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THE NUMBER OF KERATINOLYTIC MICROORGANISMS IN FEATHERS AFTER SLAUGHTER POULTRY

LICZEBNOŚĆ MIKROORGANIZMÓW KERATYNOLITYCZNYCH W PIÓRACH PO UBOJU DROBIU

Abstract: Due to demand, the industry is rapidly growing, and poultry farms and slaughterhouses increase in operation size. All kind of waste, also feather waste, must be properly managed and disposed of. However, this is not a simple process, since feathers contain keratin which is resistant to biodegradation. Therefore, the obtaining microorganisms capable of degrading this protein is indicated. The number of microorganisms in research samples has importance in the isolation of strains which are characterized by the desired properties such as high enzymatic activity. The aim of the research was to determine the colony size of microorganisms showing high keratinolytic activity to degrade chicken, duck, goose and turkey feather waste. Samples of bacteria were harvested at various times in the period from March 2015 to January 2016 in poultry slaughterhouses located in the Lubuskie and West-Pomeranian provinces. Microorganisms were grown in Mandel's and Omelianski's mineral media, with addition of keratin. The presence of keratinolytic microorganisms was confirmed in all feather samples, though colony sizes varied. Turkey feather waste was the most populated by keratinolytic microorganisms ($5.8 \cdot 10^8$ CFU·g^{-1} d.m.), followed by duck feather ($4.7 \cdot 10^8$ CFU·g^{-1} d.m.). The colony size was smaller in case of goose feather ($2.8 \cdot 10^7$ CFU·g^{-1} d.m.), and definitely the smallest for chicken feather. Since the Mandel's medium was more favourable for the cultivation of keratinolytic microorganisms, larger colonies were isolated from this substrate than from the Omelianski's medium. What is noteworthy is that only in the case of chicken feather waste, the size of keratinolytic microorganism colonies grown on both substrates was similar, and did not exceed 10^6 CFU·g^{-1} d.m. Contrary to what was expected, in the research samples bigger population of the said microorganisms was identified in the autumn and winter season.

Keywords: feathers, keratin, microorganisms, poultry industry

Introduction

More and more dynamically developing poultry industry is struggling with problems of a significant waste amount formation. Poultry facilities are facing a big challenge of a proper management of this material type, including keratin protein [1]. This is primarily due to the large technological difficulties resulting from the keratin structure, that is a part of this waste type, along with large financial outlays [2]. A major problem is the slow and difficult biodegradation of feathers resulting from their resistance to proteolytic enzymes and water insolubility [3-5]. Feathers are populated by microorganisms among which those able to degrade keratin, are found [6-9]. This is possible due to the production of appropriate proteolytic enzymes [10, 11].

Therefore, it is necessary to recognize the nature of microflora inhabiting feathers to select and isolate the active strains of microorganisms that may form the basis of a vaccine that would allow management of the waste containing protein material. Such

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bio-preparation would be useful to optimize that type of a substrate used in methane fermentation processes in the bio-gas plant or composting facility [12, 13].

The aim of the study was to determine the number of microorganisms capable of degrading the keratin in different types of feathers from poultry slaughterhouse in Poland.

Materials and methods

The study was conducted using chicken, duck, turkey, and goose feathers taken from different poultry processing plants in the Lubuskie and West-Pomeranian provinces. Chicken feathers were collected 4 times (I - 5 March 2015, II - 21 May 2015, III - 25 June 2015, IV - 14 January 2016), duck's 3 times (I - 11 May 2015, II - 20 June 2015, III - 23 November 2015), turkey's 2 times (I - 11 May 2015, II - 23 November 2015), and goose's once (20 June 2015). Microbiological assays were performed by means of plating dilutions. To isolate the keratinolytic microorganisms with different requirements in relation to the culture medium, two modified mineral substrates were applied Mandel's (MAN) and Omelianski's (OM) [14, 15], to which powdered keratin was added. The cultures of microorganisms were performed at 23-25°C for a period of 7-14 days. Assays were carried out in three replicates. Results were converted and reported in colony forming units (CFU) per one gram of dry mass.

Statistical analysis was performed using Statistica 12 software. The t-test was used for the comparison of the mean keratinolytic microorganisms number on the Mandel's and Omelianski's substrate for any kind of research material.

Results and discussions

Analysis of the results on microorganisms capable of degrading keratin and inhabiting the protein waste in the form of feathers, revealed variable number in the test feathers grown on two different mineral substrates (Fig. 1).

