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Short Communication

Influence of sugars, pre-treatment with acid and suspending medium on the interaction of *Salmonella* Typhimurium and *Salmonella* Enteritidis with concanavalin A

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ABSTRACT

The influence of experimental variables on the interactions of *Salmonella* Typhimurium L1388 (ST) and *Salmonella* Enteritidis L1225 (SE) with concanavalin A (con-A) were assessed. At a con-A concentration of 78.1 μg ml^{-1}, ST was agglutinated and SE was not. The con-A-induced agglutination of ST was completely inhibited by equal concentration of D-(+)-mannose, D-(+)-glucose, D-(+)-galactose, N-acetyl-D-glucosamine, trehalose, β-D-(+)-cellobiose and raffinose. Pre-treatment of the bacterial cells with acid did not alter the con-A reactivity of ST and SE. Change in the suspending medium from PBS to immunogold buffer did not alter the con-A reactivity of ST and SE. Interactions of lectins with microorganisms have great diagnostic clinical laboratory value in microbial typing, taxonomic classification and epidemiology.
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INTRODUCTION

The *Salmonella* serotypes Typhimurium and Enteritidis are more frequently isolated in human, swine, avian and bovine salmonellosis (Schwartz, 1999); and thus constitute a public health problem worldwide, particularly with respect to food safety (Lax et al., 1995). Typhimurium differs from Enteritidis in its O-antigenic make up: Typhimurium belongs to serogroup BO defined by the O4 antigen and Enteritidis belongs to serogroup DO as defined by the O9 antigen; both have the O12 antigen (Kaufmann, 1966; Liang-Takasaki et al., 1983).

Lectins are animal or plant proteins or glycoproteins of non-immune origin that react with specific terminal sugar residues and have more stable storage properties than antibodies. The interactions of lectins with microorganisms are significant as probes in studying carbohydrates of cell surfaces in bacteria, fungi and protozoa; and in addition, for characterizing bacterial cell components and detecting bacteriophage receptors (Etzler, 1986; Lis and Sharon, 1986; Korting et al., 1988). Consequently, lectins are used for typing of bacteria, fungi and protozoa through the detection of intra-strain variations in cell wall carbohydrate residues (Slifkin and Doyle, 1990; Moyes and Young, 1992; Annuk et al., 2001), for characterizing cellular or surface receptor sites (Damjanov, 1987) and have a role in clinical laboratory identification and taxonomic classification of many microorganisms (Slifkin and Doyle, 1990; Kellens et al., 1994; Hynes et al., 1999; Aabenhus et al. 2002; Munoz et al., 2003).

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The pattern of *Salmonella* disease is known to be determined by the infecting serotype. This makes the investigation of how these serotypes behave under changing environments very important. Since the influence of culture conditions on con-A-induced aggregation of *Salmonella* serotypes have not been elucidated, it forms the primary objective of this study. Thus, this study investigates the influence of the presence of sugars, pre-treatment of *Salmonella* with acid and change in the medium containing *Salmonella* on the in vitro interaction of *Salmonella* with lectin.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Salmonella Typhimurium L1388 and *Salmonella* Enteritidis L1225, isolated from chicken in Japan, were used in this study. The strains were propagated in either trypticase soy broth (TSB; BBL, U.S.A.) or grown on trypticase soy agar (TSA; BBL, U.S.A.). Unless otherwise indicated, all chemicals used were purchased from Wako Chemical Company, Japan.

Concanavalin A-*Salmonella* interaction assay

Concanavalin A induced aggregation of bacteria was assessed as thus described. Bacterial cells were harvested (6000 rpm, 10 min) at room temperature from 2 ml of overnight culture of bacteria grown in TSB broth at 37°C and re-suspended in 2 ml of 1.0% formalin (phosphate buffered, pH 7.0) for 4 h. The formalized cells were then recovered by centrifugation (6000 rpm, 10 min) at room temperature, washed two times with phosphate buffered saline (PBS, pH 7.4) and re-suspended in same to a final volume of 2 ml. Equal volumes (25 µl) of the bacterial suspension and con-A in PBS (156.2 µg ml^{-1}) were mixed on a white tile at room temperature for 1 h, following which binding of cells was examined under a dark field microscope (Nikon Inc., USA). Equal volume mixture of bacteria and PBS served as negative control for the assay.

Effect of sugars on concanavalin A interaction with *Salmonella*

The effect of D-(+)-mannose and other carbohydrate substances on con-A-induced bacterial cell aggregation of ST was evaluated by dark field microscopy of 1-h equal volume mixture of bacterial suspension, sugar (156.2 µg ml^{-1}) and con-A (156.2 µg ml^{-1}). Equal volume mixture of bacteria suspension and PBS, and bacteria suspension and con-A served as negative and positive controls respectively.

