

Isolation, Identification and Characterization of Feather Degrading *Raoultella electrica* from Poultry Waste Containing Soil

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Abstract: Poultry industry waste carries a major proportion of feathers, which include particularly Keratin. Keratin is an insoluble, fibrous protein, which makes the feathers proof against proteolytic enzymes. Numerous microbial isolates can carry out the degradation of such complex substrates. This study uses the ability of soil bacteria to degrade feather waste. Among the 112 isolates, 17 of them able to simultaneously hydrolyse skimmed milk and utilize chicken feather as the sole source of carbon and nitrogen. On the basis of enzyme assay, isolate 13 was found potent keratinase producers as compared to other isolates; such was expressed to 14.12U/ml of keratinase. Identification of potential keratinolytic isolate through 16S rRNA analysis revealed that it belongs to *Raoultella electrica*. Due to its potential keratinolytic action, the isolate may serve as a candidate for the management of feather waste and various biotechnological and industrial applications.

Keywords: Keratin, keratinase, protease, *Raoultella electrica*, Feather hydrolysis.

INTRODUCTION

The daily increase in the consumption of meat obtained from chicken has a serious impact on the environment because waste from chickens and poultry (especially feathers) is not properly treated. In nature, feathers degenerate slowly and produce sulfur compounds, which can cause environmental problems. Feather is almost pure keratin, which is produced in large quantities in poultry processing plants and constitutes waste by-products.

Keratin is a major component of hair, feathers and wool and is the most complex of the cytoskeletal intermediate filament proteins of epithelial cells. Keratin is an insoluble protein macromolecule with very high stability and low degradation rate. A number of physical and chemical methods were utilized for the conversion of feather meal a digestible dietary protein for animal feed. These methods destroy certain amino acids and reduce protein quality and digestibility (Manirujjaman *et al.*, 2016).

The degradation of keratin by microorganisms with keratin decomposing activity is another way to improve the nutritional value of feather waste (Williams *et al.* 1990). A group of proteolytic enzymes that is able to hydrolyze insoluble keratins more efficiently than other proteases are called keratinases. Many researchers isolated and characterized keratinases with respect to various bacterial species, for an instant, *Bacillus licheniformis* (Manczinger *et al.*, 2003; *Fervidobacterium islandicum* (Nam *et al.*, 2002); Korkmaz *et al.*, 2004; *Chryseobacterium*, *Pseudomonas aeruginosa* KS1 (Sharma *et al.*, 2010), *Microbacterium* spp. (Riffel and Brandelli, 2007), Actinomycetes such as *Streptomyces* sp (Jani *et al.*, 2014).

The Namakkal district of Tamil Nadu, India is the hub of the poultry industry. Taking into account this fact, most of the poultry industry dumps waste in the area. The current research plan is to explore keratin decomposing bacteria from soil samples in the Namakkal region and degrade feather waste. This study can help prevent the impact on environmental pollution and can also provide alternative economic support for the poultry industry.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from the regular feather dumping site of egg processing farms from Namakkal, TN, India. The samples were taken from 30 cm depth from the surface of the soil. The samples were brought to the laboratory and processed for isolation of microorganisms.

Isolation of bacterial isolates from soil samples

About 1 gm of poultry soil was suspended in 10 ml of saline solution (0.9 % NaCl). A volume of 0.1 ml of suspension is serially diluted and then spread on the nutrient agar plate. The plate was incubated at 37°C for 24 hrs. The colonies grown were screened for keratinase production.

Screening of Protease producing isolates

Skim milk agar was prepared and the above isolates were streaked on milk agar plates for testing the proteolytic activity of the isolates. Isolates were inoculated onto plates and incubated at 37±2°C for 24 h. Isolates that were producing clearing zones in this medium were selected (Sepahy *et al.*, 2011).

Screening of keratinase producing isolates

Amongst the different bacterial colonies obtained on the nutrient agar, each was inoculated onto a sterile feather meal agar plate. The Feather meal media 1 (g/l): NaCl, 0.5; K₂HPO₄, 0.3; KH₂PO₄, 0.4 and Feather, 10; and pH was maintained at 7.5 at 30°C for 7 days, isolates which exhibited the largest clearing zones were selected, identified, and grown in cultivation media for enzyme production. The potential isolates were selected based on the isolates showed a positive result on both screening methods.

Screening of potential isolates

Cultivation of the isolated cultures was performed using a 250 ml Erlenmeyer flask containing 90 ml of above-mentioned media. Isolates that exhibited the maximum enzyme production were selected. The potential isolates were selected based on the isolates showed a positive result on both screening methods.

Enzyme assays

Keratinolytic activity was measured with soluble keratin (0.5%, w/v) as substrate. Soluble keratin was prepared from chicken feathers by the method of (Wawrzekiewicz *et al.*, 1987). After the incubation period, centrifuge the broth and separate the supernatant. The keratinolytic activity was assayed 1.0 ml of crude enzyme properly diluted in phosphate buffer (0.05 M of pH 7.0) was incubated with 1 ml of keratin solution at 50°C in a water bath for 10 min, and the reaction was stopped by adding 2.0 ml 0.4M of TCA. After Centrifugation, the absorbance of the supernatant was determined at 280 nm against a control. The control was prepared by incubating the enzyme solution with 2.0 ml TCA without the addition of keratin solution. One unit (U/ml) of keratinolytic activity was defined as an increase of corrected absorbance of 280 nm (A₂₈₀) (the modified method by Gradisar *et al.*, (2005) with the control for 0.01 per minute under the conditions described above and calculated by the following equation:

$$U=4 \times n \times A_{280} / (0.01 \times T)$$

Where n is the dilution rate; 4 is the final reaction volume (ml); T is the incubation time (min).

