NOTE *Bacteriology*

Canine Intestinal Lactic Acid Bacteria Agglutinated with Concanavalin A

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ABSTRACT. Twenty-six out of 46 representative lactic acid bacteria (LAB) that we isolated from 36 dogs in a previous study were agglutinated by concanavalin A (ConA) at a concentration of 0.1563 mg/m*l*, while isolates did not agglutinate without the addition of ConA. Amongst the isolates, *L. reuteri, L. mucosae*, and *E. canintestini* agglutinated strongly, while *L. gallinarum, L. kitasatonis, L. acidophilus, L. saerimneri, B. animalis* ssp. *animalis, P. acidilactici*, and *E. hirae* did not agglutinate. ConA-agglutination of LAB was specifically inhibited by D-glucose, D-galactose, and D-mannose at a concentration of 1.563 mg/m*l*. Among the sugars, ConA-agglutination was strongly inhibited by D-mannose, while the inhibition level by D-glucose and D-galactose were lower than that of D-mannose. ConA-agglutination of all the LAB isolates was inhibited by D-mannose, except for *L. reuteri* (one species) and *L. mucosae* (two species). ConA-agglutination of *Bifidobacterium* spp. was inhibited by only D-mannose. Based on our results, ConA-agglutination of LAB seems to be strain-specific, but not species-specific.

KEY WORDS: agglutination, concanavalin A (ConA), lactic acid bacteria (LAB).

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Lectins are useful for the clinical taxonomic classification of many microorganisms because of the specific detection procedure for the binding sites on the bacterial surface [9]. Glucose, mannose, galactose, N-acetyl-D-glucosamine, and sialic acid are types of determinant sugars concerning the reaction with lectin [4].

Concanavalin A (ConA) is known as a specific probe for sugar residues [8]. The binding sites of ConA are polysaccharides with α-D-glucopyranosyl or α-D-mannopyranosyl substituents. Especially, the teichoic acid in gram-positive bacteria possesses the binding sites of ConA. Agglutination of *L. plantarum* and *L. buchneri* has been reported with ConA [5, 6, 9].

The aims of the present experiment were to characterize canine LAB isolates by ConA-agglutination and inhibition by carbohydrates.

Forty-six representative isolates of lactic acid bacteria (LAB) consisting of 24 species of the 374 LAB were used in this study and were isolated from the contents of the large intestines of 36 dogs in a preliminary study (data not shown). The LAB isolates were classified as genera *Bifidobacterium, Lactobacillus*, and *Streptococcus* morphologically. Their isolates were anaerobically grown at 37°C for 48 hr on MRS (BBL, Cockeysville, MD, U.S.A.) and BL agar (Eiken Chemical Co., Ltd., Tokyo, Japan) containing 5% (v/v) sheep blood. Species identification of these isolates was performed based on comparison of bacterial whole protein profiles by SDS-PAGE and 16S rDNA sequencing analysis as described previously [1]. The strains used in present study were registered in the DDBJ database. The accession numbers are shown in Table 1.

The LAB isolates were grown in MRS or modified trypticase phytone yeast (mTPY) [6] broth for *Bifidobacterium* species under anaerobic conditions for 24 hr at 37°C using a GasPak system (Becton Dickinson, Cockeysville, MD, U.S.A.). The harvested cells were washed three times with 1 m*l* of sterile phosphate-buffered saline (PBS) (8 g of NaCl, 0.2 g of KCl, 2.9 g of Na_2HPO_4, and 0.2 g of KH_2PO_4 per liter; pH 7.2) by centrifugation at 2,000 × g for 10 min at 20°C. The precipitated bacterial cells were suspended in PBS and adjusted to a McFarland No. 2 standard corresponding to about 6×10^8 cells/m*l*. ConA (Sigma Chemical, St. Louis, MO, U.S.A.) was resolved at a concentration of 0.1563 mg/m*l* in PBS. Twenty-five μ*l* of the adjusted bacterial cell suspension and an equal volume of ConA solution in PBS were mixed and left for 1 hr at room temperature. Agglutination of the mixtures was observed under a light microscope (Olympus, Tokyo, Japan) after Gram's staining, and was labeled as no agglutination (–), weak agglutination (+), or strong agglutination (++).

