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(RESEARCH ARTICLE)

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Isolation and characterization of proteolytic bacteria strains from poultry waste-contaminated soil

Obiageli Comfort Makuachukwu * and Uzoamaka Ogechi George-Okafor

Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology, Agbani, P.M.B 01660, Enugu, Nigeria.

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Abstract

Soil contaminated with poultry waste may harbor probiotic bacteria with beneficial traits. This study aims to isolate, characterize, and evaluate proteolytic probiotic bacteria from such soil for potential use in health and biotechnology applications. Soil samples were aseptically collected from five spots at a farmland in Enugu State and serially diluted using sterile normal saline. Isolates were cultured and screened for proteolytic activity using casein agar. Biochemical characterization and Gram staining were performed. Genomic DNA was extracted with ZR Miniprep™ kit, and 16S rRNA and ITS genes were amplified via PCR, visualized with gel electrophoresis, and sequenced. Six bacterial isolates were successfully obtained and identified morphologically and biochemically as members of the *Bacillus* and *LactoBacillus/LactiplantiBacillus* genera. All isolates were Gram-positive rods. *Bacillus* strains displayed dry, rough, opaque colonies, while *LactoBacillus* and *LactiplantiBacillus* formed moist, smooth, round colonies. Biochemical tests revealed that *Bacillus* isolates were catalase-, oxidase-, and citrate-positive, whereas *LactoBacillus* strains were generally catalase- and oxidase-negative. Sugar fermentation tests showed that all isolates fermented glucose, fructose, maltose, lactose, and mannitol, with lactic acid bacteria producing acid and gas, consistent with heterofermentative metabolism. Molecular identification via 16S rRNA confirmed species identity, and gel electrophoresis revealed bands at ~1500 bp. Proteolytic screening demonstrated that *Bacillus subtilis* strain NBT-15 and *LactoBacillus plantarum* strain ML05 exhibited the highest casein hydrolysis zones (28 mm), indicating strong protease production. The presence of robust protease-producing strains from both genera underscores the potential of poultry waste soil as a reservoir for industrially significant microorganisms. These isolates hold promise for applications in probiotic formulation, food fermentation, enzyme production, and biotechnological innovation.

Keyword: Proteolytic bacteria; *Bacillus*; *LactoBacillus*; Poultry waste; Soil microbiota; 16S rRNA; Probiotic; Industrial enzymes; Casein hydrolysis; Biochemical characterization

1. Introduction

Proteolytic enzymes (proteases) from bacteria constitute a major class of industrial biocatalysts, representing the largest segment of the global enzyme market [9][1]. These enzymes are widely used in laundry detergents, food and dairy processing, leather and textile treatment, pharmaceuticals, and other applications [1][9]. The genus *Bacillus* is a particularly prolific source of extracellular proteases: various *Bacillus* species secrete high yields of neutral and alkaline proteases with broad pH and temperature stability [1][3]. *Bacillus*-derived proteases have been exploited in detergent and textile industries, leather dehairing, and even in the generation of bioactive peptides [1]. Lactic acid bacteria (LAB), including many *LactoBacillus* species, also possess potent proteolytic systems: they hydrolyze proteins (e.g., milk casein) during fermentation to supply amino acids, releasing a variety of bioactive peptides with potential nutritional

* Corresponding author: Makuachukwu Comfort Obiageli

and health benefits [4][7]. Many *Bacillus* and *LactoBacillus* strains are generally regarded as safe (GRAS) and are used as probiotics or feed additives to improve nutrient digestion and gut health.

In view of the rising demand for microbial proteases, *Bacillus* and *LactoBacillus* strains were isolated from soil and poultry waste and characterized by standard morphological and biochemical tests. The isolates were screened on casein agar to detect proteolytic activity, and high-activity strains were selected for further study. Such protease-producing strains have potential in food processing, feed or pharmaceutical applications: their enzymes could be used to generate protein hydrolysates and bioactive peptides or added directly to improve protein digestion in industry and health contexts [1][7]. This approach highlights new bacterial candidates whose robust protease production attributes may benefit industrial biotechnology and human/animal health.

2. Materials and methods

2.1. Sample Collection

The sample collection was done in accordance with the work of Curell and Charles [2]. The soil samples were collected from five different spots from a farm land in Enugu State. Random collection of soil samples was carried out under sterile conditions to avoid contamination (using sterile soil probe). The soil surface was dug 3-5cm deep before collecting at different spots and the debris from the soil were removed before the soil sample were collected.

2.2. Sample Preparation

A total of 1g of each soil sample was suspended in 9ml of sterile normal saline in a test tube. The soil sample was serially diluted using ten-fold dilution method to decrease the microbial load of the sample. Ten-fold dilutions of the samples were made with sterile normal saline as diluent [6].

