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Research article

The use of *Yucca schidigera* and microbial preparation for poultry manure deodorization and hygienization

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ABSTRACT

The aim of this study was to determine the effectiveness of microbial preparation and *Yucca schidigera* in the removal of odorous volatile compounds from poultry manure as well as to evaluate antimicrobial properties of these amendments. It was demonstrated that the combined treatment of poultry manure (PM) with the microbial preparation and *Y. schidigera* extract can reduce the concentration of odorants by 58%–73%, depending on the tested compound. When *Y. schidigera* extract and the microbial preparation were applied at a time interval of 48 h, the deodorization efficiency was improved by 6–24%. Furthermore, *Y. schidigera* extract has antimicrobial properties, which affect poultry manure hygienization. It was found that when the microbial preparation was enriched with *Lactobacillus plantarum*, it became insensitive to the antimicrobial properties of *Y. schidigera*.

1. Introduction

For many years, there has been a significant increase in global consumption of poultry and poultry products. Despite being highly profitable, the intensification of livestock rearing leads to the production of large amounts of manure, which undergoes rapid microbial decay. Incomplete anaerobic biodegradation of animal manure (a mixture of faeces, urine and residues of food and water) generates gaseous pollutants that affect the quality of life, safety of man and the health of livestock. Odors emitted from large-scale farms are also very offensive for people living in nearby areas (Rappert and Müller, 2005).

There is little information on the chemical nature of odors deriving from animal manure, which is necessary to develop methods of their reduction. So far, it has been found that odor formed in breeding farms is a complex mixture of gases, made up of over 160 chemical components, and it mainly includes sulfur compounds, ammonia and volatile amines as well as indoles and volatile fatty acids (Yan et al., 2013). These gases can cause respiratory tract irritation, allergies, asthma, increased susceptibility to infectious diseases, irritability, stress, chronic headaches, nausea, lethargy and many others in individuals subjected to prolonged exposure. Odorous compounds also affect the health of farm animals and thus reduce the quality and efficiency of farming, which is associated with large material losses (Enticknap et al., 2006; Siegeford and Powers, 2008; Wings et al., 2008).

Research is currently conducted to develop effective methods of reducing odors in livestock production. Chemical oxidation technologies have many advantages, including high effectiveness on a wide range of malodorous compounds as well as their stability and short reaction times. However, some authors also point out considerable drawbacks of these techniques like high operating costs or formation and accumulation of potential harmful byproducts (Cantau et al., 2007; Hammouda et al.,2016; Wang et al., 2013).

Biological methods that use microorganisms capable of reducing concentrations of certain volatile chemical compounds are of great interest (Dumont et al., 2014; Jaber et al., 2014; Powers, 1991; Szynkowska and Zwoździak, 2010). Bacteria *Acinetobacter* sp., *Arthrobacter* sp. and *Alcaligenes* sp. were used by Rappert and Müller (2005) to degrade amines and volatile fatty acids, whereas sulfur bacteria *Thiobacillus* sp. were applied to treat volatile sulfur compounds (Visscher and Taylor, 1993; Hartikainen et al., 2001). Higa (1994) made a breakthrough discovery by proving that

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different groups of microorganisms can coexist, leading to favorable environmental changes towards reducing odor concentrations. This gave rise to investigations on biopreparations consisting of a consortium of microorganisms and/or enzymes.

Biopreparations have many advantages because they are safe to use and non-corrosive, they reduce excessive microbial growth and can adsorb heavy metal compounds. Contrary to other deodorization methods, biopreparations are becoming more and more popular because they do not require specialized equipment for application. They are produced in powder form of particle size, e.g., 20–160 μm, 0.5–1.5 mm, 5.0–7.0 mm, mostly in gray-beige color. The aim is to develop microbial preparations made of a variety of microbes having different properties and characterized by a broad spectrum of activity (Borowski et al., 2010; Durka et al., 2010; Gutarowska et al., 2014). It is also important that such microbial preparation would affect the hygienization of livestock premises, reducing the number of pathogenic organisms, which contribute not only to the formation of odors, but may also cause diseases in animals and humans.

A modification of fodders is one of the methods used to reduce concentrations of odorants in livestock premises. The effect of diet on the amount of odorous volatile compounds has been studied by Nahm (2002), who showed that the use of synthetic amino acids reduces the excretion of nitrogen by 10–30% and the use of phytase in feed reduces phosphorus excretion by 25–30%. Plant preparations and plant extracts can also be applied here. One of them is *Yucca schidigera*, which can lead to rapid decomposition of urea and positively influences the health of animals (Wang et al., 2001; Cheeke et al., 2006). *Y. schidigera* is a commercial source of saponins, numerous enzymes, antioxidants and resveratrol. Killeen et al. (1998) and Piacente et al. (2005) reported that the extract of this plant consumed by animals affects the nitrogen metabolism in the body, reduces urea concentration in blood and ammonia content in the air. In addition, supplementation of animal feed with *Y. schidigera* extract has a positive effect on the growth of animals and results in an increased feed intake (Cheeke and Otero, 2005). Due to the high content of saponins, this plant extract has antimicrobial properties, which has been demonstrated by Wang et al. (2001). According to the literature data, so far, *Y. schidigera* extract has been used only as an additive to animal feed and water, but there are no studies on its use for deodorization and hygienization of poultry manure as well as in the combination with microbial preparation.

