industrial processes. As a result the global industrial market is growing very fast with a current estimated value of US$7 billion. Although enzymes are found in all living organisms, most industrial enzymes currently in use are obtained from microorganisms. Worldwide over 120 companies are known to produce industrial enzymes and more than 80% of the companies controlling up to 90% of the market are located in Europe and North America with none in Africa. But Africa has a huge potential for the discovery of novel enzymes that could prove highly useful in different industrial processes [6-8].

Ethiopia is endowed with unique microbial diversity which could serve as a source of novel enzymes for industrial application. Despite its huge potential for biotechnology innovation, to date the country make no use of this resource. In addition to this protease producing bacteria have not yet been explored especially from Arba Minch University, Abaya campus. By considering this a research project has been initiated with the this objective.

2. Methodology

2.1. Description of the Study Area

Arba Minch is a city and separate woreda in southern Ethiopia; the for common name for city was Ganta Garr, located about 500 kilometer southern of Addis Ababa at an elevation of 1, 285 meters above sea level. Arba Minch city is the second biggest city in the southern nation nationality of people region next to Hawassa. It is located to the west of lake Abaya, the annual rain fall ranges from 623.5 mm to 1661mm and the temperature is 32°C. The most common vegetation type grow in Arba Minch are mango, banana, apple and so on.

2.2. Sample Collection and Isolation of Proteolytic Bacteria

Soil samples were collected from different environments around Abaya campus such as student cafeteria waste, male and female dormitory, around class room and library. Each sample was kept in clean sterile sample bottles sealed and transferred to Microbiology and Parasitology laboratory and stored at 4°C.

The soil and water samples were suspended in water by vigorous vortexing and serial dilutions were made up to 10^{-6} in sterile distilled water. 100ul of appropriate dilution were added to petri plate on skim milk agar plate containing peptone (0.1%), NaCl (0.5%), Agar (2%) and skimmed milk (10%) and incubated at 40°C for three days. Proteolytic bacteria were screened. A clear zone formation around the colonies due to skim milk hydrolysis indicated protease production by the microbes. These colonies were picked and purified by streaking on skim milk agar. The cultures were subsequently sub-cultured and used regularly. Agar slants were prepared and preserved at 7°C for short term storage for further experiments.

2.3. Biodiversity Study of Protease Producing Bacterial Isolates

The biodiversity of pure cultures of each protease producing bacterial isolate was identified on the basis of its colonial morphology, cellular morphology and biochemical characteristics according to the taxonomic scheme of

Bergey's Manual of Determinative Bacteriology [10]. Colony morphology characterized using microscope by direct observation of the overnight grown microorganisms on skim milk agar plate.

2.4. Screening and Characterization of Potential Isolate from Each Site

Many bacterial isolates with special ability of protease enzyme production (proteolytic activity) were screened depending on different growth parameters such active growth on skimmed milk agar medium and clear zone formation. They were screened by sub culturing repeatedly on skimmed milk agar medium at 40°C. These isolates were screened for further biodiversity studies. The screening took place depending on size of clear zone formed. Finally various biochemical studies were undertaken for the selected potential isolate. The selected potential isolates were also characterized by different biochemical methods; methyl red test, Catalase test (cover slip method), starch and casein hydrolysis test, citrate utilization test, and indole test.

2.5. Data Analysis

The data were analyzed using basic statistical parameters like table and percentage. In addition to this, Microsoft office Excel worksheet 2010 was used for construction and presentation tables.

