Eco-friendly and Modern Method of Poultry Waste Recycling

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Abstract. Throughout the world, along with the benefits that poultry farming brings to the economy of each individual country, there is a large-scale problem of such byproduct of its activity as feathers. In addition, 91\% of them consist of a very valuable protein source with $\beta$-keratin. Keratin itself is very poorly decomposed and further disposed component. It makes up one of the components of feathers, the part of vertebrate skin.

There is a growing preference for cost-effective and ecologically friendly poultry waste management ways. And it is the keratinase enzyme, obtained as a result of the bacteria activities, that shows great potential in this area. The selection, identification and improvement of the activities of such species of bacteria which could decompose the chicken feathers is the subject of ongoing research. About 89\% provided chicken feathers were disposed (in other words, utilized) by bacteria in 2 days at temperature of 37° C. Full disposal took place after 53 hours. This study provides new results on the activities of microorganisms in the sphere of feather composting, and could also serve as an instruction for improving the work of the poultry industry in the management of keratin waste.

1. Introduction

In addition to the positive changes that agriculture and livestock, including poultry, bring, there is a by-product that has a negative impact on the environment and needs to be addressed [1, 5].

We are talking about feathers of birds, whose volume increases every day [2]. 8.5 million tons - such an annual volume of production of only chicken feathers [1]. 125 g of feathers – from 1 bird [2, 10].

Feather consists of protein, namely $\beta$-keratin, which is mechanically strong and does not go into chemical reactions with protein [3] with custein, glutamine, proine as dominant amino acids in its structure. Elements, such as keratin, which belong to proteolytic enzymes, cannot be dissolved in water. They have high physicochemical resistance [13].

Recycling or decomposing by special microorganisms has a significant advantage, since it is a biotechnological method with lower cost, simple application conditions, favorable influence on the environment and also makes it possible to obtain useful raw materials. Bacterial keratinolytic protease are extracellular enzymes that are used for the biological utilization of keratin. Keratinolytic proteases - keratinases (EC 3.4.21/24/99.11). Bacterial keratinolytic proteases are mainly serine proteinases, less commonly metalloproteinases. Keratinase acts on peptide bonds of keratin, destroying them [4, 12].
Enzymes hydrolyze disulfide (-S-S-) and peptide bonds of keratin substrates and are widely produced mainly by some microbes: bacteria, archaea and fungi. Chicken feather, hair, nails, wool, etc. are destroyed by microorganisms, producing keratinase. Now more than 30 species of microorganisms, including fungi [3-5], actinomycetes [6-8] and bacteria [5, 9-11], show keratinolytic properties.

But the most active group in this sphere of keratinase synthesis is bacteria of the genus Bacillus: in particular B. subtilis, B. pumilus, B. cereus, B. coagulans, B. licheniformis or B. megatherium. Decomposing of keratin proteins also happen in other Gram-positive bacteria Lysobacter, Nesternokia, Kocuria, Microbacterium, and some Gram-negative bacteria, e.g. Vibrio, Xanthomonas, Stenotrophomonas and Chryseobacterium [7, 12].

In order to get the feed products from keratin products re-cycling it is used to implement mechanical, hydrothermal and thermochemical processes.

According to the results of this study, we will identify and investigate 4 strains of bacteria. It is they which have the ability to dispose of chicken features. Another of our tasks is to improve the processes of recycling proteins and amino acids with the help of microorganisms in order to achieve the optimal effect.

2. Materials and methods

2.1. Isolation
Keratinolytic strains isolated from soil and keratinous waste, respectively, were used for the present study. Isolation of bacterial strains was performed with method: feathers were washed thoroughly with detergent followed by ultrapure water 3 times. The obtained suspensions were inoculated onto meat extract agar and incubated for 72 h at 35 °C to allow the colonies to grow. The well isolated colonies were marked and colony characters and morphological characters were noted at the interval of 24 h.

2.2. Identification and molecular phylogenetic studies
16S typing rDNA was carried out for characterization of the isolates to the species level and phylogenetic analysis. Based on the 16S rDNA typing and phylogenetic analysis, the strains were designated and deposited as Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis and Streptomyces albidoflavus.

2.3. Feathers degradation by a selected bacterial isolate
Feathers were completely degraded by Bacillus licheniformis, Bacillus pumilus, Bacillus subtilis, Streptomyces albidoflavus separately from each other. Feathers with each strain were incubated for 48 h at 37°C. The feather residue was washed with distilled water and dried to a constant weight at 70°C, at which point it was weighed to determine weight loss.

Keratinolytic activity was determined in 20 minutes reaction on soluble keratin preparation, according to Gradisar [8].

Biomass concentrations were measured after 12 and 24 hours of cultivation using the Glomax Multi multi-mode reader (Promega, USA) according to protocols.

