Bacillus lentus Metabolites with Antimicrobial Activity as a New Generation of Growth Promoters for Animals

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Abstract

Bacteriocin-like inhibitory substances produced by a new strain Bacillus lentus B-7150 have shown a significant effect on broiler production in the Markinskaya research farm (B-1207, Russia), demonstrating high potentialities of getting benefits from their use. It is shown that providing broiler chickens with feed along with B. lentus bacteriocin-like substance has significantly stimulated broiler production. So, the live weight of 35 day-old broilers increased by 20.2% and 19.1%, with average daily weight gain increasing by 20.6% and 19.7%, while feed conversion decreased by 4.08% and 6.62% compared to additives-free and flavophospholipol-supplemented controls, respectively. Possible ways of stimulate growth are discussed.

Keywords: Bacteriocin; Bacillus lentus (B. lentus)

Introduction

Unlike antibiotics bacteriocins and Bacteriocin Like Inhibitory Substances (BLIS) show no toxic or negative effects and are considered as ecologically friendly additives as probiotics and phytobiotics without restrictions for their use with feed. BLIS and other biologically active substances (BAS) were produced from soil Bacillus lentus strain B-7150. We have produced samples of the BLIS-containing substance from B. lentus (BLIS-BL) for testing them with feed on animals. Experiments were carried out on farm broilers and lasted totally 35 days.

Objectives of the Study

- Derive main biologically active substances from Bacillus lentus strain B-7150.
- Prepare samples containing Bacillus lentus - derived antimicrobials to treat broilers.
- Prepare broilers for further trials.
- Determine broiler weights, feed consumption and some other selected parameters.
- Process obtained results and discuss them.

**Methods**

The strain of bacilli was cultured at 30°C for 48 hours. Technology of producing coarse fractions of these substances for testing in figure 1. Bactericidal activity was determined by placing samples of desired volumes in Petri dishes containing freshly seeded lawns of test strains of Gram-positive and Gram-negative microorganisms. The activity was expressed in arbitrary units (AU) measured for 1 ml or 1 mg of the sample depending on the level of dilution. The method of two-phase separation in the presence of organic solvent (dichloromethane, chloroform) was found to be the most effective one to isolate the product from the culture fluid [1]. Molecular identification of the bacteriocin-like substance was performed by SDS PAGE [2]. Before biological testing the gel was washed, placed in a Petri dish and overlaid with melted agar containing test cells. Molecular weight of the sample determined by MALDI-TOF Bruker Daltonics mass spectroscopy. Experiments with broilers were performed in accordance with the methodological recommendations for conducting research on the feeding of poultry.

![Figure 1: BLIS-BL production scheme.](image)

**The study of the microflora of the blind processes and litter:** After slaughtering the chickens by cutting off the head, 0.5 - 0.7g of the contents of the blind processes, litter samples were taken and introduced into sterile tubes with 4.5 ml of isotonic NaCl solution. A series of 10-fold dilutions was prepared with initially diluted (7 - 10 times) suspensions of each sample. The resulting dilutions were sown on several types of culture media: universal purpose (fish meal hydrolyzate-based nutritional agar, manufacturer SRCAMB), selectively differential nutrient (Levina, Endo-, bismuth-sulfite-, entero- and staphylococci- Agar, from SRCAMB; Perfringens Agar Base, Campylobacter Agar, MRS agar, HiMedia), and chromogenic culture media (XLD Agar, M1393 and M1295, HiMedia), followed by culturing at 30 - 37°C for 24 - 48 hours. The study of cell morphology was carried out Gram stain microscopy.

**Results and Discussion**

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**B. lentus biologically active substances**

- BSF - biosurfactant (≈900 Da) active against Gram(-) bacteria (Figure 2).
- BLIS from the cell surface (BLIS-SC) (< 10 kDa) targeting mainly Gram(+) bacteria (Figure 3b).
- BLIS from the cell-free culture fluid (BLIS-CF) ≈4 kDa targeting mainly Gram (-) bacteria (Figure 3a).
- Alkaline protease is of special interest due to its ability to inactivate both its own and foreign bacteriocins. Production of the enzyme are nearly concurrent with the dynamics of antimicrobial activity (See figure 4a).

