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Protease Producing Bacteria from Soil in Gumel Metropolis, Jigawa State of Nigeria

Umar Usman[1]*, Abba Sabo[1], Hannatu Ibrahim[1], Biliyaminu Ado[1], Buhari Suraka[2], Hassan Muhammad[3], Aminu Tijjani[4], Usman Sanusi Ismail[5] & Ni'ima Bashir Ado[6]

[1]*Jigawa State College of Education Gumel, Jigawa State - Nigeria, School of Sciences, Department of Biology.*
[2]*Federal University Dutse, Jigawa State - Nigeria, Faculty of Sciences, Department of Microbiology and Biotechnology.*
[3]*Binyaminu Usman Polytechnic, Hadejia, Jigawa State - Nigeria, School of Science, Department of Science Laboratory Technology.*
[4]*Jigawa State Polytechnic, Dutse, Jigawa State - Nigeria, College of Health Sciences, Department of Epidemiology and Disease Control.*
[5]*Usman Danfodio University, Sokoto, Sokoto State - Nigeria, Centre for Advanced Medical Research and Training (CAMRET).*
[6]*Maryam Abacha American University of Nigeria, Kano State - Nigeria, School of Health Sciences, Department of Medical Laboratory Sciences.*
Corresponding Author: Umar Usman, **E-mail**: umarusmankaz@gmail.com

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ABSTRACT

Proteases, one of the most significant industrial enzymes, account for a major share of about 70% of total global enzyme market. Microorganisms serve as a preferred source of such enzymes due to the limited space required for their cultivation, their fast growth and the simplicity with which they can be genetically manipulated to produce different enzymes with various properties that are desired for their numerous applications. The current research work was carried out to identify protease producing bacteria from soil of Gumel Metropolis, Jigawa State of Nigeria. A total of 20 bacteria were isolated from 12 soil samples collected from different sites in Gumel Metropolis. The 20 isolates were subjected for protease production by using skimmed milk agar. Among all the subjected isolates, 6 were found protease producers. The isolates were further identified through their morphological, microscopic and some biochemical examinations. The results of the study revealed that the soil of Gumel Metropolis has protease producing bacteria that exhibit proteolytic activities and biochemical features. However, intense research works are needed in the study area to reveal more reliable information.

1. Introduction

Proteases are enzymes which have characteristics of biotechnological importance and have thus become significant industrial enzymes (Masi *et al*., 2021). Different enzymes, specifically microbial proteases, are the essentially used in various corperate sectors, such as leather, detergent, food, textile and others (Razzaq *et al.,* 2019). The proteolytic enzymes can also be used in medical field offering a selective debridement, stimulating the natural curing step in the effective local management of skin ulcerations by eliminating the necrotic material in an efficiet way (Luang-In *et al*., 2019). Different species of bacteria produces protease enzyme, and the application of this enzyme has been used precisely and manipulated (Nayab *et al*., 2015). Of course, we have to investigate the cost effective way of producing industrially important enzymes in order to supply sufficient industrial proteases.

A number of protease-producing bacteria has been reported, but no study on protease-producing bacteria from soil in Gumel area has been done, though the study area seems to be rich in microbial biodiversity that can be applied for the production of industrial enzymes including protease.

2. Literature Review

Enzymes are a specific protein produced in an organism which is capable in catalyzing a particular chemical reaction (Singh *et al*., 2015). Microorganisms are known to play an important role in technology for the production of intracellular enzymes on an industrial scale (Pant *et al*., 2015). Protease is one of the most significant group of enzyme which conducts proteolysis by hydrolysis of the peptide bonds that link amino acid together in the polypeptide chain (Luang-In *et al*., 2019). Proteases have

extensive industrial applications as it account for two thirds of the total enzymes consume in many industries; and are regularly used in detergent, food, pharmaceuticals, leather and photographic industries (Nayab *et al*., 2015). Proteases are largely classified as endo or exoenzymes based on their site of action on protein substrates. They are further classified as serine protease, aspartic proteases, cysteine proteases, or metalloproteases based on their catalytic mechanism (Geethanjali and Subash, 2011).