Effect of acid treatment on concanavalin A interaction with *Salmonella*

The effect on concanavalin A agglutination, of acid treatment, was also evaluated. Bacterial cells were harvested (6000 rpm, 10 min) at room temperature from 2 ml of overnight culture of bacteria grown in TSB broth at 37°C and re-suspended in 2 ml of 1.0% formalin (phosphate buffered, pH 7.0) for 4 h. The formalized cells were then recovered by centrifugation (6000 rpm, 10 min) at room temperature, washed two times with phosphate buffered saline (PBS, pH 7.4), re-suspended in 0.2N HCl for 1 h at room temperature and neutralized by addition of few drops of 0.1N NaOH. Cells from the neutral mixture were harvested, washed three times with PBS and re-suspended in same to final volume of 2 ml. Equal volumes (25 µl) of the bacterial suspension and con-A in PBS (156.2 µg ml^{-1}) were mixed on a white tile at room temperature for 1 h, following which binding of cells was examined under a dark field microscope (Nikon Inc., USA). Equal volume mixture of acid-treated bacterial suspension and PBS, and untreated bacterial suspension and con-A served as negative and treatment controls respectively.

Effect of suspending medium on concanavalin A interaction with *Salmonella*

Bacterial cells were harvested (6000 rpm, 10 min) at room temperature from 2 ml of overnight culture of bacteria grown in TSB broth at 37°C and re-suspended in 2 ml of 1.0% formalin (phosphate buffered, pH 7.0) for 4 h. The formalized cells were then recovered by centrifugation (6000 rpm, 10 min) at room temperature, washed two times with immunogold buffer (10.4 mM Na_2HPO_4, 3.2 mM KH_2PO_4, 150 mM NaCl, 20 mM NaN_3, 0.1% bovine serum albumin; pH 7.4) and re-suspended in same to a final volume of 2 ml. Equal volumes (25 µl) of the resulting bacterial suspension and 0.1563 mg ml^{-1} of

con-A in phosphate buffered saline (PBS, pH 7.0) were mixed on a white tile at room temperature for 1 h, following which binding of cells was examined under a dark field microscope (Nikon Inc., USA). Equal volume mixture of immunogold buffered-bacterial suspension and immunogold buffer, PBS-suspended bacteria and con-A were provided for as negative and treatment controls respectively.

RESULTS

Concanavalin A induced the agglutination of ST but not of SE. Results (not shown) identical to those with formalin-fixed, phosphate-buffered saline-washed cells were obtained also with non-formalized ST and SE cells.

D-(+)-mannose, D-(+)-glucose, D-(+)-galactose, N-acetyl-D-glucosamine, trehalose, β-D-(+)-cellobiose and raffinose completely inhibited con-A-induced cell aggregation of ST. Maltose and lactose however, did not affect the agglutination of ST by con-A.

Pre-treatment of the bacterial cells with acid did not alter the con-A reactivities of ST and SE.

Change in the suspending medium from PBS to immunogold buffer did not alter the con-A reactivities of ST and SE.

DISCUSSION

Concanavalin A (hearafter called "con-A") has proven to be a useful lectin. It is extracted from Jack bean (*Canavalia ensiformis*) and reacts with non-reducing terminal α-D-Glucose and α-D-mannose. An initial evidence that con-A reacts with macromolecules that are devoid of terminal glucopyranose or mannopyranose residues was provided by Doyle et al. (1968). The conformation of concanavalin A is pH-dependent; at pH 4.5-5.6, it exists as a dimmer; above pH 7, it is predominantly tetrameric (McKenzie et al., 1972; Wang et al., 1975].

Lectin-induced microbial aggregation involves specific interactions between the lectin and cell surface carbohydrate residues. The observation that ST (and not SE) was agglutinated by con-A, suggests differences in the carbohydrate moieties of the Typhimurium and Enteritidis strains. This is in tandem with a report in which *Salmonella* Enteritidis failed to interact with concanavalin A (Calderon et al., 1997). It is also our opinion that fimbrial (H) proteins are not involved in the interaction of ST as indicated by the similar reactivity pattern for both formalized and non-formalized cells of the two serovar types tested.

Inhibition of con-A aggregation of ST by all the sugars tested (but maltose and lactose), suggests the presence, in ST, of non-reducing terminal α-D-Glucose and α-D-mannose that competes with the exogenous sugars introduced. This means that growth of the *Salmonella* Typhimurium strain used in this study in medium containing the inhibiting carbohydrates will alter the specificity of con-A in epidemiological typing studies.

Pre-treatment of bacteria with acid had no influence on the con-A agglutination of both serovar types. The main aim of the acid pre-treatment of ST and SE before reaction with con-A was to uncover additional con-A reactive sites. However, it is obvious from the results that no new sites were created by the acid treatment.

Change in suspending medium apparently also had no influence on the con-A agglutination of both serovar types. This contradicts a report on *Mycobacteria* where con-A in PBS seem to have relatively high reactivity with the bacteria than con-A in glycine buffer (Claderon et al., 1997). This has great significance in suggesting that immunogold buffer (a buffer used commonly in direct microscopical immunodetection of bacteria) does not alter lectin-*Salmonella* interaction when used in typing studies.

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