Adaptation of the Membrane Fatty Acid Composition by Growth in the Presence of n-Alkanols Influences Glycosyltransferase Expression in *Streptococcus salivarius*

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Growth of *Streptococcus salivarius* ATCC 25975 in the presence of n-alkanols in the series methanol to decan-1-ol led to a decrease in the unsaturated to saturated fatty acid ratio. Each member of the set of n-alkanols which was examined over a range of concentrations possessed a point at which extracellular glucosyltransferase (GTF) production was minimal; increasing the concentration of the n-alkanol past this point stimulated GTF production. This effect was greatest with hexan-1-ol although it was observed to a lesser extent with pentan-1-ol and heptan-1-ol. Reduced cell-associated fructosyltransferase activity was observed with increasing concentrations of each n-alkanol. Growth in the presence of 25 mM-propan-1-ol gave rise to a fatty acid profile in which 55% of the fatty acids were of an odd chain length. *S. salivarius* ATCC 25975 was shown to be able to utilize ethanol in a similar manner to propan-1-ol by growing it in the presence of 400 mM-[¹⁴C]ethanol. Analysis of the membrane lipids at the stationary phase of growth indicated that 17.6% of the carbon of the fatty acids was derived from ethanol. A leaky *adh* mutant, *S. salivarius* MJ 37501, was isolated. The leaky nature of the mutant enabled it to incorporate reduced levels of odd-chain-length fatty acids into its membrane lipids when grown in the presence of 100 mM-propan-1-ol, but not when grown in the presence of 25 mM-propan-1-ol. *S. salivarius* ATCC 25975 therefore metabolized propan-1-ol (and ethanol) via a constitutive alcohol dehydrogenase.

INTRODUCTION

The fatty acid composition of the Gram-negative organism *Escherichia coli* has been shown to alter in the presence of n-alkanols of different chain length (Ingram, 1976; Sullivan *et al.*, 1979). It has been proposed that the changes in fatty acid composition are an adaptive response to the physico-chemical interactions of the n-alkanols with the plasma membrane and that the n-alkanols exert their effect by perturbing the packing order of the lipids in the bilayer (Seeman, 1972). The resultant changes in fluidity are considered in many ways to be analogous to those induced by changes in temperature (Marr & Ingraham, 1962; Ingram, 1982). In *E. coli* K12, growth in the presence of n-alkanols of C₁–C₄ chain length causes an increase in the unsaturated to saturated fatty acid (U/S) ratio, while n-alkanols of C₅–C₁₀ chain length cause the opposite change (Ingram, 1976). However, in the case of *E. coli* ML308, the addition of butan-1-ol causes a decrease in the U/S ratio (Sullivan *et al.*, 1979). Strain differences and culture conditions notwithstanding, the increase in the U/S ratio observed when *E. coli* K12 is grown in the presence of ethanol has been the subject of much conjecture, since by increasing the degree of unsaturation in *E. coli* membranes, ethanol would be expected to further increase fluidity rather than decrease it. The apparent paradox appears to have been resolved since the lipid-to-protein

Abbreviations: ADH, alcohol dehydrogenase; GTF, glucosyltransferase; FAME, fatty acid methyl esters; FTF, fructosyltransferase; U/S ratio, ratio of unsaturated to saturated fatty acids.

ratio of the plasma membrane of *E. coli* decreases when cells are grown in the presence of ethanol, thus compensating for the fluidizing effect brought about by the increase in lipid unsaturation (Dombek & Ingram, 1984).