The highest mean number of keratinolytic microorganisms was found in turkey feathers ($5.8 \cdot 10^8$ CFU$\cdot g^{-1}$ d.m.). Predominating group of microorganisms appeared to be those grown on Mandel's substrate ($7.5 \cdot 10^8$ CFU$\cdot g^{-1}$ d.m.), that were more numerous by 46% than those grown on Omelianski's substrate. In autumn, population of microorganisms was the highest and on both substrates amounted to $8.6 \cdot 10^8$ CFU$\cdot g^{-1}$ d.m.

Smaller numbers of keratinolytic microorganisms inhabited duck feathers. The average number of microorganisms on the Mandel's substrate was at the level of $6 \cdot 10^8$ CFU$\cdot g^{-1}$, while on Omelianski's substrate, they made up by over 40% less. Like for turkey feathers, in this case of both substrates, the most numerous keratinolytic microorganisms were found in the autumn measurement ($1.0 \cdot 10^9$ CFU$\cdot g^{-1}$ d.m.).

Smaller numbers of microorganisms capable of degrading the structural protein were recorded in goose feathers, in which the average population was $2.8 \cdot 10^7$ CFU$\cdot g^{-1}$ d.m. More microorganisms were observed on Mandel's substrate (by 54%) than on Omelianski's substrate ($1.8 \cdot 10^7$ CFU$\cdot g^{-1}$ d.m.) (data were not presented on figures).

The lowest keratynolytic microorganisms populations were observed in chicken feathers. In this case, the number of these microorganisms dominated in winter ($2.4 \cdot 10^6$ CFU$\cdot g^{-1}$ d.m.). For all measurement dates, average number of basic microorganism group, both on Mandel's and Omielianski's substrate, was at similar levels of $1.0 \cdot 10^6$ CFU$\cdot g^{-1}$ d.m.

