THE PRESENCE OF AUTOAGGREGATION AND ADHESION PROPERTIES IN CHICKEN BIFIDOBACTERIA STRAINS*

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Thirty bifidobacteria strains were isolated from intestinal tract of chicken (crop, duodenum, small intestine and ceaca). Six strains (4 from crop, 1 from duodenum and small intestine) with autoaggregation and adhesion ability were determined. All strains with autoaggregation ability were able to adhere to chicken crop epithelium, while six strains without autoaggregation ability did not express adhesion activity. Our results suggest positive correlation between autoaggregation ability and adhesion in chicken bifidobacteria strains.

bifidobacteria; autoaggregation; adhesion; chicken

INTRODUCTION

Bifidobacteria are gram-positive anaerobic bacteria that are important component of the normal microflora in human and animal intestinal tract (Biavati et al., 1992; Sgorbati et al., 1995) and exert various beneficial effects on host health by controlling undesirable intestinal bacteria (Bernet et al., 1993; Blomberg et al., 1993; Gibson, Wang, 1994). The importance of these microorganisms in the gut is related to their ability to colonise the intestinal tract, and intestinal attachment is an important prerequisition for colonisation. Among reported beneficial effects of bifidobacteria belong inhibitory effect to putrefactive bacteria, reduction of faecal enzymes involved in cancer initiation, and reduction of serum cholesterol (Modler et al., 1990). Bifidobacteria are widely used as food additives, and adhesion ability to the intestinal mucus is one of the desirable properties that have to

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be selected for their specific use in commercial preparations (Del Re et al., 1998).

In previous reports, there was not confirmed the presence of adhesive factors by all strains of bifidobacteria (Ello et al., 1991; Bernet et al., 1993). However, Pérez et al. (1998) reported, that in most cases of *Bifidobacterium bifidum*, autoaggregation is related to the presence of cell adherence, evaluated by the adhesion test to human enterocyty-like Caco-2 cells (Crociani et al., 1995) in *in vitro* cultures. These data provide a basis for setting up of a very simple and rapid method for preliminary selection of bacteria potentially adherent to epithelial cells by means of autoaggregation. The ability of bacteria to cluster together is represented by the autoaggregation. On the basis of autoaggregation ability, two different phenotypes (Agg+ and Agg–) were selected from one strain (BSu895) of *B. suis*. There was a good relationship between autoaggregation and adhesion ability as only variant Agg+ had adhesion activity (Del Re et al., 1998).

The aim of this work was to isolate and test poultry *Bifidobacterium* species with autoaggregation properties and confirm whether chicken bifidobacteria species have adhesion ability to crop epithelium.

MATERIAL AND METHODS

Isolation of bifidobacteria strains

Samples of digesta of the crop, duodenum, small intestine and caecum were collected from seven-week old chicks. Trypticase Phytone Yeast Extract agar (ADSA, Spain) modified by the addition of mupirocin (100 mg/L) and glacial acetic acid (1ml/L) was used for isolation of bifidobacteria (Table I). Selective supplements were prepared according to Rada et al. (1999). Freshly collected contents were transferred to TPY broth and serially diluted in the same medium under anaerobic condition. Appropriate dilutions were transferred to sterile 100 mm Petri-dishes. The dishes were immediately filled

I. Composition of medium for isolation bifidobacteria

Modified TPY agar	
TPY agar (ADSA, Spain)	52 g/L
Tween 80	1 ml/L
Mupirocin	0.1 g/L
Acetic acid	1 ml/L
pH	5.2

with the agar and transferred to the anaerobic jars (Anaerobic Plus System, Oxoid). Anaerobic jars were equipped with the low temperature catalysts (Oxoid BR42) and filled with CO_2/H_2 (10/90%) atmosphere.

Thirty strains isolated from twenty chicken were Gram stained and screened for the presence of fructose-6-phosphate phosphoketolase to identify them as *Bifidobacterium* sp. Thereafter bifidobacteria strains were subcultivated in TPY broth (ADSA, Spain) under the anaerobic conditions at 37 °C.

Autoaggregation assay

Two phenotypes of bifidobacteria with different autoaggregation abilities have been isolated. Autoaggregating cells (Agg+), forming precipitate resulting in a clear solution and non-autoaggregating cells (Agg–), producing constant turbidity for long period of time were described. A bacterial overnight cultures (30 strains) were shaken and 1 mL upper suspension of the culture was transferred to tube where the optical density at 600 nm ($O.D._{600}$) was measured at various periods (30, 60, 90, 120, 150 min) of time. Autoaggregation ability of bacteria was expressed as autoaggregation percentage (Del Re et al., 1998):

$[1 - (O.D._{600}$ upper suspension / $O.D._{600}$ total bacterial suspension)] . 100

Two strains with autoaggregation ability (*Lactobacillus salivarius* 51R and *Lactobacillus* sp. 41) from culture collection of Department of Microbiology and Biotechnology (Czech University of Agriculture in Prague) were used as control samples.