However, Microbial proteases are preferred to enzymes from plant and animal sources, since they have almost all the characteristics desired for biotechnological applications (Sepahy and Jabalameli, 2011). Microbes function as a preferred source of these enzymes because of their rapid growth, the limited space required for their cultivation and the ease with which they can be hereditarily used to produce new enzymes with different properties that are required for their numerous applications (Josephine *et al*., 2012).

A wide variety of bacterial species produces protease enzyme, and the application of the same enzyme has been used exactly and manipulated in various biotechnological areas including industrial and environmental sectors (Masi *et al*., 2021). Bacillus species are a significant source of antibiotics, enzymes, insecticides and vitamins (Dave *et al*., 2015), and are possibly the most significant bacterial source of proteases and is capable of producing high yields of neutral and alkaline proteolytic enzymes with incredible properties, such as high stability towards extreme temperatures, pH, organic solvents, detergents and oxidizing compounds (Sepahy and Jabalameli, 2011; Contesini *et al*., 2018). Bacillus species are good-looking industrial organisms because of their high growth rates leading to short fermentation cycle times, capacity to secrete proteins into the extracellular medium and their GRAS (generally regarded as safe) status with the Food and Drug Administration for species, such as *Bacillus subtilis* and *Bacillus licheniformis* (Alcaraz *et al*., 2010). Currently there is full information about the biochemistry, genetics and physiology of the bacteria Bacillus, which could enable further development and better exploitation of these indigenous Bacillus for protease production by industries in Nigeria (Suberu *et al*., 2019).

However, several species of strains bacteria (*Bacillus licheniformis*, *B. firmus, B. alcalophilus, B. amyloliquefaciens, B. proteolyticus, B. subtilis, B. thuringiensis B. cereus, B. sterothermophilus, B. mojavensis and B. megaterium*) are reported to produce proteases (Patil and Jadhav, 2017).

The main aim of our study was to identify protease producing bacteria from soil in Gumel Metropolis, Jigawa State of Northern Nigeria.

3. Materials and Methods

31 Study area

The current study was carried out on the soil samples of Gumel Metropolis, Jigawa state of northern Nigeria. Jigawa State is one of the warmest region in Nigeria with an average daily high temperature of 36 degrees centigrade. It is yearlong warm or hot.

3.2 Samples collection

Total 12 different soil samples were collected from the different sites of Gumel Metropolis (Agricultural fields, gardens, hills and slaughter places) with aid of sterile spatula from 4-5 cm depth in to sterile plastic bags. Soil samples were air dried at room temperature prior to the samples processing.

3.3 Cultivation of Bacteria

One gram soil of each sample was serially diluted by using sterilized distilled water, and 100µl diluted soil sample were spread on nutrient agar plates. The inoculated plates were incubated at 37ºC for 24 hours to determine different colonies of bacteria.

3.4 Purification of the isolates

The isolated bacterial colonies were purified by subsequent sub-culturing on nutrient agar by following streak plate method at 37°C for 24 hours. The pure culture of the isolates was confirmed through microscopic examination.

3.5 Screening of the protease producers

Protease producers were identified by streaking the pure culture of the isolates on skimmed milk agar plate and incubated at 37oC for 24 hours. The Formation of clear zone around the bacterial colonies indicated the proteolytic activities of bacteria resulting from milk protein hydrolysis.

3.6 Identification of protease producers

Bacterial colonies showing protease activity on skimmed milk agar plate were further characterized using cultural characteristics (colony color, shape, and transparency), microscopic examinations (Gram staining and spore staining) and some biochemical characteristics (catalase, oxidase and starch hydrolysis test).

4. Results and Discussion

A total of 12 soil samples from different sites of Gumel Metropolis (Agricultural fields, gardens, hills and slaughter places) were screened for protease producing bacteria. The soil samples recorded two different consistency (hard and sandy). Colour of the soil were also brownish and red. The pH and temperature of the collected various soil samples showed no significant difference as ranged from 7.44-7.86 and 26-29^{o}C respectively (Table. 1).

Isolation of enzyme producers from the collected soil samples was first conducted using nutrient agar media and further subjected for protease production screening on skim milk agar plates. Formation of clear zones around the bacterial colonies served as indication of protease producing bacteria. In 12 soil samples collected, various types of bacterial colonies (20) with different culture characteristics were isolated. However, out of 20 colonies isolated 06 isolates indicated zone around the colonies. Those isolates which clearly showed zone of enzyme production were subjected for culturing, microscopic examinations (Gram staining and spore staining), and some biochemical tests (Catalase, oxidase and starch hydrolysis) to record their culture, microscopic and some biochemical characteristics respectively. The results of morphological and biochemical tests carried out are shown in Table 2 below.