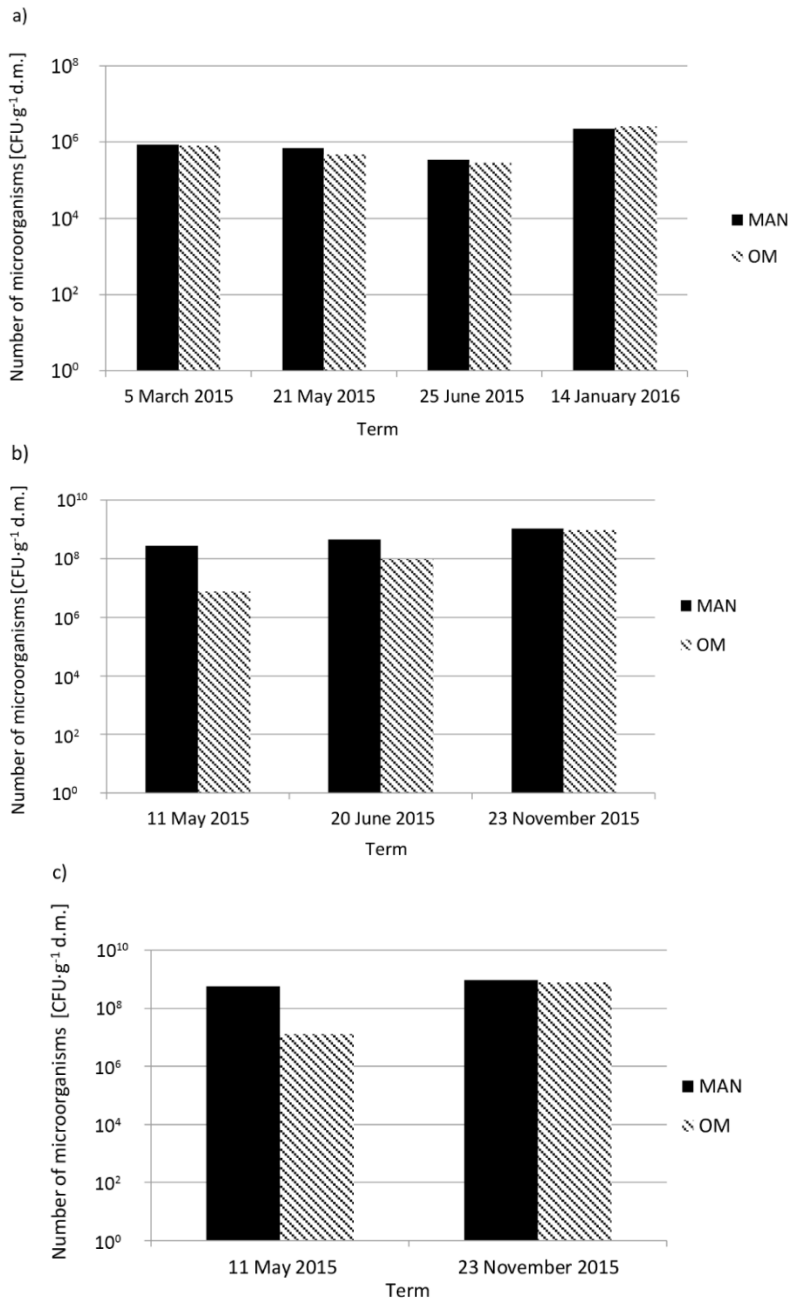
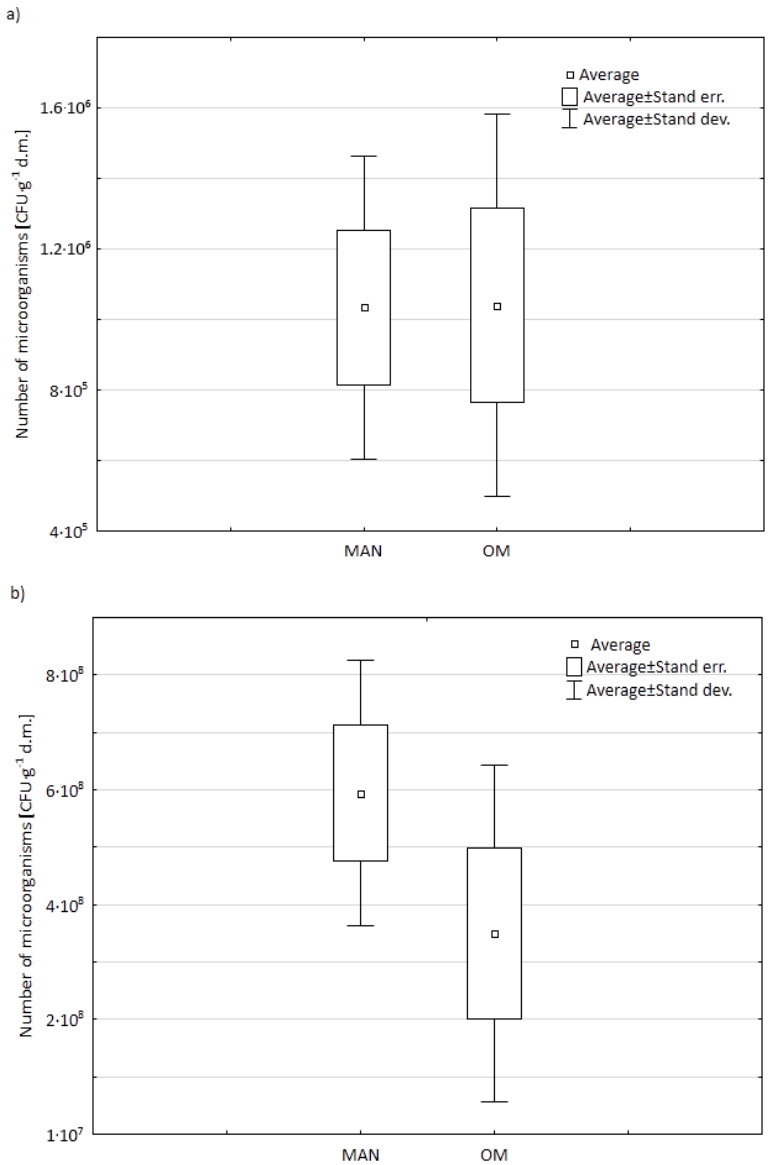


Fig. 1. The number of keratinolytic microorganisms in analyzed term: a) chicken feathers, b) duck feathers, c) turkey feathers, MAN - Mandel's medium, OM - Omelianski's medium

In the analyzed samples, keratinolytic microorganisms most frequently colonized turkey feathers, by 18% less duck feather, and by 94% goose feathers. A small percentage of the population of keratin-decomposing microorganisms were found in chicken feathers (Fig. 2).



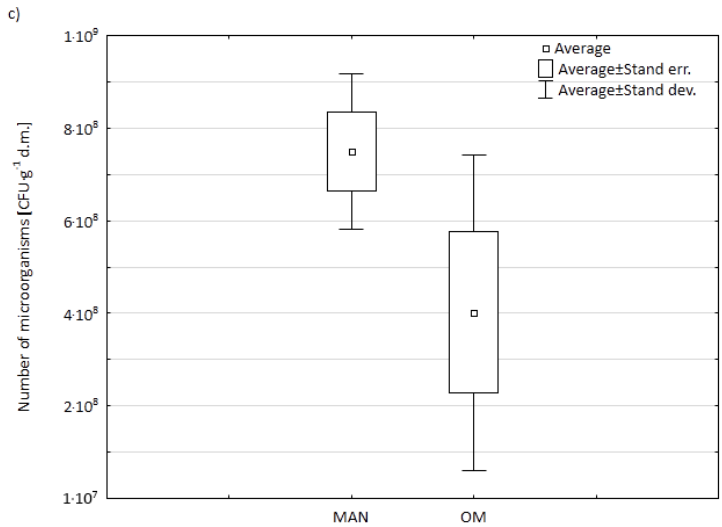


Fig. 2. The number of keratinolytic microorganisms on various medium: a) chicken feathers, b) duck feathers, c) turkey feathers, MAN - Mandel's medium, OM - Omelianski's medium

