



## INVESTIGATION OF INHIBITORY ACTIVITY OF ENTEROCOCCI ISOLATED FROM THE DIGESTIVE TRACT OF CHICKENS AGAINST SOME ENTEROPATHOGENIC BACTERIA

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### ABSTRACT

Inhibitory activity of 96 strains of enterococci, isolated from chicken's digestive tract is studied. 52 strains of them are isolated from chickens reared in industrial conditions and 44 strains – from chickens reared in natural domestic conditions. Antimicrobial activity of isolated enterococci against 4 strains of enteropathogenic *E. coli* and 3 strains of *Salmonella* bacteria (*S. typhimurium*, *S. enteritidis* and *S. gallinarum*) is determined. Higher inhibitory activity against tested enteropathogenic strains is observed for enterococci, isolated from digestive tract of chickens, reared in natural domestic conditions. The maximum inhibitory activity was 86.00% against *E. coli* and 84.82%, 84.00% and 83.46% against *S. typhimurium*, *S. enteritides* and *S. gallinarum* respectively.

**Key words:** enterococci, inhibitory activity, enteropathogenic bacteria

### INTRODUCTION

The normal intestinal microflora plays an important role for the macroorganism health. The microorganisms in the human and animal intestinal tract form a complicated ecosystem, staying in dynamic balance. This balance may be disturbed by accepting of antibiotics, stress, changes in the diet and too great hygiene. In that case the organism gets ill.

One of the basic functions of the normal intestinal microflora is inhibition of the pathogenic bacteria (9, 12, 13).

The efforts for controlling the composition of microbial population in the intestinal tract of chickens and their state of health have been orientated to increasing or detention the number of the beneficial microorganisms. The lactic acid bacteria -lactobacilli, streptococci, pediococci and enterococci are of great significance. They are normal inhabitants of the intestine of chickens, they can attach to the intestinal mucosa and form colonies over it (2, 10, 14, 17). They also synthesize a great number of antimicrobial substances, inhibiting the growth of pathogenic bacteria, and influence on increasing the natural resistance of the organism (8, 15, 19).

The enterococci are a significant part of the normal intestinal microflora of birds. They

could be found several hours after hatching of chicken in its intestinal tract, whereas the lactobacilli appear only after the 7th day (1, 17).

The enterococci in the intestinal microflora synthesize antimicrobial substances and possess antimicrobial activity against some pathogenic bacteria, including enteropathogenic *E. coli* and *Salmonella* bacteria (7, 18). They are one of main species of microorganisms having probiotic activity together with lactobacilli and bifidobacteria (5, 16, 17).

The purpose of this study was to isolate some strains of enterococci from the digestive tract of chickens, reared in domestic and industrial conditions and to study their inhibitory activity against enteropathogenic *E. coli* and *Salmonella* bacteria with regard to the further use in the composition of probiotics for birds.

### MATERIALS AND METHODS

In the research two groups chickens were used: 1. hatched in an incubator and reared in industrial conditions; 2. hatched by brood-hen and reared in domestic conditions.

### ***Isolation of Enterococci from Various Parts of Digestive Tract of Chickens***

The strains of enterococci were isolated from citrat azid agar plates from the crop, glandular stomach, small intestine, cecum and large intestine. The strains were isolated in hydrolyzed milk and incubated at 37°C for 24h.

### ***Determination the Inhibitory Effect of Isolated Strains***

1. Test organisms: 4 strains of enteropathogenic *E. coli* and 3 strains of *Salmonella* - *Salmonella typhimurium*, *Salmonella enteritidis* and *Salmonella gallinarum* were used.

2. Examination for Inhibitory activity (IA): The method described by Gilliland (11) was

carried out and the percent of inhibition was calculated.

### ***Identification of Selected Strains with High Inhibitory Activity***

The identification was carried out according to Sherman's indexes (3) and fermentative ability in relation to lactose, arabinose, melezitose and sorbitol was studied according to Bergey (4).

## **RESULTS AND DISCUSSION**

Only 22 from all determined strains enterococci had an inhibitory activity (IA) over 50% against used test-bacteria of *E.coli*. (Table 1)

**Table 1.** Inhibitory activity of enterococci isolated from industrial and domestic reared chickens with value over 50%.

Number of the strains	<i>E. coli</i> O1	<i>E. coli</i> O2	<i>E. coli</i> 9C	<i>E. coli</i> 10C	<i>S. typhi-murium</i>	<i>S. enteritidis</i>	<i>S. gallinarum</i>
Enterococci, isolated from industrial reared chickens							
1.234	76.90	77.48	78.00	79.92	65.82	65.45	64.00
1.332	82.20	82.32	81.90	83.40	79.90	79.50	79.00
7.534	79.00	78.40	79.00	79.70	68.99	68.30	65.70
7.631	78.95	78.00	79.02	81.82	67.50	67.30	66.99
7.632	77.14	77.82	78.40	80.45	68.90	68.00	67.50
7.635	78.95	78.55	79.00	80.55	65.48	65.00	64.90
14.632	76.30	77.00	79.12	81.00	66.32	65.99	65.00
14.931	78.00	78.20	79.00	80.63	67.75	67.30	67.00
30.933	82.00	82.20	81.99	82.50	79.52	78.99	77.50
60.633	81.89	81.80	80.59	82.89	79.18	79.00	78.92
60.831	82.50	82.90	82.00	83.44	80.25	80.00	80.00
Enterococci, isolated from domestic reared chickens							
1.334	84.99	85.48	85.90	86.00	84.82	84.00	83.46
1.631	81.00	81.44	80.00	84.25	82.71	81.32	81.00
1.632	82.58	82.00	81.40	84.00	81.82	82.21	82.08
1.731	71.43	72.08	72.90	80.24	70.00	72.40	71.00
1.732	73.00	72.00	71.99	80.00	69.85	70.00	69.51
1.931	83.90	83.00	83.48	84.80	83.90	82.99	82.40
1.932	83.33	82.90	83.62	84.99	83.72	82.97	82.83
1.933	84.00	84.70	85.00	85.62	84.00	93.90	83.12
7.531	71.05	71.48	72.00	78.90	72.20	70.90	70.00
7.733	69.90	71.42	72.00	79.80	71.22	70.33	69.00
14.733	70.50	70.23	72.14	74.00	70.80	69.99	68.42