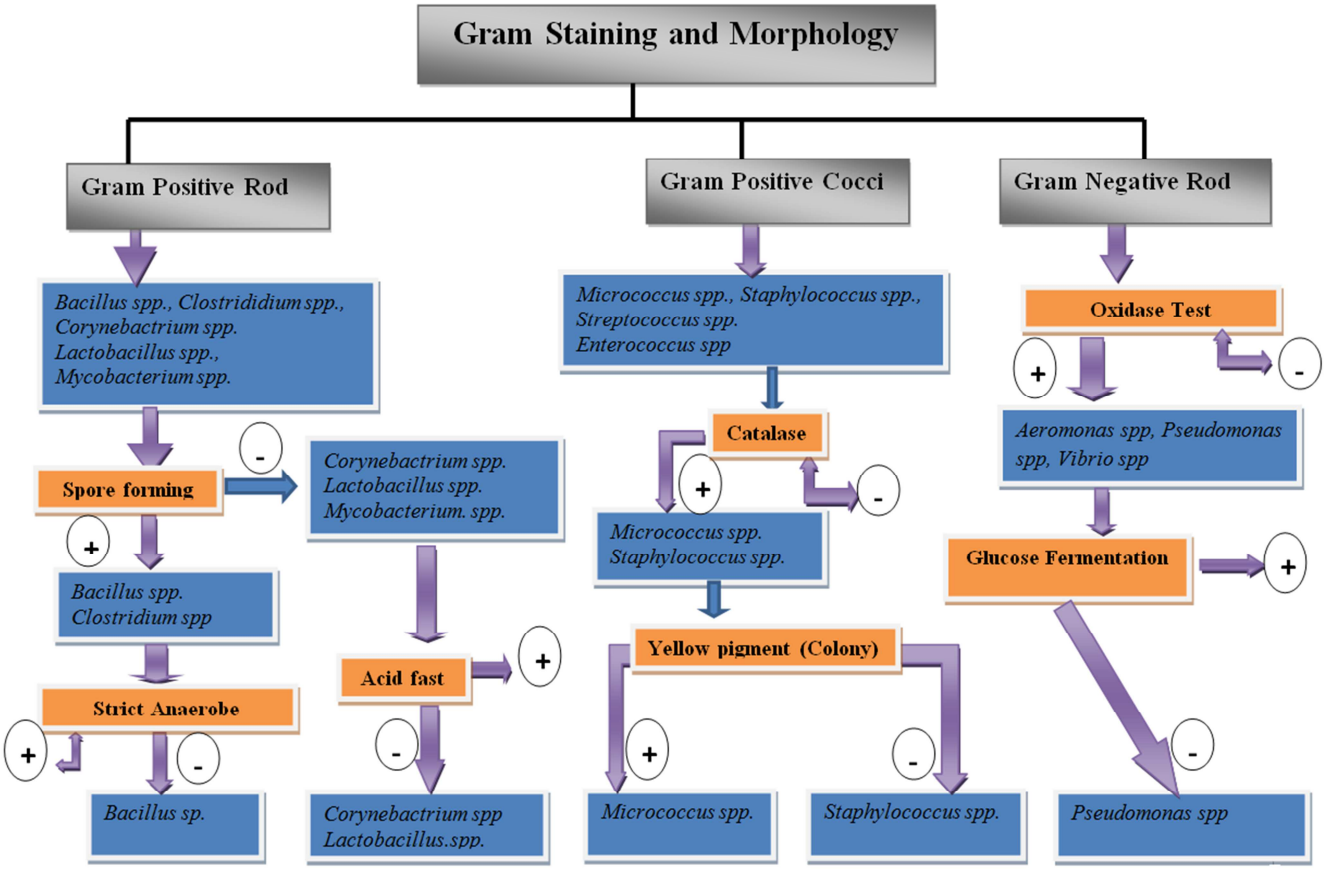

Figure 1. Identification flow charts Used in the present study (Bergey's Manual of Determinative Bacteriology.

3. Results and Discussion

Proteolytic bacteria are wide spread in nature and are able to isolate them from any ecological entities like soil, water, leaves, marine environment and waste and wastewater, however, soil is Vast reservoir of diverse microorganism. Isolation of protease producing bacteria was performed by the serial dilution spread plate technique [2].

One hundred fifty seven (157) bacterial isolates were obtained from composite soil sample from four different site in Abaya campus. One of the main concerns of the present investigation was to isolate and identify protease producing bacterial diversity in the study area. Out of 157, 38 bacterial isolates were found to be protease producer since they form clear zone around the colony in skimmed milk agar medium (Table 1). Formation of clear zone around the bacterial colony indicated the protease positive strains hydrolysed the skim milk present in the media. Four potential isolates were selected one from each site on the basis of clear zone size for further study. The use of skim milk agar for the isolation of protease producing bacteria has earlier been reported by some workers [11].

Table 1. Biodiversity of protease producing bacterial isolates in the study area.