Adhesion assay

Adhesion ability was tested according to Fuller (1973). The tested cultures were centrifuged and resuspended in 10 mL of buffered saline (BS: NaCl 0.8%; K_2HPO_4 0.121%; KH_2PO_4 0.034%; pH 7.2). Freshly collected crop was washed in BS. The crop epithelium from fasted chicken was scraped off with the edge of laboratory knife, washed in BS and finally suspended in BS. The 0.4 mL of this epithelial cell suspension was added to 0.1 mL of bacterial suspension. The mixture was rotated for 30 minutes at 37 °C and then the epithelial cells were examined by phase-contrast microscope. The adhesion ability was expressed as number of bacterial cells per one epithelial cell. Thirty epithelial cells were counted for each strain.

Statistic analysis

The calculation of correlation between autoaggregation and adhesion ability in chicken bifidobacteria strains was expressed in the correlation coefficient by means of Microsoft Excel 97.

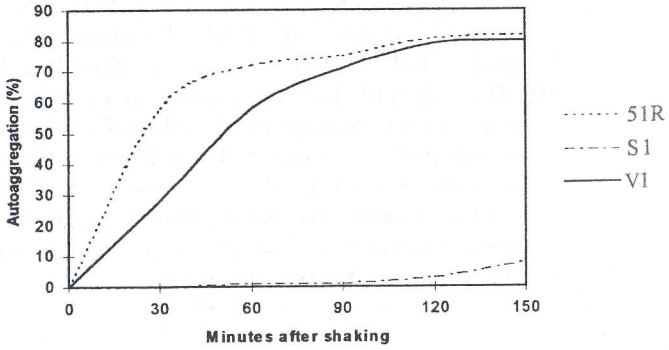
RESULTS AND DISCUSSION

Thirty chicken bifidobacteria strains were selected for two phenotypes with different autoaggregation abilities. Six autoaggregating (Agg+) and twenty four non-autoaggregating (Agg–) strains were obtained. Agg+ bifidobacteria strains were isolated from crop (V1, V2, V3, V4 strains), duodenum (Dv strain) and from small intestine (Ts strain). Agg– strains were isolated from duodenum (Dv1, Dv3, Dv5), small intestine (Ts3) and ceaca (S1, S5). Autoaggregation abilities in two Agg+ strains (*Lactobacillus salivarius* 51R and *Bifidobacterium* sp. V1) and the example of Agg– strain (Bifidobacterium sp. S1) are presented in Fig. 1.

In the adhesion test six Agg+ and six Agg– bifidobacteria strains were investigated. Only Agg+ strains expressed adhesion activity to crop epithelial cells in vitro (Table II).

Pérez et al. (1988) found correlation between autoaggregation and adhesion ability in human strains belonging to *B. bifidum* and they raised the question of whether this correlation could be found in bifidobacteria isolated from other sources. Our results give a positive answer to this question by selecting six chicken strains for autoaggregation and adhesion ability. According to determination of correlation coefficient (r_{xy} = 0.97), the positive correlation was confirmed between autoaggregation (X) ability and adhesion (Y) in chicken bifidobacteria strains.

The results are in line with data showed by Del Re et al. (1998) as only Agg+ phenotypes expressed adhesion ability. In addition, the adhesion test according to Fuller (1973) is simply used and lends to capable results.



1. Autoaggregation ability (Agg+) in two strains *Lactobacillus salivarius* 51R and *Bifidobacterium* V1. The variant of *Bifidobacterium* S1 is an example of the strain without autoaggregation ability (Agg–)

II. Six autoaggregating (Agg+) bifidobacteria strains and the example of six non-aggregating bifidobacteria strains isolated from chicken intestinal tract

Agg+ strains	Autoaggregation (%)	Adhesion[1)]	Agg– strains	Autoaggregation (%)	Adhesion[1)]
V1	77	46	Dv3	20	2
V2	75	45	Dv1	15	0
V4	72	57	S5	7	1
Dv	72	53	Dv5	5	5
Ts	69	58	Ts3	4	6
V3	68	48	S1	3	0

Autoaggregation data are means from triplicate determination

[1)] Adhesion data were expressed as number of bacterial cells per one epithelial cell. Data are means from thirty determinations

Our data suggest that autoaggregation is an important trait that contributes to the ability of bacteria to colonise the chicken intestinal tract. It may be important for chicken to become colonized with these bifidobacteria strains as soon after hatching as possible to protect the newly hatched chick against pathogen microorganisms.

References

BERNET, M. F. – BRASSART, D. – NEESER, J. R. – SERVIN, A. L.: Adhesion of human bifidobacterial strains to cultured human intestinal epithelial cells and inhibition of enteropathogen-cell interactions. Appl. Environ. Microbiol., *59*, 1993: 4121–4128.

BIAVATI, B. – SGORBATI, B. – SCARDOVI, V.: The genus *Bifidobacterium*. In: BALOWS, A. – TRUPEN, H. G. – DWORKIN, M. – HARDER, W. – SCHLIFER, K. H. (ed.): The Prokaryotes. A Handbook on Habitats, Isolation and Identification of Bacteria. Springer-Verlag 1992: 816–833.