Note: The results are arithmetical mean of values obtained from three contiguous experiments.

The IA of strains isolated from chickens reared at industrial conditions against *E.coli* O<sub>1</sub> varied from 76,30% to 82,50%; against

*E.coli* O<sub>2</sub> - from 77,00% to 82,99% ; against *E.coli* 9C - from 78,00% to 82,00% and against *E.coli* 10C - from 79,70% to 83,44%.

In larger limits varied the IA of enterococci isolated from chickens reared at natural domestic conditions: against *E.coli* O<sub>1</sub> - from 70,50% to 84,99%; against *E.coli* O<sub>2</sub> - from 70,23% to 85,48%; against *E.coli* 9C - from 71,99% to 85,90% and against *E.coli* 10C - from 74,00% to 86,00%.

Some differences in inhibitory activity of determined strains according to the type of used test-bacteria were observed.

All strains enterococci possessed the highest inhibitory activity against *E.coli* 10C. The dependence between the IA of enterococci and the part of chicken digestive tract, from which they were isolated, also was observed. The strains isolated from the epithelium of chicken crop and caecum were with the highest IA (strains 1.332, 1.334 and 60.831). The IA of strain 1.332 varied from 81,90% (against *E.coli* 9C) to 83,40% (against *E.coli* 10C). This strain was isolated from the epithelium of the crop of one-day old chick reared at industrial conditions. The strain 1.d.334, isolated from crop epithelium of one-day old chick, reared at natural domestic conditions had the highest IA - from 84,99% (against *E.coli* O<sub>1</sub>) to 86,00% (against *E.coli* 10C). The other strain with high IA is strain 60.831 isolated from the caecal epithelium of industrial reared 60-day old chick. Its IA varied from 82,00% (against *E.coli* 9C) to 83,44% (against *E.coli* 10C). The strains 30.933 and 1.d.933 had high IA too. They were isolated from the content of the large intestine. Strain 30.933 from one-month old chick reared at industrial conditions has an inhibitory activity from 81,99% (against *E.coli* 9C) to 82,50% (against *E.coli* 10C). The strain 1.d.933, with IA from 84,00% (against *E.coli* O<sub>1</sub>) to 85,62% (against *E.coli* 10C), was isolated from one-day old chick reared at natural domestic conditions.

11 strains (21.15%) of all isolated from industrial reared chickens possessed inhibitory activity over 50% against all used *Salmonella* bacteria (Table 1). The inhibitory activity was smallest against the strain of *Salmonella gallinarum* - from 64% for strain 1.23 to 80% for strain 60.831.

There were significant differences in the inhibitory activity of the strains depending on the part of the intestinal tract they were isolated. The highest inhibitory activity showed the strain 60.831. It was isolated from the cecal epithelium of a one-day old chicken, reared in industrial conditions.

In the group of domestic reared chickens the inhibitory activity of the isolated enterococci

reached 84.82%; 84.00% and 83.46% against *Salmonella typhimurium*, *Salmonella enteritides* and *Salmonella gallinarum* respectively.

The strain 1d 334 isolated from crop epithelium of one-day old domestic reared chicken was with the highest inhibitory activity against the all three strains of *Salmonella*. Similar results have been obtained by Carina Audisio et al. (6) with a strain of *Enterococcus faecium*, isolated from intestinal tract of a free-ranging chicken and producing antagonistic compounds against *Salmonella spp.* Our data showed that the inhibitory activity of the enterococci isolated from domestic reared chickens was from 2% to 10% higher in comparison to the inhibitory activity of the enterococci, isolated from industrial reared chickens.

The data for identification of the isolated from the digestive tract of chickens enterococci with IA over 50% show that they belong to *Enterococcus faecalis* (strains 1.234, 1.332, 7.631, 1.d.334, 1.d.631, 1.d.632, 1.d.732, 7.d.531, 7.d.733, 14.632, 14.931, 30.933 and 60.633) and a species near to *Enterococcus faecium* (strains 1.d.731, 1.d.931, 1.d.932, 1.d.933, 7.534, 7.632, 7.635, 14.d.733 and 60.831).

## CONCLUSION

1. The digestive tract of chickens, reared in natural domestic conditions and precisely the ceca and the crop are an exceptionally source for isolation of enterococci with strongly expressed inhibitory properties towards enteropathogenic *E. coli* and *Salmonella* bacteria.
2. *Enterococcus faecalis* - strain 1.d.334 was the strain with the highest inhibitory activity and most suitable for preparation, in combination with other microorganisms (such as lactobacilli) of probiotics for chickens to protect them of *E.coli* and *Salmonella* infections.

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