Lecithinase-positive Clostridia Isolated from Human Feces on Consumption of Lactulose and Lactosucrose

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Lecithinase-positive clostridia including *Clostridium perfringens* isolated from feces of nine healthy adults each fed lactulose (3 g/day for 2 wk) or lactosucrose (2 g/day for 1 wk) were examined. Before the consumption of lactulose or lactosucrose, lecithinase-positive clostridia in fecal specimens were detected in 9/9 (mean 3.9×10^5 /g) and 8/9 (4.5×10^5 /g) specimens, respectively. On consumption of lactulose or lactosucrose, they were detected in 4/9 (4.2×10^3 /g after 2 wk) or 6/9 (2.9×10^4 /g after 1 wk) specimens, respectively.

Clostridia isolated from 18 fecal specimens before consumption were identified as *C. perfringens* (10^2 – 10^8 /g) from 17/18 specimens, *C. sordellii* (10^3 – 10^5 /g) and *C. baratii* (10^2 /g) from 2 specimens each, and *C. bifermentans* (10^3 /g) from 1 specimen, but only *C. perfringens* from 4/9 (10^2 – 10^5 /g) and 6/9 (10^2 – 10^7 /g) specimens on lactulose and lactosucrose consumption, respectively.

Key words: Lactulose, Lactosucrose, Human feces, Lecithinase-positive clostridia.

Introduction

Lecithinase-positive (L(+)) clostridia including *Clostridium perfringens* are one of the harmful bacteria in the intestine of humans and animals¹⁷. Especially, *C. perfringens* is etiologically related to food poisoning, and enteric infections in humans and animals^{8, 22}. Heat treatment of fecal specimens has been recommended for the recovery of clostridia from food poisoning caused by heat-resistant bacteria⁸ and animals¹³. TERADA *et al.*²⁶ recommended that specimens be treated at 80°C for 10 min in parallel with unheated specimens with both selective and non-selective media for the enumeration of L(+) clostridia in fecal specimens, when the numbers are less than 10^4 /g.

The enumeration of L(+) clostridia in human feces on oligosaccharide consumption has not been reported previously. Therefore, in the present study we examined by this recommended method the incidence and enumeration of L(+) clostridia including *C. perfringens* from human feces during the consumption of lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose) and lactosucrose (4-G-β-D-galactosylsucrose or O-β-D-galactopyranosyl-(1→4)-O-α-D-glucopyranosyl-(1←2)-β-D-fructofuranoside).

Materials and Methods

Subjects and diet

Two groups of nine subjects were chosen from healthy male students aged 18 to 28 years. They consumed the normal free-choice diet for 2 wk prior to the consumption of oligosaccharides. During the experiment, one group of subjects were given once a day a drink supplemented with lactulose at 3 g/day for 2 wk, and the other group were given lactosucrose at 2 g/day for 1 wk. They did not receive any medication or foods with abundant viable bacteria for 2 wk prior to or during the experiment.

This work was performed in accordance with the Helsinki Declaration as updated in Tokyo, 1975.

Bacteriological analysis

Fecal specimens were collected from each subject before oligosaccharide consumption, and on days 14 and 7 during consumption. L(+) clostridia including *C. perfringens* were analyzed by the method of TERADA *et al.*²⁶⁾ Two grams of feces were dissolved in 18 ml of anaerobic diluent solution described by Mitsuoka¹⁵⁾, and serial 10-fold dilutions (10^{-2} – 10^{-8}) were prepared. Aliquots of 0.05 ml of each dilution were inoculated onto BL (glucose blood liver) agar and EG (Eggerth-Gagnon) agar plates in non-selective media¹⁵⁾ and NN (neomycin-Nagler) agar plates¹⁵⁾ and CW (*Clostridium welchii*) agar (Nissui Seiyaku Co., Ltd, Tokyo) in selective media. Then, the dilutions (10^{-1} – 10^{-5}) were placed at 80°C for 10 min in a water bath for isolating spores of clostridia, and 0.1 ml of each dilution was inoculated onto the surfaces of BL agar and/or EG agar and NN agar and/or CW agar. The plates were incubated at 37°C for 48 h in an anaerobic steel wool jar²¹⁾ filled with 100% CO gas. The results are expressed as the number of bacteria per gram of feces. The isolates were identified by the method described by MITSUOKA¹⁵⁾.

Results and Discussion

The detection rate and the counts of L(+) clostridia including *C. perfringens* before and after lactulose consumption are shown in Table 1. Before lactulose consumption, L(+) clostridia was isolated in the order of 10^3 – 10^8 /g in 6/9 unheated specimens, and of 10^2 – 10^6 /g in all the heated specimens. On day 14 of lactulose consumption, *C. perfringens* was detected at levels of 10^3 – 10^5 /g in 2/9 unheated specimens, and of 10^2 – 10^4 /g in 4/9 heated specimens.