*Figure 2: Molecular weight of the B. lentus biosurfactant sample as determined by MALDI-TOF Bruker Daltonics mass spectroscopy.*

*Figure 3: SDS PAG Electrophoresis of B. lentus BLIS samples. Line: 1 - marking PageRuler™, 2 - BLIS-CF, 3 - BLIS-SC.*
Figure 4: Molecular weights (lanes) of B. lentus substances as determined by SDS PAG electrophoresis (a); antimicrobial effect of the substances on the growth of the test-strain; visualized influence of the enzyme on the medium (b). M - marking, kDa; № 2, 3 - BAS.

Subject and conditions of trials

- The effect of BLIS-BL on broiler production was evaluated. The activity unit is an Arbitrary Unit (AU), with 1 kg of dry BLIS being equal to 1,000,000 AU.

- The dosage of BLIS-BL of approximately 1,000 AU/kg of complete feed that is equal to 900g of this BLIS per one ton of feed used in the trial.

- What we expected from BLIS-BL application: antimicrobial action (especially against Clostridia perfringens, Campylobacter jejuni, Salmonella spp. and E. coli) and harmonization of intestinal microflora.

- Broilers (males) Ross-308 were used; 80 broilers/one treatment (4 replica, 20 broilers/pen, floor penning - see figure 5).

Figure 5: Distribution of birds in poultry farms Hall.

• Measurements: Live weight of broilers, feed intake.
• Floor litter and cecal samples were taken after the trial for microbiological analysis.
• 100 ml of manure were taken from each pen and homogenized for further analysis.
• For comparison with BLIS-BL (T7) we used conventional premixes: antibiotic (T2), probiotics (T3-T6), organic acid (T8), copper sulfate (T9) served as a control forage without additive T1.

Conclusion

• Strain B. lentus B-7150 produces several biologically active substances, properties and conditions of which have been explored [3]. BLIS-SC adsorbs on the surface of cells and is released by means of polar solvents. BLIS-CF and alkaline protease are released into the interphase film under influence of non-polar solvents. Biosurfactant attaches to the walls of the bioreactor and can be removed with hot water with alkaline pH. Solutions BLIS and protease are stabilized with polyvinylpyrrolidone followed by spray-drying (Figure 1). Then dry powder is normalized to obtain the specific antimicrobial activity. The dry mix is added to broiler feed.

• Trials have shown that (1) the live weight of 35-day-old broilers increased by 20.2 and 19.1 percent, (2) the average daily gain weight was 20.6 and 19.7%, (3) feed conversion decreased by 4.08 and to 6.62% vs additive-free and flavophospholipol-added controls, respectively (Figure 6 and table 1-4).

• Unlike antibiotics bacteriocins and BLIS produce neither toxic nor negative effects and are considered as ecologically friendly additives as probiotics and phytobiotics without restrictions for their use with feed.

• No significant changes in bacterial profiles of broiler ceca or litter samples were observed. It was also shown that BLIS-BL is not harmful for useful E. coli and Enterococcus faecium (Table 5). However, this question should be clarified.

• It quite possible that intestinal toll-like receptors are involved in these actions [4,5]. This effect of bacterial cells and their separate components has been actively studied in medicine, especially in pediatrics.

• Alkaline protease acts from jejunum throughout colon and cecum, masking digestion more efficient. Production of the enzyme are nearly concurrent with the dynamics of antimicrobial activity (See figure 4).

• This effect of bacterial cells and their separate components is widely investigated in human medicine, especially in pediatrics.

Figure 6: Dynamics of gain weight by broilers provided with BLIS-added (T7) feed compared to the other groups.
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Table 5: Data analysis of the microbial composition of the contents of the blind processes.

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<tr>
<th>Trials</th>
<th>Total number of aerobes</th>
<th>E. faecalis</th>
<th>E. faecium</th>
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<th>E. coli</th>
<th>Other coliforms</th>
<th>Salm. spp.</th>
<th>Bac. spp.</th>
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<td>4.4. 10⁵</td>
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<td>HO</td>
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*(*): Not Detected; Bac.: Bacillus; Lb.: Lactobacillus; Cl.: Clostridium; C.: Campylobacter.

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Bibliography


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