CELL WALL COMPOSITION IN THE
CLASSIFICATION OF VIBRIO FETUS¹

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ABSTRACT. Cell walls of most Biotype 1 strains of V. fetus contained galactose and mannose; one type 1 strain had galactose and glucose. Cell walls of most Biotype 2 strains also had galactose and mannose; however, some strains had galactose, glucose and mannose, some galactose alone and some galactose and rhamnose. Cell walls of most Biotype 3 strains had galactose and glucose; a few had galactose, glucose and mannose, or galactose alone. Whole cells of V. fetus contained small amounts of mesodiammopimelic acid (DAP), but meso-DAP could not be detected in purified cell walls.

Vibrio fetus is an organism that is responsible for abortion in cattle and sheep. It also causes clinical disease in man with symptoms of fever, chills, respiratory involvement and diarrhea.

We have studied about 250 phenotypic characteristics of over 200 strains of V. fetus isolated from man, animals, and birds. Results indicate that V. fetus may be divided into three distinct groups on the basis of phenotypic characteristics, Table 1. The first, called biotype 1, is composed of organisms infecting cattle by way of venereal transmission. These organisms usually do not produce hydrogen sulfide. Strains

Table 1. Differential Characteristics of Vibrio fetus

Biotype	mode of Transmission	H ₂ S*	Growth		
			1% glycine	25C	45C
Biotype 1	Venereal	V **	-	+	-
Biotype 2	Intestinal	+	+	+	-
Biotype 3	Intestinal	+	+	-	+

* = lead acetate impregnated paper strips.

** = these strains are usually negative, but some (Type 1A) will cause slight grayish coloring to the lead acetate paper strips after 7 days incubation.

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that are weakly H_2S positive in 7 days have been called Type 1A by Bryner *et al.* (2). They fail to grow in a medium with 1% glycine. Growth occurs at 25° C, but not at 42° C. The second and third groups called biotypes 2 and 3 represent strains infecting cattle, sheep, birds and man as well as isolates from the intestinal tracts of normal animals and birds. These organisms are transmitted to animals and probably to man by way of the oral route and can establish themselves in the intestinal tract of animals. Biotype 1 strains, however, will not grow in animal intestinal tracts. The biotype 2 and 3 strains are designated *V. fetus* var. *intestinalis* and the type 1 strains *V. fetus* var. *fetus*. Biotype 2 strains causes abortion in cattle and sheep, and infect man. They are all H_2S positive, grow in 1% glycine and grow at 25° C. The biotype 3 strains are involved in abortion in sheep and also infect man. The human strains are the "related vibrios" described by King (6). This biotype also represents organisms isolated from the intestinal tracts of normal sheep, cattle, swine, chickens, turkeys, starlings, sparrows, and pigeons (8, 9). These strains are all H_2S positive, grow in 1% glycine, and grow at 42° C. but not at 25° C. In addition, most of these strains (70%) are RNase and or DNase positive, while those of biotypes 1 and 2 are all negative for these characters.

Vibrio fetus has a DNA base composition (G + C) of 32-35 moles percent (1, 7). In contrast, *Vibrio cholerae* and the EL-Tor vibrios base composition is 46 to 48% GC (3). *Spirillum* spp. have a base composition of 49 to 61% GC (7). *Vibrio fetus* is not phenotypically similar to *V. cholerae* and has a different GC percentage in its DNA composition. Because of these differences Sebald and Vernon (7) have suggested that *V. fetus* should be renamed *Campylobacter fetus*.

In a recent article by Basden, *et al.* (1), a bovine biotype 1 strain and a human and ovine biotype 2 strain were shown to be homologous (100%) using DNA-RNA hybridization. An avian biotype 3 strain had a very low homology when compared to the bovine type 1 strain (15.4%).

The cell wall composition of the 3 biotypes of *V. fetus* is now under investigation. Our interest in cell wall chemistry is to determine if wall composition can be applied to the classification of *V. fetus*.

Hydrolysates of whole cells of 50 strains of *V. fetus* representing the 3 biotypes contained small amounts of *meso*-diaminopimelic acid (DAP). Cell wall material was prepared from *V. fetus* in two ways. Cell wall fractions from a biotype 3 human strain (5749) of *V. fetus* were prepared in a Braun disintegrator, and the crude walls digested with pronase, washed, and lyophilized. Weighed amounts of walls were hydrolyzed and amounts of hydrolysate equivalent to 0.5, 1.0 and 2.0 mg of cell walls were chromatographed. No DAP could be detected in the hydrolysates, although whole cell hydrolysates (representing 1.8 and 3.6 mg of cells) of the same strain contained a small amount of *meso*-DAP. In addition, DAP was not found in cell walls prepared in the same way from 4 biotype 1 strains and 9 biotype 2 strains.

In another experiment whole cells from one strain of each biotype (482, 1925 and 5749) were lysed with sodium lauryl sulphate, and the residual material treated with phenol and finally digested with pronase. No DAP could be detected in these cell wall hydrolysates from any of the 3 strains, although *meso*-DAP was readily detected in similar material from one strain each of *Escherichia coli* and *Spirillum serpens*. Hydrolysates of whole cells of the 3 strains of *V. fetus* contained small

amounts of meso-DAP. Weighed amounts of lyophilized whole cells of strains 5648, 942 and UM were hydrolyzed and amounts of hydrolysate equivalent to 1.3 mg, 2.7 mg and 2.2 mg respectively were chromatographed. Meso-DAP was readily detected in material from all 3 strains.