Inhibition testing of bacterial agglutination with ConA was carried out using D-glucose, D-galactose, and D-mannose. All carbohydrates were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The bacterial cells were adjusted to a McFarland No. 2 standard. After mixing with 25 μ*l* of the carbohydrate and an equal volume of ConA, the mixture left for 10 min at room temperature. Twenty-five μ*l* of the bacteria suspension was added into the mixture, and then the mixture was left for 1 hr at room temperature. The reaction was observed and labeled as mentioned above for ConA-agglutination of LAB. The assay was repeated three times.

As shown in Fig. 1, at first, the minimum concentration of ConA required to complete bacterial agglutination was determined to be 0.1563 mg/m*l*. Twenty-six out of 46 isolates tested agglutinated with ConA, a rate of 57%. No self-agglutination was observed in our experiment. Seven of the strains strongly agglutinated (Table 1). Although three *L. reuteri*, one *L. mucosae*, and two *E. canintestini* isolates

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Table 1. Agglutination of the isolates with ConA and inhibition of the agglutination by carbohydrates

Species[a)]	Strains[b)]	Accession no. based on DDBJ	ConA	ConA-agglutination in the presence of[c)]		
				Glucose	Galactose	Mannose
L. reuteri	DLM0802	AB186341	++[d)]	++	++	–
L. reuteri	DLM1712	AB186323	++	–	+	–
L. reuteri	DLC0512	AB186338	++	++	++	–
L. reuteri	DST0113	AB186318	+	+	+	–
L. reuteri	DST2702	AB186330	+	+	+	+
L. mucosae	DLS1003	AB186315	++	++	++	–
L. mucosae	DLC0203	AB186326	+	+	+	+
L. mucosae	DLC1110	AB186317	+	+	+	+
L. johnsonii	DLL0902	AB186343	+	–	–	–
L. salivarius ssp. salicinus	DLM2206	AB186325	+	–	+	–
L. murinus	DLM3108	AB186332	+	+	–	–
L. animalis	DLM3405	AB186334	+	+	+	–
B. pseudolongum	DBF0412	AB186304	+	+	+	–
B. animalis ssp. lactis	DBF1307	AB186296	+	+	+	–
B. animalis ssp. lactis	DBF1905	AB186297	+	+	+	–
B. longum bv. infantis	DBF0315	AB186299	+	+	+	–
B. subtile	DBF3208	AB186301	+	+	+	–
B. pseudocatenulatum	DBF0309	AB186303	+	+	+	–
B. pseudocatenulatum	DBF3210	AB186300	+	+	+	–
B. catenulatum	DBF3507	AB186302	+	–	–	–
S. bovis	DST1607	AB186306	+	–	+	–
E. canintestini	DLS0702	AB186313	++	–	–	–
E. canintestini	DLS3002	AB186308	++	–	–	–
E. faecalis	DST0401	AB186310	+	–	–	–
E. avium	DST0703	AB186314	+	+	–	–
E. faecium	DST3502	AB186309	+	–	–	–
L. johnsonii	DLM1701	AB186322	–	–	–	–
L. johnsonii	DLM2502	AB186328	–	–	–	–
L. gallinarum	DLH0804	AB186329	–	–	–	–
L. gallinarum	DLH3501	AB186335	–	–	–	–
L. kitasatonis	DLM0607	AB186339	–	–	–	–
L. kitasatonis	DLL0302	AB186333	–	–	–	–
L. kitasatonis	DLL1105	AB186319	–	–	–	–
L. acidophilus	DLM0206	AB186342	–	–	–	–
L. acidophilus	DLM1612	AB186321	–	–	–	–
L. acidophilus	DLH2306	AB186327	–	–	–	–
L. saerimneri	DLM0501	AB186337	–	–	–	–
L. saerimneri	DLM1401	AB186320	–	–	–	–
L. saerimneri	DLM2908	AB186331	–	–	–	–
L. salivarius ssp. salicinus	DLS0811	AB186340	–	–	–	–
L. murinus	DLS0306	AB186336	–	–	–	–
L. animalis	DLM0202	AB186324	–	–	–	–
L. animalis	DLM1101	AB186316	–	–	–	–
B. animalis ssp. animalis	DBF1007	AB186294	–	–	–	–
P. acidilactici	DST0701	AB186311	–	–	–	–
E. hirae	DST2010	AB186307	–	–	–	–