Determination of feather degradation

The feather degradation study was carried out according to Son and Park (2008) procedure. After inoculation of each isolate in sterile FMM broth and incubation at 30°C on a shaker for 6 days, visual observation showed that the feathers were degraded. After the incubation period, the residue of the feather was harvested by filtration of feather meal media (FMM). The percentage of feather degradation was calculated from the differences in residual feather dry weight between a control (feather without bacterial inoculation) and a treated sample.

16 s rRNA sequence analysis

The 16s rRNA genes were amplified using PCR with the universal primer FD1 and RP2. The sequence of FD1 and RP2 were 5'- AGAGTTTGATCTGGCTCAG-3' and 5'-AAGGAGGTCATCCAGCC3' respectively. A search of the GenBank Nucleotide library for a sequence similar to those determined was made by using BLAST (Altschul *et al.*, 1990), through National Center for Biotechnology information (NCBI) internet site <http://www.ncbi.nlm.nih.gov/BLAST>. Sequences with more than 98% identity with a Genbank sequence were considered to be of the same species as the highest score matching sequence on the public sequence databases.

RESULT AND DISCUSSION

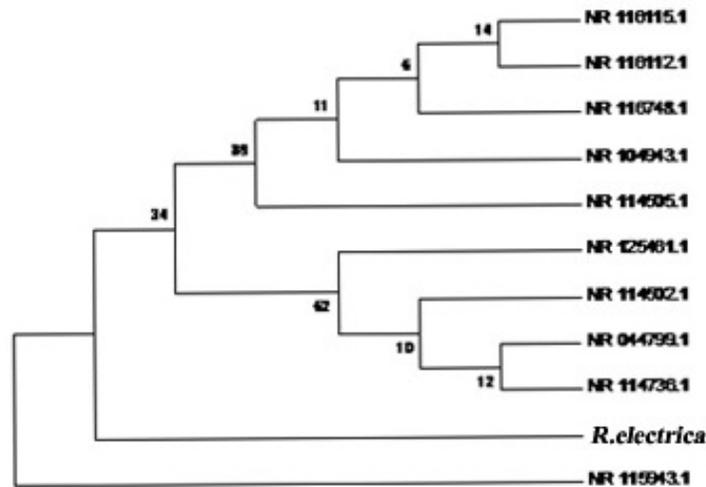
Green technology promotes environmental sustainability and greatly stimulated the continuity of finding microorganisms with industrial and biotechnology prospects from various ecological niches. Therefore, a series of bacterial strains were isolated from the waste dump using chicken feathers as the only carbon and nitrogen source of the basal medium. Bacteria with remarkable keratinolytic activity are recorded from soil samples with keratinous waste deposition (Nnolim *et al.*, 2020; Barman *et al.*, 2017).

A total of 112 morphologically well-formed single colonies were selected from 21 different poultry waste containing soil samples on the basis of their morphological difference in nutrient agar plates. Among the tested bacterial isolates, 26 were found as protease producers based on their clear zones formation on skim milk agar. The preliminary screening of the protein hydrolysis in skimmed milk is an important step to identify the protease producing isolates (Nnolim *et al.*, 2020).

Subsequently, only 17 out of the 112 bacterial isolates were able to simultaneously hydrolyse skimmed milk and utilize chicken feathers as the sole source of carbon and nitrogen. On the basis of enzyme assay, isolate 13 was found potent keratinase producers as compared to other isolates; such was expressed to 14.12U/ml of keratinase.

The selected keratinolytic bacterium was identified based on the 16S rRNA sequence. Sequence analysis revealed a DNA fragment about 1500 bp confirming the 16S rRNA genes. The 16S rRNA sequence was analyzed with the blast program in NCBI and was identified as *Raoultella electrica* strain with about 99% homology to available sequences. Also phylogenetic analysis of 16S rRNA gene confirmed it (Fig. 1).

Fig.1 Phylogenetic tree analysis



Diverse groups of microorganisms are reported to produce keratinase, among bacteria, particularly *Bacillus subtilis* (Grohs Ferrareze, *et al.*, 2016). Moreover, actinomycetes from the Streptomyces genus are known to produce keratinases (Allure, 2014). The degradation of feathers by *Raoultella electrica* in FMM shows that it can use feathers as the only carbon source. Based on the residual feather dry weight it was concluded that *Raoultella electrica* had the best feather degradation potential. It was observed that only 58 % of initial feather weight remained after 6 days of cultivation. Degradation begins with the adhesion of bacteria to feathers. It was found that the creature first penetrated the feathers of the barbs, and the barbs were broken down into a powdery gel-like substance. Then spread to the shaft area, causing it to rupture. The shaft broke down into a needle-like structure before the sixth day. A similar observation was made in a previous study of Lakshmi *et al.*, 2013.

The conclusion of the present study, *Raoultella electrica* identified via 16S rRNA gene sequence showed excellent keratinase production potential, which is suitable for the bacterial degradation of keratin waste. Further studies on the media optimization and purification of the keratinolytic enzyme(s) will be needed to eventually improve the keratin-degrading capacity of *Raoultella electrica*.

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