Furthermore, out of 06 isolates showed clear zone of protease production 04 were Gram positive rods. All these 04 isolates were negative for catalase and oxidase test but positive for starch hydrolysis test. The isolates revealed spore formation (Table 2). The 02 isolates were Gram negative rods and non-spore forming bacteria. These isolates were positive for catalase and oxidase tests while negative for starch hydrolysis (Table. 2).

Table 1: Characteristics of Soil samples collected from different sites of Gumel metropolis.

S/N	Sample Site	Consistency	Colour	Temperature	pH
1	Agricultural Field (A)	Hard	Brownish	29^0C	7.44
2	Garden (A)	Sandy	Brownish	26^0C	7.55
3	Slaughter Place (A)	Sandy	Gray	29^0C	7.85
4	Hill (A)	Hard	Brownish	28^0C	7.75
5	Agricultural Field (B)	Hard	Brownish	29^0C	7.45
6	Garden (B)	Sandy	Brownish	26^0C	7.50
7	Slaughter Place (B)	Sandy	Gray	29^0C	7.86
8	Hill (B)	Hard	Brownish	28^0C	7 .74
9	Agricultural Field (C)	Hard	Brownish	29^0C	7.44
10	Garden (C)	Sandy	Brownish	27^0C	7.54
11	Slaughter Place (C)	Sandy	Gray	28^0C	7.85
12	Hill (C)	Hard	Brownish	28^0C	7.75

Table 2: Morphology and Biochemical Characteristics of Protease Producing Bacteria isolates from different soil samples of Gumel Metropolis.

S/N	Isolate	Morphology	Microscopy (Gram Staining)	Biochemical Test			
				Spore staining	Catalase	Oxidase	Starch hydrolysis
1	Garden (B1)	Green, rough & opaque	Gram -ve rods	-ve	+ve	+ve	-ve
2	Garden (C3)	Green, smooth & opaque	Gram -ve rods	-ve	+ve	+ve	-ve
3	Garden (C3)	Yellow & irregular	Gram +ve rods	+ve	-ve	-ve	+ve
4	Hill (C1)	Yellow & irregular	Gram +ve rods	+ve	-ve	-ve	+ve
5	Slaughter Place (B2)	Yellow, irregular & opaque	Gram +ve rods	+ve	-ve	-ve	+ve
6	Slaughter Place (C3)	Yellow, circular & opaque	Gram +ve rods	+ve	-ve	-ve	+ve

Different research works have been performed for screening novel protease producing bacteria. A previous study done by Rani *el al*., (2010) revealed significant number of protease producing bacteria including *Bacillus licheniformis*. Patil and Jadhav (2017) isolated numerous protease producing bacteria and revealed that among the 28 *Bacillus species* 3 isolates (*Bacillus cereus, Bacillus licheniformis and Bacillus megaterium)* showed significant protease production after 24hours. According to their study, *Bacillus cereus* showed significant protease enzyme production after 72h followed by *Bacillus licheniformis* and *Bacillus megaterium* which was 35mm, 26mm and 18mm respectively. Another study carried on Isolation of alkaline protease producing bacteria from leather industry effluent revealed *Bacillus cereus* as best protease activity exhibiting bacteria (Masi *et al.,* 2021). However, study done by Luang-In *et al*., (2019) stated that *Bacillus thuringiensis* was found to exhibit the highest protease enzyme production of 3.72 ± 0.08 U/mg protein at the optimal conditions of 65°C and pH 8.0 after 30 min incubation with 1% casein in 0.05 M PBS buffer. From the results obtained in the present study, 06 out of 20 isolates were found protease producers.

5. Conclusion

From this study, it is therefore concluded that the soil of Gumel Metropolis has protease producing bacteria that exhibit proteolytic activities and biochemical features, though further intense studies are required for more baseline information. These bacteria can be used for protease production and applied locally and nationally in food processing and agriculture.

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Conflicts of Interest: The authors declare no conflict of interest.

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