The number of microorganisms can be affected by technology applied in the processing facility, the poultry species, and hygiene conditions in livestock farms [16, 17]. Microorganisms frequently occurred on mineral substrates, for which keratin as a component, was a source of carbon and energy. The ability of environmental microorganisms to produce keratinase and to use keratin as a carbon source was confirmed on an example of *Bacillus subtilis* bacteria [18, 19]. Isolated microorganisms were characterized by different growth rate, which can be attributed to their generic or species differences. The ability of keratin degradation is often a feature of thermophilic microorganisms, which [20] associated with the need to supply large amounts of energy to effectively degradation of the substrate. On average for all of the analyzed samples, 45% less keratinolytic microorganisms was grown on Omelianski's than on Mandel's substrate ($3.5 \cdot 10^8$ CFU·g^{-1} d.m.). Smaller number on Omelianski's substrate confirms that it is the substrate much poorer in nutrients. These differences demonstrate the need to ensure appropriate conditions for growing microorganisms that can be used in biodegradable waste from, among others, the poultry industry.

Conclusions

1. Analyzed types of feathers were numerously colonized by keratinolytic microorganisms. The poultry species, slaughterhouse type, and date of sampling had a great impact on this fact.
2. Turkey feathers were the most often inhabited by microorganisms capable of degrading keratin, then duck and goose feathers, while the lowest numbers were found in chicken feathers.

3. Mandel's rather than Omelianski's appeared to be the preferred mineral substrate with keratin as the sole carbon source.
4. Keratinolytic microorganisms colonized feathers of different poultry kinds more in autumn and winter than in other periods.

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Abstrakt: Stale powiększająca się koncentracja produkcji mięsa drobiowego powoduje powstawanie ogromnych ilości produktów odpadowych. Jak każdy materiał odpadowy, również pierze musi zostać odpowiednio zagospodarowane. Nie jest to jednak prosty mechanizm, gdyż w skład piór wchodzi odporna na degradację keratyna. Z tego też względu wskazane jest poszukiwanie drobnoustrojów zdolnych do degradacji tego białka. Liczebność drobnoustrojów w badanych próbach ma istotne znaczenie w izolacji szczepów cechujących się pożądanymi właściwościami, tj. wysoką aktywnością enzymatyczną. Celem badań było określenie liczebności drobnoustrojów zdolnych do rozkładu keratyny w piórach kurzych, kaczych, gęsich oraz indyczych pozyskanych w wyniku uboju. Zostały one pobrane w różnych terminach badawczych w okresie od marca 2015 do stycznia 2016 roku, w ubojniach drobiu zlokalizowanych w województwach lubuskim i zachodniopomorskim. Hodowle prowadzono w oparciu o mineralne podłoża Mandela i Omeliańskiego, uzupełnione o keratynę. We wszystkich analizowanych piórach stwierdzono występowanie drobnoustrojów keratynolitycznych. Były one w różnym stopniu zasiedlone przez te mikroorganizmy. Pióra indycze były najliczniej reprezentowane przez mikroorganizmy zdolne do rozkładu keratyny ($5{,}8 \cdot 10^8$ jtk·g^{-1} s.m.), następnie pióra kacze ($4{,}7 \cdot 10^8$ jtk·g^{-1} s.m.). Mniejsze liczebności stwierdzono w piórach gęsich ($2{,}8 \cdot 10^7$ jtk·g^{-1} s.m.), z kolei najmniejsze w piórach kurzych. Ze względu na lepsze dostosowanie składu pożywki Mandela do potrzeb keratynolitycznych udało się wyizolować na tym podłożu większą ich liczebność w porównaniu do podłoża Omeliańskiego. Jedynie w przypadku piór kurzych liczebność drobnoustrojów keratynolicznych na obu podłożach była zbliżona i nie przekraczała 10^6 jtk·g^{-1} s.m. Wbrew oczekiwaniom w badanych materiałach w okresie jesienno-zimowym stwierdzono większą liczebność analizowanej grupy drobnoustrojów.

Słowa kluczowe: pióra, keratyna, mikroorganizmy, przemysł drobiarski