By comparison with *E. coli*, studies of the effects of n-alkanols on Gram-positive organisms have been far less rigorous and have involved species in which unsaturated fatty acids are either minor constituents of or absent from the plasma membrane (Rigomier *et al.*, 1980; Herrero *et al.*, 1982). In *Bacillus licheniformis*, long-chain n-alkanols preferentially suppress normal extracellular enzyme formation (Fishman *et al.*, 1980). Amphiphilic molecules which perturb the membrane of *Streptococcus salivarius* also alter the degree of expression of extracellular glucosyltransferases (GTF) as well as cell-associated fructosyltransferases (FTF) (Jacques, 1983, 1985). The stimulation of extracellular GTF production in oral streptococci either by non-ionic surfactants containing oleic acid residues (Umesaki *et al.*, 1977; Shimamura *et al.*, 1983; Jacques *et al.*, 1985), or by variations in K^+ concentrations, which also influence membrane composition (Markevics & Jacques, 1985), has implicated membrane lipids containing C_{18} -unsaturated fatty acids in the secretion of the enzyme. It was therefore of interest to observe alterations in the membrane fatty acids of *S. salivarius* by growing the bacterium in the presence of a series of n-alkanols, especially as this bacterium contains primarily linear saturated and unsaturated fatty acids similar to *E. coli*. Alterations in the expression of GTF and FTF under these conditions were also determined.

METHODS

Chemicals. [U - ^{14}C -glucosyl]sucrose, [U - ^{14}C -fructosyl]sucrose, [1 - ^{14}C]ethanol and the liquid scintillation cocktail Aquasol-2 were purchased from New England Nuclear. Prop-2-en-1-ol (allyl alcohol) was obtained from Merck, BSA, Tris and NAD from Sigma, and alumina from BDH. The muramidase enzyme preparation, M-1, from *Streptomyces globisporus* 1829 was kindly supplied by K. Yokogawa, Dainippon Pharmaceutical Co., Osaka, Japan (Yokogawa *et al.*, 1975). The n-alkanols were of the highest grade available; all other reagents were of analytical reagent grade.

Organisms and growth. *S. salivarius* ATCC 25975 and *S. salivarius* MJ 37501 (this study) were stored or grown to late-exponential or early-stationary phase in semi-defined medium (SDM) containing 1 mM- $CaCl_2$ (Jacques, 1983). Initial data collection for analysis of fatty acid composition and measurement of glucosyltransferase activities involved experiments with the various n-alkanols in the series methanol to decan-1-ol, in which *S. salivarius* ATCC 25975 was grown in 250 ml Pyrex screw-top bottles containing 55 ml medium. As samples of these cultures were aseptically removed and replaced at 15 min intervals in order to monitor growth, conditions were more aerobic than in 5.5 ml or 11 ml volume cultures grown in capped test tubes (14 mm i.d.).

Isolation of prop-2-en-1-ol resistant mutants. Spontaneous mutants of *S. salivarius* ATCC 25975 resistant to prop-2-en-1-ol (allyl alcohol) (Rando, 1974) were selected by plating cells on SDM agar containing 200 mM-prop-2-en-1-ol. Individual colonies were isolated and cultured overnight in SDM containing 20 mM-prop-2-en-1-ol. After replating and reisolating twice more, mutants were grown in SDM and assayed for alcohol dehydrogenase (ADH) activity. One of these mutants, designated MJ 37501, was used for further study.

Enzyme assays. Cell-associated and extracellular glucosyltransferase activities were assayed as previously described (Jacques, 1985).

In order to assay ADH activity, cells were grown in 110 ml batches to mid-exponential phase, harvested by centrifugation (10000 g, 4 °C, 10 min), washed twice and resuspended to 2 ml in 50 mM-potassium phosphate buffer pH 6.5. The resuspended cells were lysed by incubating them at 37 °C for 1 h after the addition of 100 μ l M-1 enzyme in water (10 mg ml $^{-1}$). The crude supernatant fraction was obtained by centrifugation (50000 g, 4 °C, 15 min). ADH activity in the soluble fraction was assayed spectrophotometrically at 37 °C by following the production of NADH at 338 nm (Racker, 1955). The cuvette contained NAD (1 μ mol), ethanol or propan-1-ol (20 μ l), enzyme preparation (100 μ l) and 20 mM-Tris/ KH_2PO_4 buffer pH 8.0 to give a final volume of 1.0 ml. The amount of protein added to the assay mixture was determined by the method of Bradford (1976) using BSA as standard. One unit of ADH activity was defined as that capable of reducing 1 μ mol NAD min $^{-1}$ (mg protein) $^{-1}$.