Sample Area	Strain Code	Colony Color	Morphology and Gram Reaction	Strict Anaerobe	Catalase Test	Probable Identity
	AMUa1	Yellow	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa2	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa3	Yellow	Gram positive cocci	Negative	Positive	*Micrococcus sp.*
	AMUa4	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa5	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa6	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa7	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa8	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa9	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa10	Yellowish	Gram positive cocci	Negative	Positive	*Micrococcus sp.*
	AMUa11	White	Gram positive cocci	Negative	Negative	*Streptococcus sp.*
	AMUa12	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa13	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa14	White	Gram positive rod	Negative	Positive	*Corynebactrium sp.*
	AMUa15	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa16	White	Gram positive cocci	Negative	Negative	*Staphylococcus sp.*
	AMUa17	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa18	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
Arba Minch	AMUa19	White	Gram positive cocci	Negative	Negative	*Staphylococcus sp.*
University,	AMUa20	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
Abaya	AMUa21	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
campus	AMUa22	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa23	White	Gram negative rod	Negative	Positive	*Psuedomonas sp.*
	AMUa24	White	Gram positive cocci	Negative	Positive	*Micrococcus sp.*
	AMUa25	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa26	White	Gram negative rod	Negative	Positive	*Psuedomonas sp.*
	AMUa27	Yellow	Gram negative rod	Negative	Positive	*Psuedomonas sp.*
	AMUa28	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa29	Yellow	Gram positive cocci	Negative	Positive	*Micrococcus sp.*
	AMUa30	White	Gram negative rod	Negative	Negative	*Psuedomonas sp.*
	AMUa31	White	Gram negative rod	Negative	Positive	*Psuedomonas sp.*
	AMUa32	White	Gram positive rod	Negative	Positive	*Corynebactrium sp.*
	AMUa33	White	Gram positive rod	Negative	Negative	*Lactobacillus sp*
	AMUa34	White	Gram positive rod	Negative	Positive	*Lactobacillus sp*
	AMUa35	White	Gram positive rod	Negative	Positive	*Bacillus sp.*
	AMUa36	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa37	White	Gram positive cocci	Negative	Positive	*Staphylococcus sp.*
	AMUa38	White	Gram positive rod	Negative	Positive	*Bacillus sp.*

Fifteen (15) *Bacillus sp.*, 9 *Staphylococcus sp.*, 5 *Psuedomonas sp.*, 4 *Micrococcus sp.*, 2 *Corynebacterium sp.*, 2 *Lactobacillus* and 1 *Streptococcus sp*. were obtained from soil samples collected from the study area (Figure 2).

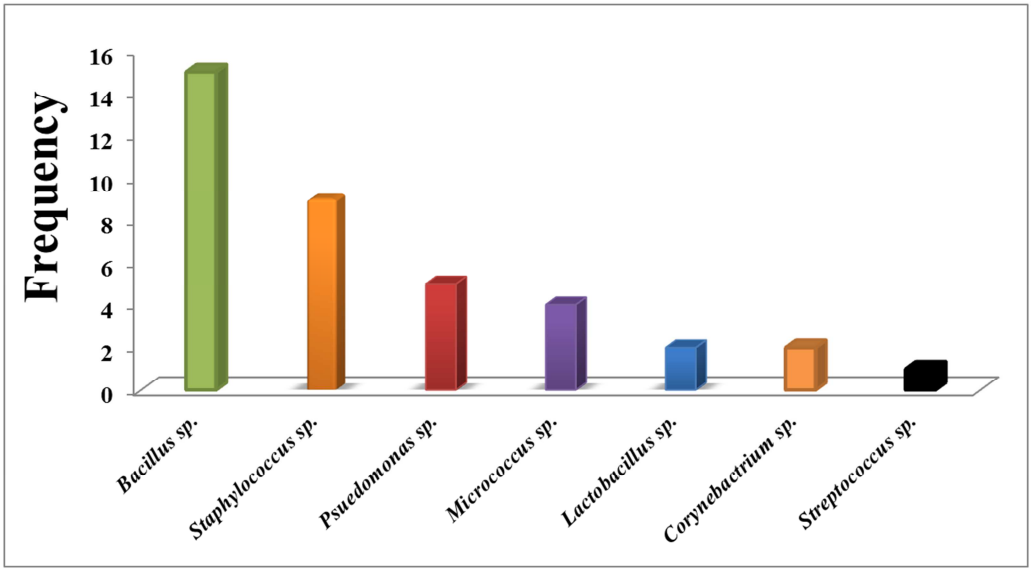

Figure 2. Distribution and biodiversity of protease producing bacteria isolated from the study area.

Among the 38 protease producing bacterial isolates, four potential isolate were selected for further characterization. Isolate AMUa 12 and AMUa31 have produced relatively considerable quantity of extracellular protease which represents clear zone diameter of 16 and 26.5mm respectively.

