ANALYSIS OF GUANINE PLUS CYTOSINE CONTENTS OF DNA OF *Butyrivibrio fibrisolvens* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

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Summary

High performance liquid chromatography (HPLC) analysis was applied for determination of guanine plus cytosine (G + C) contents of DNA of *Butyrivibrio fibrisolvens*. By values of G + C contents, a reference strain and 20 wild strains of *B. fibrisolvens* were classified into at least two distinct subgroups, i.e. G + C contents of 18 strains were 38-40 mol% and those of 3 strains including the reference strain were 43-45 mol%. Clear relationships were not observed between G + C contents and biological properties of 21 strains of *B. fibrisolvens*.
(**Key Words**: *Butyrivibrio fibrisolvens*, G + C Contents, HPLC, Rumen Bacteria)

Introduction

Guanine plus cytosine (G+C) contents of DNA have been accepted as one of the most important criteria for bacterial taxonomy and is required for identification of new species of bacteria. Recently, high performance liquid chromatography (HPLC) was applied to determine G+C contents of aerobic and facultative anaerobic bacteria, yeast, and phage (Katayama-Fujimura et al., 1984; Tamaoka and Komagata, 1984; Katoh et al., 1986). A number of previous studies have clearly shown that *Butyrivibrio fibrisolvens* strains are quite variable in phenotypic traits. Recent studies have indicated that *B. fibrisolvens* is collection of genetically diverse strains that differ in the hybridization level between DNAs (Mannarelli, B. M., 1988) and in the composition of the extracellular polysaccharides produced by these strains (Stack, R. J., 1988). These data suggest that *B. fibrisolvens* consist of several different species.

In the present study, we applied HPLC to investigate the G + C contents of a refrence strain and strains of *B. fibrisolvens* to clarify the relationship between genetic diversity and biological properties.

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Materials and Methods

Bacterial strains

Butyrivibrio fibrisolvens A-38 and 20 wild strains of *B. fibrisolvens* that were isolated in this laboratory were used.

DNA base composition assay

Bacterial strains were cultured in Medium 2 broth (Hobson, 1969). DNA was isolated from bacterial cells in logarithmic phase. The purified nucleotide preparation was treated with nuclease P1 (Yamasa Shoyu Co.,) as described by Suzuki et al. (1987) with some modification. One mg of DNA in the preparation was first mixed with 2.0 ng nuclease P1 in 2.0 ml of 10 mM sodium acetate buffer contained Zn^{2+}, pH 5.3, and was incubated at 50℃ for 30 min. The resultant solution was heated in a boiling bath for 15 min and rapidly cooled in an ice bath. The solution was treated again with 2.0 μg of nuclease P1 at 50℃ for 2 hr. After centrifugation, 5-20 μl of the solution containing a standard mixture of authentic dNMPs (dAMP, dCMP, dGMP and dTMP) was subjected to HPLC analysis.

HPLC liquid chromatograph, PU-880 (JASCO, Japan), with a reversed phase column (4.6 mm i.d. by 15 cm), Crestpak C18S (Japan Spectroscopic Co.,) was employed. dNMPs were eluted out with 0.2 M $NH_4H_2PO_4$, at 1.0 ml/min at 27℃ and detected with spectrophotometric detector, UV-870 (JASCO, Japan) at 260 nm. The retention time and area were measured with a SIC chromatopak, chromatocorder 12 (System Instrument Co.,).

Analysis of VFA of *B. fibrisolvens*

Analysis of VFA was carried out as described by Holdeman et al. (1977).

Analysis of metabolism of carbohydrate substrates by *B. fibrisolvens*

B. fibrisolvens was cultured in PY + 1% carbohydrate substrates at 37℃ for 5 days, and the pH of the medium was measured. Strains which showed less than pH 6.0 in the media were judged to fermentate added carbohydrate substrates.