2.3. Isolation of Organisms on Nutrient Agar and MRS agar

A total of 0.1ml of the different dilutions of the different soil samples were inoculated into the petri dishes with nutrient agar and MRS agar media using pour plate method and were allowed to solidify. The plates were incubated at 37°C for 24hrs. After incubation the representative colonies on the plates were sub-cultured into fresh medium. [6].

2.4. Proteolytic screening of the isolates

The isolates were screened for proteolytic activity using casein rich agar medium. Each isolate was inoculated into casein agar using spot plate method and incubated at 37°C for 24hrs. After 24hrs clear zone showing proteolytic activity was observed. The isolates that exhibited proteolytic activity were sub-cultured and characterized [6].

2.5. Characterization and Identification of Bacteria Isolates

2.5.1. Phenotypic identification

The isolates were characterized by Gram staining and biochemical test which include catalase, coagulase, citrate, oxidase, indole, methyl red and sugar fermentation tests.

2.5.2. Genomic identification

The isolates were subjected to genomic identification. The DNA isolation and other procedures were adopted as described by [8]

3. Results

Table 1 Phenotypic identification scheme for the Bacterial Isolates

S/N	Growth Appearance on Media	Gram reaction	Cat test	Oxi test	Cit test	Coa test	Methyl red test	Indole test	Glu	Fru	Mal	Man	Lac	Suspected Organisms
1	Dry, rough, opaque colonies on Nutrient agar	+ve rods	+v	+v	+v	-v	+v	-v	A	A	A	A	A	*Bacillus sp.*
2	Creamy, rough colonies on Nutrient agar	+ve rods	+v	+v	+v	-v	+v	-v	A	A	A	A	A	*Bacillus sp.*
3	Small, round, white colonies on MRS agar	+ve rods	-v	-v	-v	-v	+v	-v	AG	AG	AG	AG	AG	*LactoBacillus sp.*
4	White, raised, moist on MRS agar	+ve rods	-v	-v	-v	-v	+v	-v	AG	AG	AG	AG	AG	*LactoBacillus sp.*
5	White, convex, smooth on MRS agar	+ve rods	-v	-v	-v	-v	+v	-v	AG	AG	AG	AG	AG	*LactiplantiBacillus sp.*
6	White, flat, sticky colonies on MRS agar	+ve rods	-v	-v	-v	-v	+v	-v	AG	AG	AG	AG	AG	*LactiplantiBacillus sp.*

KEY: Cat=Catalase test, Cit= Citrate test, Coa=Coagulase test, Ind=Indole test, Oxi=Oxidase test, Gl=Glucose, F=D-Fructose, Ml=Maltose, Ma=Mannitol, La=Lactose, +=positive, - =negative, A=Acidic, AG=Acidic and Gas, G=Gas, +ve=positive, —ve = negative.