The aim of this study was to determine the degree of removal of odorous volatile compounds and the reduction in the number of selected groups of microorganisms from poultry manure after using mineral – microbial preparation and *Y. schidigera* extract as well as to evaluate the antimicrobial properties of *Y. schidigera* extract against microorganisms contained in the preparation, test strains and isolates from poultry manure.

2. Materials and methods

2.1. Poultry manure

The poultry manure, which was used in this study, was collected from a poultry farm (cage system; Zgierz, Poland) at which 27,800 laying hens are kept.

2.2. Yucca schidigera

The study was conducted using a water extract of *Y. schidigera* plant ("Melissa", Brzeziny, Poland) in a concentration of 40%.

2.3. Microbial preparation

The microbial preparation consisted of a consortium of six strains of microorganisms on a carrier being a mixture of perlite and bentonite (1:1): *Pseudomonas fluorescens* (0961), *Enterococcus faecium* (0965), *Bacillus subtilis* (0962), *Bacillus megaterium* (0963), *Leuconostoc mesenteroides* (0964) and *Streptomyces rutgersensis* (0967). The affiliation to the microbial species from the microbial preparation was identified based on their nucleotide sequences of the 16S rRNA gene using a polymerase chain reaction (PCR) with 98–100% of compatibility. The identified microorganisms were deposited in the Pure Culture Collection ŁOCK 105 (ITFM, LUT), and their nucleotide sequences in the NCBI GenBank, the numbers KJ919967–KJ919972.

Microbes contained in the microbial preparation were selected on the basis of the previous study on their ability to reduce the content of volatile odorous compounds from poultry manure and their antagonistic interactions (Gutarowska et al., 2009). The microbial preparation used in the study was prepared according to the procedure described by Gutarowska et al. (2014), and described in Patent No. P393863. In the present study, the preparation was enriched with two strains of bacteria: *Lactobacillus plantarum* 0995 and *Lactobacillus plantarum* 0981 from the Collection ŁOCK 105, and their accession numbers in GenBank National Center for Biotechnology Information database were as follows: KP773475 and KP773478, respectively.

2.4. Tested microorganisms and isolates from poultry manure

The antimicrobial activity of *Y. schidigera* extract was determined using microorganisms contained in the microbial preparation (Section 2.3), and thirteen tested strains, which were potentially pathogenic for poultry, including eight strains of microorganisms from the American Type Culture Collection (ATCC) and NCAIM (National Collection of Agricultural and Industrial Microorganisms): *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 10536, *Listeria monocytogenes* ATCC 19115, *Salmonella enterica* serovar Typhimurium ATCC 14028, *Enterococcus faecalis* ATCC 29212, *B. subtilis* NCAIM 01644 and *Clostridium difficile* ATCC 9689, and five strains isolated from poultry manure: *E. coli, S. enterica, Enterococcus avium, E. faecalis and Clostridium butyricum*. The species affiliation of isolates was determined using API tests: API 20 E with a compatibility of 91% and 75% for *E. coli* and *S. enterica*, API 20 Strep with a compatibility of 98% and 99.3% for *E. avium* and *E. faecalis*, and API 20 A with a compatibility of 99.9% for *C. butyricum*.

Microorganisms isolated from the microbial preparation and tested microorganisms were grown in De Man, Rogosa and Sharpe (MRS Merck, Germany) media – *L. mesenteroides*, *Lb. plantarum* 0995, *Lb. plantarum* 0981, and the other microorganisms in Tryptic Soy Broth and in Tryptic Soy Agar (TSB, TSA, Merc, Germany).

2.5. Evaluation of the deodorizing effectiveness of the microbial preparation and Y. schidigera

The effectiveness of *Y. schidigera* extract and the microbial preparation in reducing odorous volatile compounds from poultry manure was determined using an installation specially constructed for this purpose. It consisted of four separate chambers, each with an active volume of 0.8 dm^3. Aerobic conditions were provided with the use of a membrane blower (Secoh Air Pump), and the air flow rate was controlled by rotameters. Manure samples of approx. 100 g were collected from five different locations in the laying hen farm and thoroughly mixed. Each chamber was filled with 0.5 kg of this material. Then the manure was sprinkled with 50 ml of the