BLOMBERG, L. – HENRIKSSON, A. – CONWAY, P. L.: Inhibition of adhesion of *Escherichia coli* K88 to piglet ileal mucus by *Lactobacillus* spp. Appl. Environ. Microbiol., *59*, 1993: 34–39.

CROCIANI, J. – GRILL, J. P. – HUPPERT, M. – BALLONGUE, J.: Adhesion of different bifidobacteria strains to human enterocyty-like Caco-2 cells and comparison with *in vivo* study. Lett. Appl. Microbiol., *21*, 1995: 146–148.

DEL RE, B. – BUSETTO, A. – VIGNOLA, G. – SGORBATI, B. – PALENZONA, D. L.: Autoaggregation and adhesion ability in a *Bifidobacterium suis* strain. Lett. Appl. Microbiol., 27, 1998: 307–310.

ELO, S. – SAXELIN, M. – SALMINEN, S.: Attachment of *Lactobacillus casei* strain GG to human colon carcinoma cell line Caco-2: comparison with other dairy strains. Lett. Appl. Microbiol., *13*, 1991: 154–156.

FULLER, R.: Ecological studies on the *Lactobacillus* flora associated with the crop epithelium of the fowl. J. Appl. Bacteriol., *36*, 1973: 131–139.

GIBSON, G. R. – WANG, X.: Regulatory effects of bifidobacteria on the growth of other colonic bacteria. J. Appl. Bacteriol., *77*, 1994: 412–420.

MODLER, H. W. – MCKELLAR, R. C. – YAGUCHI, M.: Bifidobacteria and bifidogenic factors. Can. Inst. Fd. Sci. Tech. J. J., 1990: 29–41.

PÉREZ, P. F. – MINNAARD, Y. – DISALVO, E. A. – DE ANTONI, G. L.: Surface properties of bifidobacterial strains of human origin. Appl. Environ. Microbiol., *64*, 1998: 21–26.

RADA, V. – SIROTEK, K. – PETR, J.: Evaluation of selective media for bifidobacteria in poultry and rabbit caecal samples. J. Vet. Med., *46*, 1999: 369–373.

SGORBATI, B. – BIAVATI, B. – PALENZONA, D.: The genus *Bifidobacterium*. In: WOOD, B. J. B. – HOLZAPFEL, W. H. (eds.): The Lactic Acid Bacteria. Vol. II. Chapman and Hall 1995: 279–306.

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Přítomnost autoagregačních a adhezivních vlastností u bifidobakterií izolovaných z trávicího ústrojí kuřat.

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Bifidobakterie hrají významnou roli v intestinálním traktu zdravých organismů (Biavati et al., 1992; Sgorbati et al., 1995). Bifidobakterie patří mezi G^+ nesporulující, nepohyblivé tyčinky různých tvarů. Zvláště typické jsou kyjovité amforovité tvary a tyčinky tvarované do podoby **Y**. Bifidobakterie jsou striktní anaeroby a jen některé jsou tolerantní ke kyslíku, a to pouze v přítomnosti oxidu uhličitého. Vysoké počty bifidobakterií jsou stanovovány v tlustém či slepém střevě člověka a hospodářských zvířat. Přítomnost vysokého počtu bifidobakterií v trávicím traktu může chránit hostitele před kolonizací patogenů, může mít pozitivní účinek na intestinální peristaltiku, podporuje imunitní systém hostitele, má preventivní účinky proti rakovině a snižuje hladinu cholesterolu (Modler et al., 1990).

V naší práci bylo na základě fruktózo-6-fosfoketolázového testu identifikováno 30 kmenů bifidobakterií izolovaných z volete, dvanáctníku, tenkého střeva a slepého střeva drůbeže. Všech 30 získaných kmenů bylo podrobeno měřením optické denzity na spektrofotometru. Na základě naměřených hodnot byla procentuálně vyjádřena autoagregační schopnost jednotlivých izolátů podle autorů Del Re et al. (1998). Autogregace je schopnost bakterií vytvářet shluky buněk. Ze získaných výsledků jsme autoagregační schopnost (Agg+) stanovili u šesti kmenů bifidobakterií, z toho čtyři kmeny byly z volete (V1, V2, V3, V4), jeden z dvanáctníku (Dv) a jeden z tenkého střeva (Ts). Jako vzor k porovnání výsledků měření nám sloužily dva sbírkové kmeny rodu *Lactobacillus sallivarius*, u kterých byly již dříve prokázány autoagregační vlastnosti (Agg+). Na základě adhezivního testu *in vitro* (Fuller, 1973) byla zjištěna adhezivní schopnost u šesti kmenů (Agg+) bifidobakterií. Bakteriální buňky bifidobakterií byly schopny adherence na epiteliální buňky odebrané z povrchu sliznice volete. Tím se potvrdila kladná korelace mezi autoagregační a adhezivní schopností u kmenů bifidobakterií.

bifidobakterie; autoagregace; adheze; kuřata

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