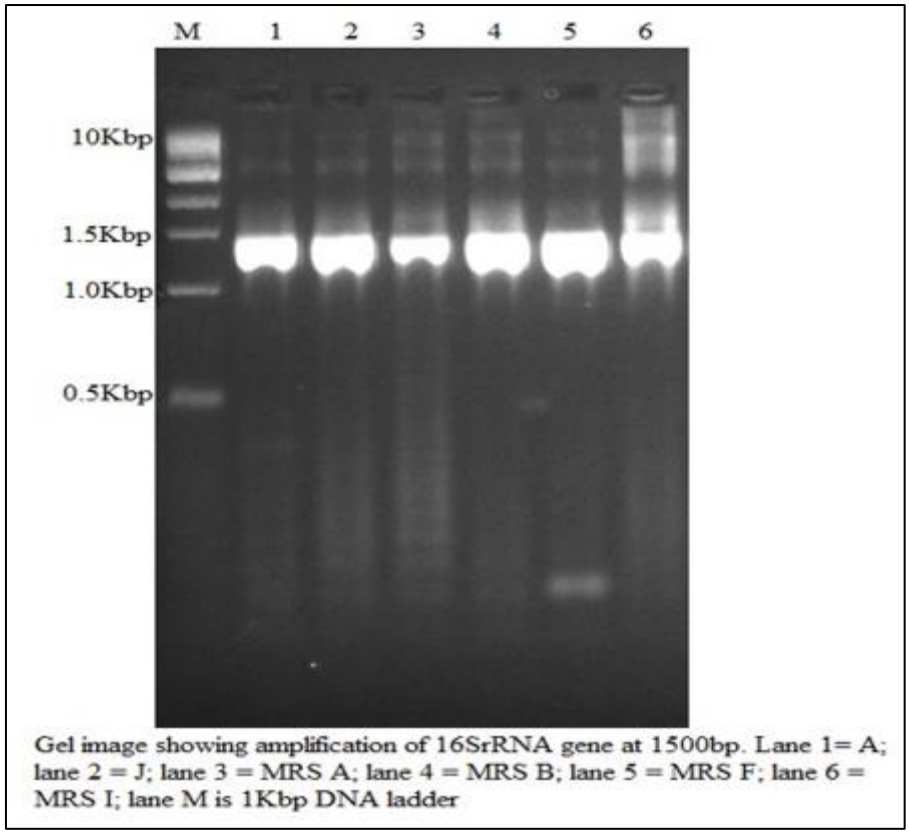

Figure 1 Gel image showing amplification of 16SrRNA gene at 1500bp

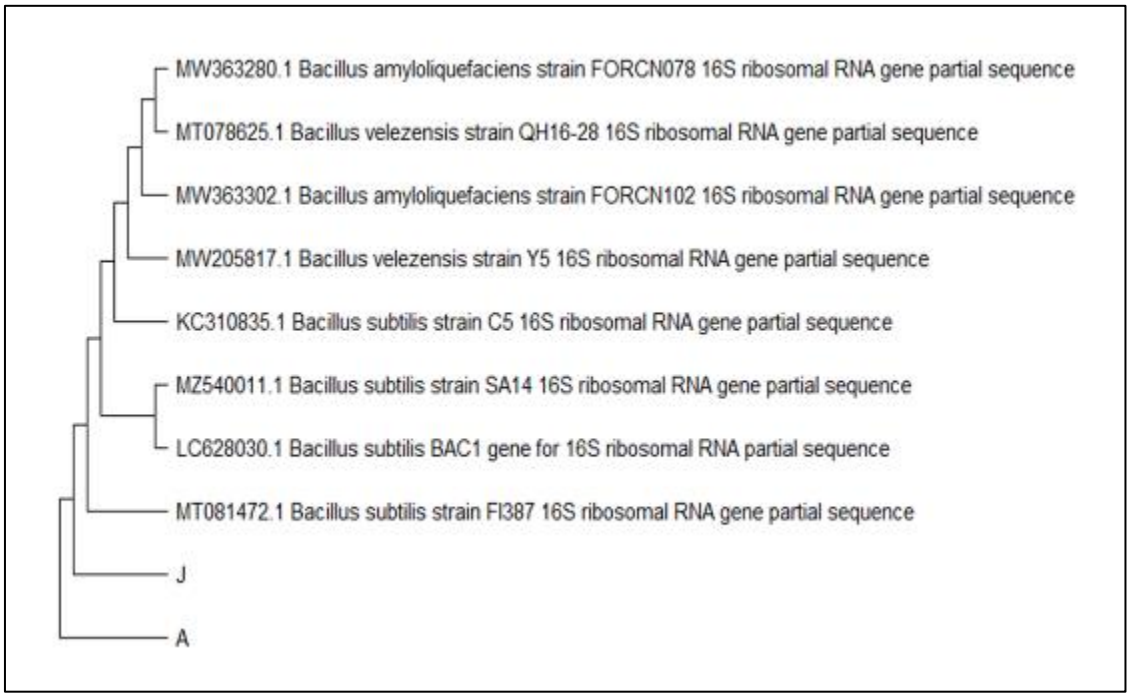

Figure 2 Phylogenetic scheme of bacterial isolates

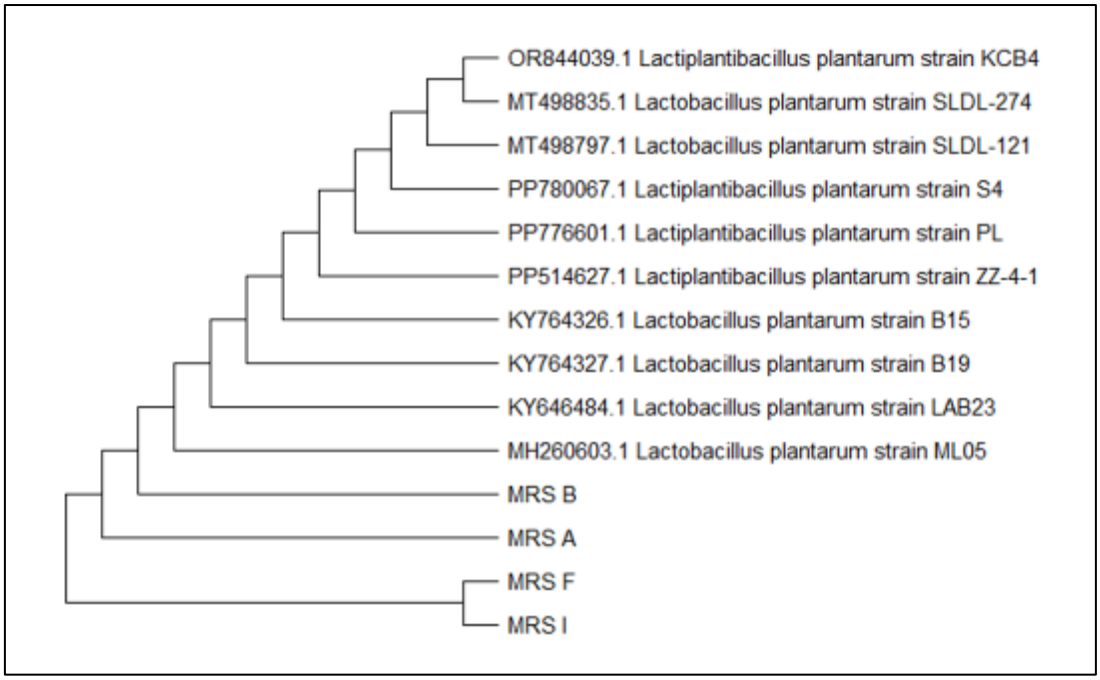

Figure 3 Phylogenetic scheme of bacterial isolates

Table 2 Primary proteolytic screening of the isolated isolates

S/N	Isolate ID	Zone of Hydrolysis (mm)
1	*Bacillus subtilis* strain NBT-15	28
2	*Bacillus amyloliquefaciens* strain FORCN102	26
3	*LactoBacillus plantarum* strain ML05	28
4	*LactoBacillus plantarum* strain B19	26
5	*LactiplantiBacillus plantarum* strain KCB4	26
6	*LactiplantiBacillus plantarum* strain S4	24

4. Discussion

The successful isolation and biochemical characterization of six bacterial isolates from soil and poultry waste revealed the presence of two major genera: *Bacillus* and *LactoBacillus*/*LactiplantiBacillus*. All isolates were Gram-positive rods, consistent with the morphology of these genera. *Bacillus* isolates exhibited dry, rough, opaque, and creamy colony appearances on nutrient agar, while *LactoBacillus* and Lactiplanti*Bacillus* isolates formed round, moist, and smooth colonies on MRS agar. These morphological features align with reports by Danilova and Sharipova [3], who noted that *Bacillus* spp. often display irregular, dry colonies due to their spore-forming capabilities, whereas *LactoBacillus* spp. typically form small, round colonies suited to acidic environments. Biochemical testing showed that *Bacillus* isolates were catalase-, oxidase-, and citrate-positive but coagulase- and indole-negative, while *LactoBacillus* and *LactiplantiBacillus* were generally negative for catalase, oxidase, and citrate utilization. This biochemical behavior is consistent with the metabolic profiles described by Contesini, Melo, and Sato [1], who noted that *Bacillus* species exhibit versatile enzyme systems, contributing to their high protease productivity. In contrast, *LactoBacillus* species rely on fermentation-based metabolism and are typically catalase-negative due to their anaerobic or microaerophilic nature [4]. Sugar fermentation patterns revealed that all isolates fermented glucose, fructose, maltose, mannitol, and lactose, with the *LactoBacillus* and *LactiplantiBacillus* strains producing acid and gas (AG) – a characteristic feature of heterofermentative lactic acid bacteria [7]. These fermentation capabilities are crucial for probiotic functionality, particularly in gastrointestinal environments where carbohydrates are abundant.

