By

ZEINAB ELNAYED HAMADA ELWANY
B. Sc. Agric. Sci. (Animal Production Program), Fac. Agric., Fayoum Univ., Egypt, 2016

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5. SUMMARY AND RECOMMENDATION

Every year, millions of tons of feathers are produced as a waste product by chicken processing all over the world. Accumulation of this feather constitute a sizable waste disposal problem and will lead to environmental pollution. Degradation of the feather is the promising solution to outcome these problems. Furthermore, it is economically important since chicken feather consists of approximately 90% keratin which can be converted to valuable byproducts. Biodegradation of keratin requires keratinase enzyme which can be produced by specific bacteria as an extracellular product. The present study was carried out in Genetics Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt, during the period from 2017 to 2021 aiming to:- (1) Isolation of keratinolytic bacteria from chicken feather and soil at Fayoum region. (2) Selection of bacterial isolates that have high efficiency to degrade feathers. (3) Characterization and identification of the bacterial isolates using morphological and biochemical testes. (4) Molecular identification of isolates using 16S rDNA. (5) Molecular characterization of strains using, RAPD-PCR. (6) Amplification of the keratinase gene from bacteria strains.

Results of the present study are summarized as follows:-

1. Thirty two bacterial isolates were obtained from feather waste and soil, and their keratinolytic activity was examined. FAK13, FAK25, FAK18, FAK16, and FAK27 were the best isolates, with distinct hydrolysis zones of 28.7mm, 27.5mm, 27.2mm, 27.1mm, and 25.4mm, respectively, with feather degrade activity rate FAK25 giving 5Units/ml followed by isolates FAK13, FAK16, FAK27 and FAK18, giving 3.16, 2.34, 2.1 and 2 U/ml respectively.
2. Morphological tests revealed that all isolates were long rods, gram-positive, motile and spore formers.

3. Biochemical tests proved that all isolates were positive for producing caseinase, catalase and gelatinase enzymes. Also, all isolates except FAK18 produced amylase. As well, all the isolates fermented glucose, lactose, and fructose. However, FAK25 was the only isolate which could not ferment mannitol. Similarly, all the isolates did not hydrolyze fat except FAK25.
4. The five isolates were identified based on their molecular characterization in comparison with other strains. Phylogenetic tree was constructed based on the nucleotide sequences of 16S rDNA gene of the strains. Isolates FAK13, FAK16, FAK18, FAK25 and FAK27 were identified as *Bacillus thuringiensis*, *Bacillus Velezensis*, *Bacillus Megaterium*, *Bacillus Subtilis* and *Bacillus licheniformis* respectively. They were registered at GenBank with accession numbers MK788291, MK788292, MK788293, MK788294 and MK788295 respectively.
5. Genomic DNA of the five strains was extracted and were analyzed for their RAPD profiles using eight random primers. All the primers generated different and distinct profiles specific to their species origin and relationships among strains. Fifty eight polymorphic and monomorphic bands were generated. The dendrogram showed that strains FAK13 and FAK18 were closely related in one linage, while strains FAK27 and FAK16 were highly distant.
6. Keratinase-specific primer pairs KerNma and KerAwt were used to detect the keratinase gene. Amplification with KerNma revealed that strain FAK27 has a Ker gene with a fragment length of 1190 bp, while amplification with KerAwt revealed that strain FAK25 has a fragment length of 1050 bp.

Recommendations

1. Biodegradation is likely to be applied for efficient and economic recycling of chicken feathers.
2. For this purpose the following five native *Bacillus* isolates (*Bacillus thuringiensis, Bacillus Velezensis, Bacillus Megaterium, Bacillus Subtilis and Bacillus licheniformis*) are highly recommended since they have high keratinase activities.
3. Keratinase enzymes are genetically distinct from one another since they are coded for by many genes.
4. Two keratinase genes were isolated using two pairs of primers, and it is recommended in the future to transfer these genes to other species to improve enzyme production and to benefit from them in practice.