Protein was determined according to the Barnstein method.

The degree of hydrolysis of keratin was determined as the ratio of amino nitrogen to the total.

3. Results and discussion
As a result of the isolation procedure, a total number of 4 isolates of keratinolytic bacteria were obtained from 10 original samples. The choice of strains stemmed from the availability of components included in nutrient growth medium, high keratinolytic activity, shorter periods of cultivation and the duration of biosynthesis of enzymes. Due to the ability of keratin degradation, all tested bacterial strains were capable of growth in the medium containing chicken feathers as a main source of carbon.

Figure 1 shows phylogenetic analysis one of the strains - Bacillus pumilus.
Phylogenetic analysis of 16S rRNA gene sequence of Bacillus isolates was provided by State Research Institute of Genetics and Selection of Industrial Microorganisms (GosNIIGenetika institute, Moscow). The strain was designated and deposited as *Bacillus pumilus* AL-16. Other strains were designated and deposited like *Bacillus licheniformis* 34, *Bacillus subtilis* S7, *Streptomyces albidoflavus* B45.

Figure 1. Phylogenetic analysis of 16S rRNA gene sequence of Bacillus isolates.

Important properties of keratinolytic microorganisms are the following: keratinolytic activity, biomass concentration, degree of hydrolysis of keratin, protein content (table 1).

**Table 1.** Properties of keratinolytic strains.

| Strain                  | Keratinolytic activity (U/mg) | Biomass concentrations (CFU/g·dm³) | Degree of hydrolysis of keratin (%) | Protein (%) |
|-------------------------|-------------------------------|-----------------------------------|-------------------------------------|-------------|
| *Bacillus licheniformis*| 36.0±1.8                      | 370.4±22.1                        | 80.1±1.5                            | 69.4±4.2    |
| *Bacillus. pumilus*     | 37.7±1.7                      | 451.3±25.6                        | 79.4±0.9                            | 76.5±3.4    |
| *Bacillus subtilis*     | 9.7±0.2                       | 112.0±6.5                         | 41.1±1.6                            | 23.1±1.2    |
| *Streptomyces albidoflavus* | 22.9±1.9                     | 232.0±14.7                        | 78.6±1.6                            | 65.1±4.8    |

These strains have a high level of keratinolytic activity – an average of 30 E/mg of protein. This is more than three times higher than other strains. Strains *Bacillus pumilus* AL-16, *Bacillus licheniformis* 34, *Bacillus subtilis* S7, *Streptomyces albidoflavus* B45 are characterized by high rates of protein accumulation in biomass (from 65.19 to 76.50 %) and the degree of protein hydrolysis (from 78.32 to 79.44 %).

Figure 2 shows hydrolysis of chicken feather by strains *Bacillus pumilus* AL-16, *Bacillus licheniformis* 34, *Bacillus subtilis* S7, *Streptomyces albidoflavus* B45.

Figure 2. Keratin degrading by keratinolytic strains: *Bacillus pumilus* AL-16, *Bacillus licheniformis* 34, *Bacillus subtilis* S7, *Streptomyces albidoflavus* B45 in the ratio of 1:1:1:1.

a – immediately after adding strains; b – after 24 h; c – after 48 h.
In scientific terms, according to the data obtained from Kornillowicz-Kowalska’s research [7], microbial keratinolytic abilities in the sphere of reducing the keratin substrate volumes proved to be the best. About 89% provided chicken feathers were disposed (in other words, utilized) by bacteria in 2 days at temperature of 37 °C. Full disposal took place after another 5 hours.

4. Conclusion
The problem of industrial waste disposal is the most acute in modern society. And the presence of a large number of chicken feathers as by-product of agricultural activity became obvious. Thus, the above-mentioned studies have shown - it is the effective use of the keratinolytic abilities of bacteria which could significantly facilitate the solution of this issue of recycling large volumes. And what is very important is not only in a single economy, but also in the entire economy of the whole country.

In the research, it was Bacillus pumilus AL-16, Bacillus licheniformis 34, Bacillus subtilis S7, Streptomyces albidoflavus B45 that showed the best keratinolytic abilities and activity. These microorganisms demonstrated and were able to produce gigantic volumes of keratinolytic enzymes. It made possible for these bacteria to reproduce and as a result to utilize chicken feathers, despite the fact that this product is quite difficult to decompose.

It will be reasonable to use this experience and the abilities of Bacillus pumilus AL-16, Bacillus licheniformis 34, Bacillus subtilis S7, Streptomyces albidoflavus B45 in the development and modernization of biotechnological spheres.

The disposal of keratin waste from the poultry industry through the keratinolytic properties of these microorganisms could also be used in other areas, requiring high energy consumption. And it also provides the industry with the necessary raw materials for valuable food additives. Testing these results in production will be our next priority phase of the research.

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