Preparation and analysis of fatty acids. The membrane lipids were extracted and the fatty acid methyl esters (FAME) of the acyl lipids prepared and analysed as reported previously (Markevics & Jacques, 1985). The crude FAME were dissolved in hexane and further purified on 8 $\times$ 1 cm alumina columns packed in hexane. The purified FAME were eluted from the columns with 5% (v/v) diethyl ether in hexane and evaporated to dryness under a stream of N_2 at <25 °C. By comparing the amount of radioactivity present in the purified FAME obtained from a 5 ml culture grown in the presence of 400 mM- ^{14}C ethanol (19.5 Ci mol $^{-1}$; 722 GBq mol $^{-1}$) with the amount and composition of FAME obtained from a 1.5 litre culture grown in the presence of the same

concentration of non-radioactive ethanol, the amount of carbon in the fatty acids of the membrane lipids derived from the added ethanol could be estimated.

Analysis of data. Data from individual experiments are reported unless otherwise indicated (see below). Where appropriate, results are presented as the mean and standard deviation (n = number of experiments).

RESULTS

*Preliminary observations with *S. salivarius* grown in the presence of n-alkanols of different chain length*

In batch cultures of *E. coli* K12, the toxic concentration of a given n-alkanol is related to its chain length. Furthermore, the higher the concentration of a given n-alkanol, the greater the degree of change in the pattern of the membrane fatty acids (Ingram, 1976; Sullivan *et al.*, 1979). Experiments were therefore done with 5.5 ml batch cultures of *S. salivarius* to determine what concentration of each n-alkanol would approximately double the generation time (t_d) observed in the absence of any added alkanol (data not shown). Applying this knowledge to 55 ml batch cultures of *S. salivarius* gave rise to lesser and greater effects on the t_d than had been observed in the preliminary experiments (Table 1). Because of this problem and the fact that each n-alkanol was not examined over a range of concentrations, it could not be stated unequivocally that the toxic concentration of a given n-alkanol was related to its chain length as was the case for *E. coli*. However, the data were useful in directing attention towards certain n-alkanols and their effects on the physiology of *S. salivarius*. For instance, an increase in extracellular GTF activity appeared to be favoured by the addition of hexan-1-ol to the culture medium (Table 1). Second, cell-associated FTF activity appeared to be suppressed in all cultures to which an n-alkanol had been added (Table 1). Third, the U/S ratio of the fatty acids of the membrane lipids decreased regardless of the chain length of the n-alkanol added to the culture medium (Table 2). Fourth, the odd-chain-length n-alkanols gave rise to odd-chain-length fatty acids; this was particularly apparent for cells grown in the presence of propan-1-ol, when odd-chain-length fatty acids represented 75% of the total fatty acid profile (Table 2).

After these preliminary investigations, all subsequent results were obtained from 11 ml batch cultures. Despite attempts to keep conditions of inoculation and growth rigidly controlled, significant variations in t_d were sometimes observed between experiments regardless of the use of the same n-alkanol at the same concentration. Generally, for a given concentration of a given n-alkanol, a longer t_d was associated with a lower U/S ratio. Because of this difficulty, typical data from individual experiments are given, unless the t_d values for the same experimental condition were equivalent (± 3 min).

Glycosyltransferase activity of cells grown in the presence of hexan-1-ol

As the concentration of hexan-1-ol in the medium was increased to 17.5 mM, the U/S ratio of the membrane lipids steadily decreased to a value of 0.37. The most notable changes were in the levels of hexadecanoic acid (16:0), which increased by 65%, and in octadecenoic (18:1) and eicosenoic acids (20:1), which decreased by 47% and 78% respectively (Table 3).