The 16S rRNA gene amplification (as visualized by the gel image at 1500 bp) and phylogenetic analysis further confirmed the identity of these isolates, supporting their classification into well-characterized industrially relevant species. Molecular identification using 16S sequencing has become a gold standard for taxonomic placement and is critical for biosafety and regulatory approval in commercial applications [5].

The proteolytic screening further distinguished the isolates based on their enzyme activity. *Bacillus subtilis* strain NBT-15 and *LactoBacillus plantarum* strain ML05 showed the highest zones of hydrolysis (28 mm), indicating strong protease production. These results are significant, as B. *subtilis* is widely recognized for its secretion of alkaline proteases used in detergent and leather industries [9], and *L. plantarum* is increasingly employed in food fermentations and nutraceuticals due to its ability to release bioactive peptides during protein degradation [4]. The presence of robust protease-producing strains from both genera in soil and poultry waste suggests these environments are rich reservoirs for industrially useful microbes. The combination of enzymatic potential and probiotic attributes makes these isolates ideal candidates for applications in fermented foods, probiotic supplements, and bio-catalytic processes in biotechnology [1][9].

5. Conclusion

This study successfully isolated and characterized proteolytic bacterial strains—primarily *Bacillus* and *LactoBacillus*—from poultry waste-contaminated soil. The isolates demonstrated distinct morphological and biochemical traits, confirmed through 16S rRNA gene sequencing. Notably, several strains exhibited strong protease activity, highlighting their potential as industrial enzyme producers. Given their probiotic characteristics and enzymatic capabilities, these isolates present promising candidates for applications in food processing, biotechnology, and health-related industries.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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