ABSTRACT

Clostridium perfringens bacteria pose a serious threat to livestock animals. Until recently, antibiotics were the main tool to combat them. However, excessive antibiotic use in animal production has led to the emergence of strains displaying antibiotic resistance. Due to the growing concern, in 2006, the use of antibiotics for preventive and growth-promoting purposes in animal breeding was banned. Consequently, there was a need to develop alternative methods to limit the development of pathogenic *C. perfringens*.

In response to this need, this dissertation focuses on developing probiotic feed additives capable of antagonistic action against *C. perfringens* strains causing acute infections in animals raised in our country. To create practical preparations, clinical isolates obtained from animals were chosen as indicators for activity tests. First, their genotyping was conducted. Based on the presence of genes encoding α, β, ε, iota, and β2 toxins, it was determined that they belonged to five toxic types of C. perfringens: A, B, C, D, and E, among which two subtypes were also identified - A β2 positive and E β2 positive. Type C was the causative agent of necrotic enteritis in pigs, while D and E types caused enterotoxemia in cattle and pigs. Types A β2 positive, B, and E β2 positive induced enterotoxemia and necrotic enteritis in poultry. Subsequently, LAB strains were isolated.

From 69 samples collected from healthy piglets, calves, and slaughtered chickens, a total of 912 isolates were obtained, which were tested for their ability to antagonize individual toxic types of C. perfringens. The sought-after activity was found in 75.7% of calf isolates, 50.4% of piglet isolates, and nearly 30% of slaughtered chicken isolates. The obtained isolates exhibited a high selectivity towards *C. perfringens*. Only 7% of them exhibited antagonistic activity against all toxic types of *C. perfringens*. However, none of the isolated strains showed strong activity against all toxic types of *C. perfringens*.

A key step in developing probiotic formulations was the selection of LAB strains. For this purpose, 41 strains most active against *C. perfringens* were identified and their probiotic potential was determined. To understand the mechanisms underlying the anti-*C. perfringens* activity, factors responsible for this action were initially identified. Ultimately, three strains were chosen as components of probiotic preparations: *E. faecium* 10t, *L. paracasei* 11c, and *L. plantarum* 12k.

Strain E. faecium 10k exhibited activity against *C. perfringens* types A, A β2 positive, B, C, and D, with strong activity against type C. The activity of *L. paracasei* 11c encompassed *C. perfringens* types C, D, E, and E β2 positive, with strong activity against types C and D. Strain *L. plantarum* 12k exhibited weak activity against all toxic types of *C. perfringens*. All selected strains exhibited strong adhesion properties to pig mucin and survived simulated intestinal passage. They did not cause bile salt hydrolysis and were sensitive to the main groups of antibiotics used in zootechchics. Moreover, their extracellular metabolites active against *C. perfringens* were identified.

In addition, their most active extracellular metabolites against *C. perfingens* were identified. In the case of *L. paracasei* 11c, such a metabolite was class IIa bacteriocin with a molecular weight of about 4.9 kDa, while the *L. plantarum* 12k strain were two bioactive peptides with molecular weights of 2.2-2.3 kDa.

Subsequently, the production of probiotic preparations and their storage stability assessment were conducted. Probiotic preparations were produced using conventional and modified methods. The conventional method involved culturing selected strains in liquid medium, biomass separation by centrifugation, and preservation using freeze-drying. During this stage, optimal conditions for the growth of studied strains were determined, and the composition of a typical feed medium for LAB was optimized. The proposed modification of probiotic production involved replacing liquid medium with a cost-effective semi-solid medium based on wheat, oat, and corn flour. Biomass separation was eliminated, and spray drying was used instead of freeze-drying. The corn flour medium with glucose, inulin, yeast extract, and soy extract proved most suitable for industrial probiotic production. All strains grew better in this medium compared to the original MRS liquid medium. Strains *L. paracasei* 11c and *L. plantarum* 12k multiplied to levels above 10^{10} CFU/ml, and strain *E. faecium* 10t reached over 10^{9} CFU/ml. Protein supplements from insect sources further increased biomass production. Importantly, semi-solid culture-based production allowed for waste-free preparation. This modification was also ecologically advantageous as it reduced biological waste. In addition, drying biomass with whole culture media allowed to obtain products with a higher weight, which were therefore easier to dose and mix with feed. However, cell stability during storage was lower in preparations obtained from semi-solid cultures than in those produced conventionally. Nevertheless, they exhibited stronger activity against *C. perfringens* due to the additional source of metabolites active against *C. perfringens*.

Based on strains with defined activity, preparations targeting specific toxic types of C. perfringens can be created. They can also be used to construct multi-strain preparations with a broad range of activity against *C. perfringens*. The results of this work may contribute to the development of new strategies for promoting the health of livestock animals and thereby the improving the quality and safety of animal origin products.

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