Results

G + C contents of a reference strain and 20 wild strains of *B. fibrisolvens* were measured by HPLC (table 1). G + C contents of the reference strain was 44.4 mol% and wild strains were from 37.8 to 43.6 mol%. They were classified into at least two distinct subgroups. Eighteen strains belonged to a group with G+C contents of 38-40 mol% and the reference strain and 2 wild strains belonged to a group with 43-43%.

TABLE 1. G + C CONTENT OF *B. fibrisolvens* STRAINS BY HPLC

Strain No.	G + C content (mol%)
1	39.1
2	39.9
3	37.8
4	39.0
5	39.5
6	38.6
7	38.9
8	38.6
9	38.5
10	39.5
11	38.8
12	39.5
13	38.5
14	38.5
15	39.3
16	38.6
17	38.9
18	38.9
19	43.6
20	43.6
A-38[1]	44.4

[1]Reference strain

Production of VFAs (acetic acid, n-butyric acid, lactic acid and succinic acid) by *B. fibrisolvens* were determined (table 2). Although phenotypic diversity among these strains was observed in the production of acetic acid, lactic acid and succinic acid, obvious relationship between the production and G + C contents were not recognized.

Fermentation of carbohydrates by *B. fibrisolvens* was examined (table 3). Although phenotypic diversity of strains in metabolism of mannose, lactose, raffinose, trehalose and mannitol were observed, direct relationships between the fermentation and G + C contents were not shown.

Discussion

Analysis of G + C contents of the strains of *B. fibrisolvens* showed that there is considerable genetic diversity. A number of studies have shown that strains that differ in their G + C contents more than 2 mol% can be expected to be different species (Marmur et al., 1963). Mannarelli (1988) reported that G + C contents of 39 strains of *B. fibrisolvens* ranged from 39.0 to 49.2 mol% by determination from their buoyant density. Therefore, the value of G + C contents observed in this study indicate that 21 strains of *B. fibrisolvens* including reference strain are classified into at least two distinct subgroups. In recent studies Hudman and Gregg (1989) reported that 8 strains of *B. fibrisolvens* could be divided into four homology groups by DNA hybridization studies. Mannarelli (1988) also reported that DNA reassociation among 39 strains present an even more complex pattern and actually comprise a number of distinct species and, possibly, several genera. Therefore, DNA-homology analysis should be performed to clarify genetic diversity of strains of *B. fibrisolvens*.

In the present study, we investigated biological diversities among the strains in fermentation of carbohydrates and production of VFAs. Although several phenotypic diversities were observed in both experiments, direct relationships were not recognized between G + C contents and those diversities. Diversity among strains of *B. fibrisolvens* has been reported in size, shape, flagella, motility, substrates fermented or hydrolyzed, products, biochemical tests, cell wall compositions and nutritional require-

TABLE 2. PRODUCTION OF VOLATILE FATTY ACIDS BY *B. fibrisolvens*

VFA Production	Strain number																				
	38-40%[1]																		43-45%		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	A38[2]
Acetic acid	−	−	+	−	−	−	+	−	−	+	+	−	+			−	+	+	+	−	+
N butyric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Lactic acid	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Succinic acid	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+

[1] G + C content.
[2] Reference strain.

TABLE 3. CARBOHYDRATE SUBSTRATES METABOLIZED BY *B. fibrisolvens*

Carbohydrate	Strain number																				
	38-40%[1]																		43-45%		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	A38[2]
Fuructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	−	+
Rhaffinose	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	
Trehalose			+			−	−	+	+		+	−	−	+	+	+	+	−	−	−	−
Mannitol	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Sorbitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Glycerol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[1] G + C content.
[2] Reference strain.

ments (Cheng and Costern, 1977; Bryant and Small, 1956; Hazlewood and Dawson, 1975; Shane et al., 1969). Therefore, *B. fibrisolvens* should be investigated in detail for phenotypes, DNA homology and G + C contents.

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