The results of L(+) clostridia before and after lactosucrose consumption are shown in Table 2. Before lactosucrose consumption, L(+) clostridia was isolated at counts of 10^4 – 10^7 /g in 6/9 unheated specimens, and of 10^2 – 10^6 /g in 8/9 heated specimens. On day 7 of lactosucrose consumption, *C. perfringens* was isolated in numbers of 10^3 – 10^7 /g in 3/9 unheated specimens, and of 10^2 – 10^5 /g in 6/9 heated specimens. L(+) clostridia isolated from the fecal specimens before consumption of either oligosaccharide were identified as *C. perfringens* from 17/18 specimens, *C. sordellii* and *C. baratii* from 2 specimens each, and *C. bifementans* from 1 specimen, but only *C. perfringens* from 4/9 and 6/9 specimens after lactulose and lactosucrose consumption, respectively. It is possible that the decrease tendency of L(+) clostridia may have been due to a lowering of the intestinal pH as a result of metabolism of the oligosaccharides by increased levels of bifidobacteria.

Before oligosaccharide consumption, the counts of L(+) clostridia were 10^1 – 10^2 /g higher on EG and BL agar plates inoculated without heated specimens than on NN and CW agar plates, when they were present in 10^7 /g or more. When L(+) clostridia was present under 10^6 /g, they were

Table 1. Lecithinase-positive clostridia isolated from human feces during lactulose (3 g/day) consumption

Medium	Before consumption				Lactulose consumption			
	Unheat treatment		Heat treatment		Unheat treatment		Heat treatment	
	BL & EG	NN & CW	BL &/or EG	NN &/or CW	BL & EG	NN & CW	BL &/or EG	NN &/or CW
Sample No.								
1	$2.0 \times 10^{8*1a}$	4.0×10^{6c}	3.8×10^{6a}	2.5×10^{5c}	ND	ND	1.2×10^{4a}	1.2×10^{4c}
2	2.0×10^{7b}	1.6×10^{5c}	2.0×10^{4b}	1.0×10^{5d}	ND	ND	ND	ND
3	2.0×10^{7b}	8.0×10^{6c}	2.2×10^{5b}	6.0×10^{4c}	ND	ND	ND	ND
4	ND	4.0×10^{6c}	3.0×10^{5a}	4.2×10^{5c}	ND	1.2×10^{5c}	8.0×10^{3b}	1.0×10^{4d}
5	ND	2.4×10^{5d}	2.4×10^{5a}	2.0×10^{5d}	ND	ND	ND	ND
6	ND	8.0×10^{3d}	2.0×10^{3b}	1.0×10^{3c}	ND	1.0×10^{3c}	1.0×10^{3b}	2.0×10^{2c}
7	ND	ND	7.6×10^{4b}	2.0×10^{3c}	ND	ND	ND	ND
8	ND	ND	1.3×10^{4b}	7.2×10^{3c}	ND	ND	2.0×10^{2a}	2.0×10^{2c}
9	ND	ND	4.0×10^{2b}	ND	ND	ND	ND	ND

*1 Data are expressed as the counts of *Clostridium perfringens* per gram feces that grew on ^aEG (modified Eggerth-Gagnon) agar, ^bBL (Glucose blood liver) agar, ^cNN (Neomycin-Nagler) agar, and ^dCW (*Clostridium welchii*) agar. *Clostridium bifementans* (1.0×10^3 /g), *C. baratii* (4.0×10^2 /g) and *C. sordellii* (2.0×10^5 /g) were isolated from specimens No. 2, No. 4 and No. 1 before the administration, respectively.
ND; Not detected.

Table 2. Lecithinase-positive clostridia isolated from human feces during lactosucrose (2 g/day) consumption

Medium	Before consumption				Lactosucrose consumption			
	Unheat treatment		Heat treatment		Unheat treatment		Heat treatment	
	BL & EG	NN & CW	BL &/or EG	NN &/or CW	BL & EG	NN & CW	BL &/or EG	NN &/or CW
Sample No.								
1	$4.6 \times 10^{7*1b}$	2.0×10^{7c}	2.0×10^{6b}	2.0×10^{6c}	ND	4.0×10^{6c}	1.6×10^{5b}	3.0×10^{5c}
2	2.8×10^{7b}	2.0×10^{6c}	2.0×10^{6a}	4.0×10^{5d}	1.4×10^{7b}	1.0×10^{7c}	3.2×10^{5b}	1.8×10^{5c}
3	2.6×10^{7a}	2.0×10^{6d}	2.0×10^{6b}	1.0×10^{5d}	ND	ND	ND	ND
4	ND	7.8×10^{5c}	2.0×10^{5b}	1.0×10^{5c}	ND	5.6×10^{3d}	2.0×10^{4a}	2.0×10^{4d}
5	ND	3.2×10^{5d}	2.0×10^{5b}	1.2×10^{5c}	ND	ND	ND	ND
6	ND	8.0×10^{4c}	3.6×10^{3a}	2.0×10^{4d}	ND	ND	3.0×10^{3b}	6.8×10^{3d}
7	ND	ND	3.0×10^{3b}	2.0×10^{2c}	ND	ND	2.0×10^{2a}	ND
8	ND	ND	8.0×10^{2a}	6.0×10^{2c}	ND	ND	ND	ND
9	ND	ND	ND	ND	ND	ND	4.0×10^{2b}	2.0×10^{2c}