Table 2 shows results of morphological characteristics and colonial appearance of the selected potential isolates. All potential isolate have circular in shape of colony and flat in height of colony. Motile, Non-motile and elliptical or circular shaped endospore were observed under light microscope with oil immersion of objective lense (1000X magnification) (Table 2). Except AMUa31, all potential protease producing isolates were found to be Gram positive, however, all of them have rod shaped morphology (Figure 3).

Table 2. Results of colony characteristics of potential isolate.

Potential Isolate	Colony surface	Colony Size	Shape of Colony	Elevation	Shape of Spore	Motility test
AMUa8	Rough	Large	Circular	Flat	Elliptical	Non-motile
AMUa12	Smooth	Small	Circular	Flat	Elliptical	Motile
AMUa31	Smooth	Medium	Circular	Flat	Circular	Motile
AMUa38	Rough	Medium	Circular	Flat	Elliptical	Motile

Table 3 shows the result of biochemical characteristics of all the potential isolates, except AMUa8, all isolates were able to hydrolyse starch. All potential strains were found to be indole positive and able to hydrolyse casein.

Table 3. Biochemical characteristics of potential isolates.

Potential Isolate	Starch Hydrolysis	Citrate Utilization	Methyl Red	Casein Hydrolysis	Indole Tests
AMUa8	Negative	Positive	Negative	Positive	Positive
AMUa12	Positive	Positive	Negative	Positive	Positive
AMUa31	Positive	Positive	Negative	Positive	Positive
AMUa38	Positive	Positive	Negative	Positive	Positive

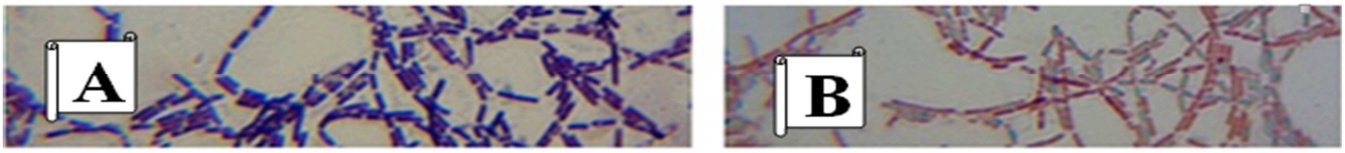

Figure 3. Gram reaction result (+ve) (A) and Endospore test (+ve) of AMUa38 on skimmed milk agar plate at 40°C.

The diversity study of these proteolytic bacteria was performed by colony morphology, colony color, Gram staining, endospore staining, motility, biochemical tests. These organisms were then identified in accordance with the Bergey's Manual of Determinative Bacteriology. As shown in Table 1, the morphological and biochemical characterization of all the isolates lead to their identification up to genus level. As indicated on Figure 2, Bacillus spp. is the most highly distributed bacterial species in the study area having high capability of producing extracellular proteases.

In the present study, almost all of the selected potential strain were gram positive and spore producer except AMUa31 strain. This indicate that they have thick peptidoglycan layer in their cell wall structure. In addition to this, structural, staining and biochemical activity results revealed that the three of four active enzyme producing potential bacteria are belongs to *Bacillus spp*, however, AMUa31 isolate was found to be *Psuedomonas sp*. Members of the genus Bacillus produce a large variety of extracellular enzymes (proteases) which have significant industrial application. This finding was in accordance with well documented reports in literature for protease producing bacteria isolation and characterization [12-15].

Enzymes can be derived from several sources such as plants, animals and microorganisms, however, enzymes from microbial sources generally meet the industrial demands. Therefore, screening of microorganisms with higher enzymatic activities could facilitate the discovery of novel enzymes that are suitable for industrial application. Among these industrial enzymes, protease is the most important hydrolytic group of enzymes which have diversified application in a various industries. However, 90% of this enzymes are produced from microorganism. Similar idea were reported in the previous study [16-17].

4. Conclusions

Based on the results obtained from this study, the following conclusions could be drawn:

Isolation and selection of potential microbial organisms is the primary step for production of industrially important product from microorganisms. Soil is an ideal source of many unique microorganisms to for production of novel enzymes like

proteases. Protease enzymes are produced by culturing different microbial species which have number of technical and economic advantages. Enzymes produced from such microorganism are widely used in a various industries for different purpose like hair removal in leather industry, removal protein stain in detergent industry, protein degradation in food processing industry and so on.

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