a) *L. Lactobacillus* spp.; *B. Bifidobacterium* spp.; *S. Streptococcus* spp.; *E. Enterococcus* spp.; *P. Pediococcus* spp.
b) The first two digits of the four-digit bacterial number (i.e. DLS*00***) express the dog sample number.
c) D-glucose, D-mannose, and D-galactose were adjusted at a concentration of 1.563 mg/m*l* of ConA for the inhibition test of ConA agglutination.
d) Agglutination was judged based on three scores as follows: strong agglutination (++), weak agglutination (+), and no agglutination or inhibition of ConA agglutination (–).

strongly agglutinated, other isolates agglutinated weakly, and the other 20 isolates did not agglutinate with ConA. In particular, *L. gallinarum, L. kitasatonis, L. acidophilus, L. saerimneri, B. animalis* ssp. animalis, *P. acidilactici*, and *E. hirae* did not agglutinate with ConA.

The results of inhibition tests of ConA-agglutination with canine LAB using three sugars are shown in Table 1. In the tests, the ConA-agglutination of all LAB isolates except for one *L. reuteri* isolate DST2702, and two *L. mucosae* isolates, DLC0203 and DLC1110, was completely inhibited by D-mannose at a concentration of 1.563 mg/m*l*. The ConA agglutination of 17 and 18 isolates was not inhibited by D-