*1 Data are expressed as the counts of *Clostridium perfringens* per gram feces that grew on ^aEG (modified Eggerth-Gagnon) agar, ^bBL (Glucose blood liver) agar, ^cNN (Neomycin-Nagler) agar, and ^dCW (*Clostridium welchii*) agar. *Clostridium sordellii* (2.0×10^3 /g) and *C. baratii* (3.0×10^2 /g) were isolated from specimens No. 6 and No. 5 before the administration, respectively.
ND; Not detected.

isolated on NN and CW agar plates inoculated without heated specimens, or EG, BL, NN and CW agar plates inoculated with heated specimens, but not on EG or BL agar plates inoculated without heated specimens. After lactulose and lactosucrose consumption, the detection rates (44.4% and 66.7%, respectively) of spores of *C. perfringens* were increased in comparison with the numbers (22.2% and 33.3%) of vegetative forms of *C. perfringens*, with no correlation to media used. These results suggested that heat treatment for isolating L(+) clostridia including *C. perfringens* must be used to determine the composition and metabolic activity of the human fecal flora on oligosaccharide consumption.

With heat treatment, the detection of L(+) clostridia on days 4 and 7 of lactulose consumption (3 g/day) showed a tendency to decrease compared with that on day 14, and the results on day 14 of lactosucrose (1 g, 2 g or 3 g/day) consumption were decreased more effectively than on day 7 (data not shown).

Although the numbers of clostridia were 10–20 times lower in heated specimens than those in unheated specimens, the detection rate of L(+) clostridia in heated specimens was double that in unheated specimens. These findings suggest that heat treatment may severely damage bacterial spores, and that clostridia are inhibited by quick multiplication of coexisting organisms when present in low numbers. Decreasing tendencies of *C. perfringens* in fecal specimens have been observed during the consumption of raffinose²⁾ and fructooligosaccharides⁷⁾. In the present study, L(+) clostridia including *C. perfringens* were decreased during lactulose and lactosucrose consumption as in our previous reports^{5, 18, 23–25)}. Some investigators^{3, 6, 9–12, 19, 20)}, however have found no definite decrease in the frequency of occurrence or the levels of *C. perfringens* during consumption of oligosaccharide. This discrepancy may be considered to be related to the methodological differences for isolation of L(+) clostridia²⁶⁾, or to individual differences in the subjects studied^{1, 4, 14, 16, 17)}.

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ラクチュロースおよび乳果オリゴ糖摂取時におけるヒト糞便内 レシチナーゼ陽性 *Clostridium*

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ラクチュロース (3 g/日, 2 週間), または乳果オリゴ糖 (2 g/日, 1 週間) を摂取させた各 9 人の健康成人の糞便内レシチナーゼ陽性 *Clostridium* の菌種, 検出率と菌数を測定した結果, ラクチュロースまたは乳果オリゴ糖摂取前にはそれぞれ 9 例中 9 例 (平均 3.9×10^5 /g) と 9 例中 8 例 (4.5×10^5 /g) から本菌が検出されたが, ラクチュロースまたは乳果オリゴ糖摂取後はそれぞれ 9 例中 4 例 (4.2×10^3 /g) と 9 例中 6 例 (2.9×10^4 /g) と減少傾向を示した. なお, レシチナーゼ陽性 *Clostridium* の菌種としてはオリゴ糖摂取前で *Clostridium perfringens* ($10^2 \sim 10^8$ /g) が 18 例中 17 例, *C. sordellii* ($10^3 \sim 10^5$ /g) と *C. baratii* (10^2 /g) が各 2 例, *C. bifermentans* (10^3 /g) が 1 例から検出されたが, ラクチュロースと乳果オリゴ糖摂取では *C. perfringens* のみがそれぞれ 9 例中 4 例 ($10^2 \sim 10^5$ /g) と 9 例中 6 例 ($10^2 \sim 10^7$ /g) 検出された.