Fung and Winter (4) studied the chemical composition of uridine nucleotides recovered from penicillin and glycine treated cultures of V. fetus. They reported that some of the uridine-diphosphate-acetyl muramic acid nucleotides contained DAP. The amino acids of the uridine nucleotides were glutamic acid, glycine, and alanine. These results are different than ours since we did not find DAP in the cell wall preparations of V. fetus but only in hydrolysates of whole cells. One of the strains (UM) used by Fung and Winter was also used in the present study.

Keeler *et al.* (5) in their study of cell walls of V. fetus reported that cell wall material contained a small amount of either cysteine or DAP. They were in doubt as to the identity of the compound. Three of the strains (436, 482 and 653) used in their study were also used in the present investigation. Cell walls prepared from strains 482 and 653 did not contain DAP.

Results of examination of the carbohydrate content of whole cell hydrolysates of 7 biotype 1 strains are in Table 2. All seven strains contained galactose. Six strains contained galactose and mannose, while one strain contained galactose and glucose. Cell walls prepared from 482 contained galactose and mannose.

Table 2. Carbohydrate composition of Cell Walls of Biotype 1 strains of Vibrio fetus.

Strain No.	Biotype	galactose	glucose	mannose	rhannose
482	1A*	+	-	+	-
21085	1	+	-	+	-
942	1	+	-	+	-
UM	1	+	-	+	-
998	1	+	-	+	-
66-312	1	+	-	+	-
490	1A	+	+	-	-

* 1A = strains that are weakly H₂S positive.

Whole cell hydrolysates of 22 biotype 2 strains were studied (Table 3). All 22 strains contained galactose. Six of 7 human isolates also had mannose, one strain contained only galactose, while another contained glucose in addition to galactose and mannose. Eight of 10 strains from ovine abortion contained galactose and mannose, while 2 of the 10 strains contained only galactose. Two of 3 strains from bovine abortion

Table 3. Carbohydrate composition of Cell Walls of Biotype 2 strains of Vibrio fetus

Strain No.	Source	Galactose	Glucose	Mannose	Rhamnose
1734	Man	+	-	+	-
1886	Man	+	-	+	-
1134	Man	+	-	+	-
1925	Man	+	-	+	-
PB 1	Man	+	+	+	-
PB 35	Man	+	-	-	-
PB 76	Man	+	-	±	-
6533	ovine abortion	+	-	+	-
62-654	ovine abortion	+	-	+	-
63-39	ovine abortion	+	-	+	-
63-38	ovine abortion	+	-	+	-
63-10	ovine abortion	+	-	+	-
5090	ovine abortion	+	-	+	-
653	ovine abortion	+	-	+	-
12351	ovine abortion	+	-	+	-
4440	ovine abortion	+	-	-	-
2221	ovine abortion	+	-	-	-
436	bovine abortion	+	-	-	+
3530	bovine abortion	+	-	-	+
5648	bovine abortion	+	-	-	-
709	bovine bile	+	-	+	-
811	bovine bile	+	-	-	-

had galactose and rhamnose while 1 strain contained only galactose. One from bovine bile contained galactose and mannose, while another contained only galactose. A cell wall hydrolysate of one of the human strains (1925) contained galactose and mannose. These results agree with those from whole cell hydrolysates.

Twenty-one biotype 3 strains were examined (Table 4). Whole cell hydrolysates of all strains contained galactose. Nineteen of 21 strains contained galactose and glucose. Two of these strains contained a small amount of mannose in addition to galactose and glucose. Two of the 21 strains contained only galactose. The type 3 strains were isolated from human disease, ovine abortion, normal sheep intestines, normal cow and pig feces, and from turkey, chicken, and sparrow intestines. A cell wall hydrolysate of a human type 3 strain (5749) contained both galactose and glucose. This was in agreement with the results of whole cell hydrolysates.

Table 4. Carbohydrate Composition of Cell Walls of Biotype 3
Strains of Vibrio fetus

Strain No.	source	Galactose	Glucose	Mannose	Rhamnose
7046	Man	+	+	-	-
8355	Man	+	+	-	-
5749	Man	+	+	-	-
6397	Man	+	+	-	-
4849	ovine abortion	+	+	+	-
68-128	ovine abortion	+	+	-	-
M-6	ovine intestine	+	+	-	-
L-111	ovine intestine	+	+	-	-
32	bovine intestine	+	+	-	-
431	bovine intestine	+	+	-	-
76	bovine intestine	+	-	-	-
5	bovine intestine	+	+	-	-
549	swine intestine	+	+	-	-
Davis	swine intestine	+	+	-	-
16	swine intestine	+	+	-	-
1261	turkey intestine	+	+	-	-
1223	turkey intestine	+	+	-	-
73181	turkey intestine	+	-	-	-
T16A	chicken intestine	+	+	-	-
Star. 1	starling intestine	+	+	+	-
Star. 10	starling intestine	+	+	-	-

SUMMARY

It appears that meso-DAP is found in whole cell hydrolysates of V. fetus but not in the isolated cell walls. The DAP may be located in the cytoplasm and may not be a part of the cell wall mucopeptide.

Cell wall carbohydrate composition appears to be useful in the classification of V. fetus when compared to results based on phenotypic characteristics. Biotypes 1 and 2 usually do not contain glucose in their cell walls. Only 2 of 29 strains contained glucose in their cell walls. Most type 1 and 2 strains contain galactose and mannose whereas only 5 of 29 strains contained only galactose and 2 of 29 had galactose and rhamnose. Biotype 3 strains usually contain galactose and glucose (19 of 21). Only 2 of 21 strains had only galactose in their cell walls.¹

Biotypes 1 and 2 may be more closely related to each other than to biotype 3 strains. Results of Basden *et al.* with DNA-RNA hybridization studies indicate this as well as our results with cell wall carbohydrate composition. Additional studies should indicate the correlation of surface antigens with the chemical composition of the cell wall of V. fetus.

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