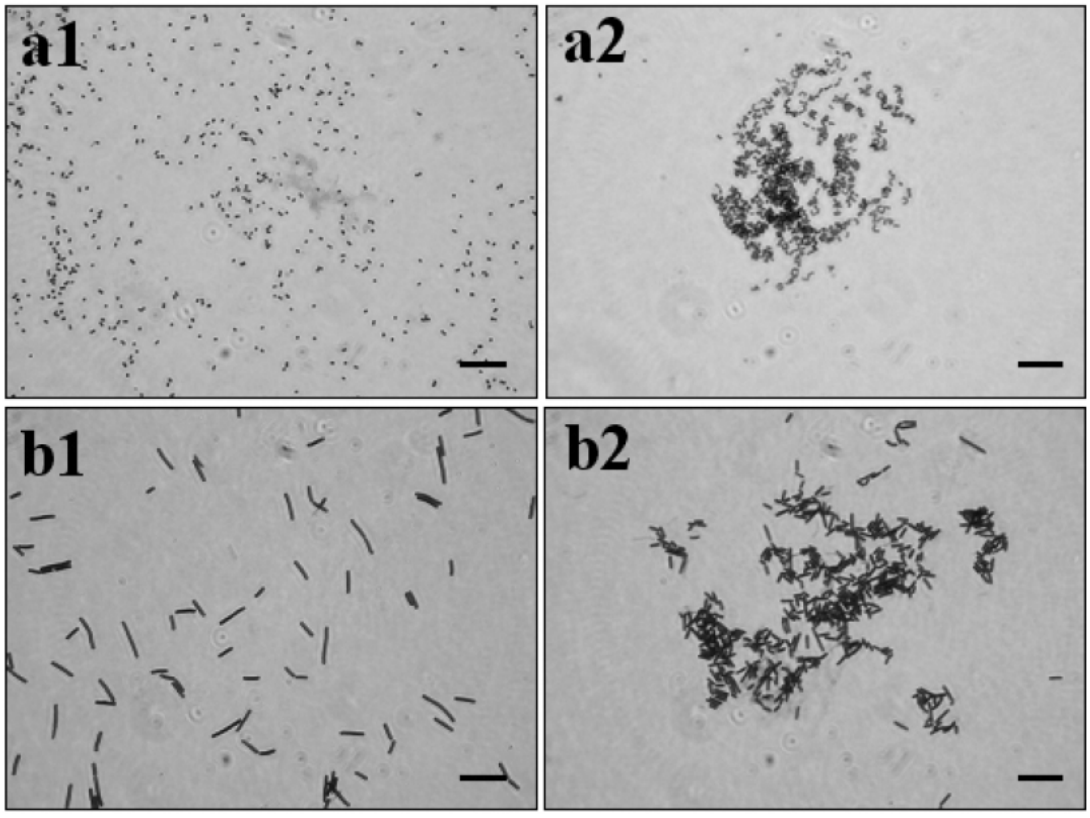

Fig. 1. ConA-agglutination of representative LAB isolates from dogs observed by Gram's staining. a1: No agglutination of *P. acidilactici* DST0701 with ConA. a2: Agglutination of *S. bovis* DST1607 with ConA. b1: No agglutination of *L. acidophilus* DLM0206. b2: Agglutination of *L. animalis* DLM3405. The concentration of ConA used for the agglutination test was 0.1563 mg/m*l*. Scale bar = 15 μm.

glucose or D-galactose, respectively. The ConA-agglutination of the isolates from genus *Bifidobacterium* was only inhibited by D-mannose.

ConA has been studied in regard to cancer cell surfaces and the identification and differentiation of microorganisms because of the presence of a ConA-binding site on the cells [3, 8]. In our experiment, ConA-agglutination of LAB and inhibition were used in an *in vitro* assay for adherence of LAB to epithelial cells. This technique may reflect the role of adherence of LAB to epithelial cells [5]. As shown in Table 1, LAB with strong ConA-agglutinability were found to be the *L. reuteri, L. mucosae*, and *E. canintestini* isolates. However, the agglutination features of ConA were different between the *L. johnsonii* and *B. animalis* ssp. *animalis* isolates. Therefore, the ConA-agglutination of LAB was isolate-specific, but not species-specific.

On the other hand, amongst the sugars used in the inhibition tests, D-mannose was the most effective inhibitor as shown in previous reports [2, 3]. Only D-mannose inhibited ConA-agglutination of the *B. animalis* ssp. *lactis* isolate. Accordingly, we speculate the presence of three types of binding sites based on our limited results from the inhibition test, *man-glu-gal, man-glu*, and *man*, contributed to the arrangement of strong to weak inhibition. This experiment may be useful for investigation of the determinants of binding sites on bacterial surfaces with ConA. We also speculate that it may be possible to reclassify LAB based on the carbohydrate arrangement of the binding sites in regard to ConA-agglutination. The ConA-agglutination of LAB was intimately related to bacterial surface materials, such as capsules, fimbriae, and polysaccharides that comprise the glycocalyx of LAB. Therefore, we believed there was interaction between the surface material of LAB and mucoidal materials on the epithelial cell surface [8]. Since ConA binding sites are located on the surface materials of LAB, their materials may play a role of LAB adhesion to epithelial cells in the intestinal tract. Therefore, ConA could be useful for detection of mannose-specific sites of LAB for research of the adhesion of LAB to the epithelial cells of the intestinal tract as described previously [4].

Accordingly, the biological characteristics of each LAB isolate in regard to the dog specificity of the isolates should be considered before usage for probiotic LAB.

REFERENCES

1. An, Y., Adachi, Y. and Ogawa, Y. 2004. *Ant. Sci. J.* **75**: 245–252.
2. Archibald, A. R. and Coapes, H. E. 1971. *Biochem. J.* **123**: 665–667.
3. Fuller, A. L. and MacDougald, L. R. 2002. *Parasitol. Res.* **88**: 118–125.
4. Fuller, R. 1975. *J. Gen. Microbiol.* **87**: 245–250.
5. Hamada, S., Gill, K. and Slade, H. D. 1977. *Infect. Immun.* **18**: 708–716.
6. Hassan, A. N., Frank, J. F. and Qvist, K. B. 2002. *J. Dairy Sci.* **85**: 1705–1708.
7. Kandler, O. and Weiss, N. 1986. Bergey's Manual of Systematic Bacteriology. Williams and Wilkins. Baltimore.
8. Savage, D. C. and Fletcher, M. 1985. Bacterial Adhesion. Plenum Press, New York.
9. Slifkin, M. and Doyle, R. J. 1990. *Clin. Microbiol. Rev.* **3**: 197–218.