No GTF activity was detected on the surface of cells grown in the presence of hexan-1-ol (Table 4). However, compared with cells grown in the absence of added alkanol, extracellular GTF activity declined by 84% in the presence of 7.5 mM-hexan-1-ol. As the concentration of hexan-1-ol was increased above this value, GTF production increased (Table 4). When the hexan-1-ol concentration in the medium was 17.5 mM, extracellular GTF activity was 307 mU (mg dry wt)⁻¹, some 31-fold greater than GTF activity in the presence of 7.5 mM hexan-1-ol. The t_d of the cells, however, increased by 2.5-fold when the hexan-1-ol concentration was increased from 7.5 mM to 17.5 mM. Although this would allow a greater time for the accumulation of GTF in the culture fluid (Jacques, 1983), the increase in time alone was not sufficient to account for the 31-fold greater GTF activity.

Both the extracellular and the cell-associated FTF activities of *S. salivarius* decreased with increasing concentrations of hexan-1-ol in the growth medium. The cell-associated activity fell more rapidly than the extracellular activity (Table 4).

Table 1. *Effect of n-alkanols on the doubling times and expression of GTF and FTF activities in cultures of S. salivarius*
Cells were cultured in 55 ml batch cultures in 250 ml screw-capped bottles.

Alkanol	Concn (mM)	<i>t_d</i> (min)	Extracellular GTF [mU (mg dry wt) ⁻¹]	Cell-associated FTF [mU (mg dry wt) ⁻¹]
None	—	33	112	1582
Methanol	800	48	127	475
Ethanol	400	54	165	416
Propan-1-ol	110	51	107	977
Butan-1-ol	80	125	43	213
Pentan-1-ol	40	114	166	62
Hexan-1-ol	13	105	439	77
Heptan-1-ol	3	46	34	845
Octan-1-ol	0.85	41	41	1291
Nonan-1-ol	0.50	94	107	416
Decan-1-ol	0.19	66	61	1203

Table 2. *Fatty acid profiles of S. salivarius grown in the presence of various n-alkanols*

Cells were cultured in 55 ml batch cultures in 250 ml screw-capped bottles.

Alkanol	Concn (mM)	Fatty acid composition (% w/w)										U/S ratio
		14:0	15:0*	16:0	16:1	17:0*	17:1*	18:0	18:1	19:1*	20:1	
None	—	5.1		37.3	9.4			5.6	32.2		10.4	1.08
Methanol	800	5.3		44.6	8.6			6.0	29.0		6.5	0.79
Ethanol	400	7.8		48.0	8.0			6.6	24.4		5.3	0.60
Propan-1-ol†	110	2.3	19.9	11.3	1.8	27.7	18.7	2.3	5.6	5.7	2.4	0.52
Butan-1-ol	80	12.2		52.3	8.5			8.7	18.3		T	0.37
Pentan-1-ol	40	10.0	3.4	48.1	6.7	4.6	T	6.9	16.0		4.3	0.37
Hexan-1-ol	13	9.2		50.2	5.4	T	T	9.1	20.1		6.1	0.46
Heptan-1-ol	3	6.6		47.9	7.7			7.2	23.4		7.1	0.62
Octan-1-ol	0.85	5.8		44.5	7.9			6.6	26.0		9.1	0.76
Nonan-1-ol	0.5	7.9	2.0	42.6	5.9	3.9	T	7.4	20.9		9.5	0.57
Decan-1-ol	0.19	4.5		41.4	7.1			8.0	27.5		11.6	0.86

T, Trace amount (<1%).

* Identification based on relative retention time at a constant temperature of 180 °C.

† 2.5% 13:0 and trace amounts of 19:0 were observed in this sample.

Table 3. *Fatty acid profiles of S. salivarius grown in the presence of hexan-1-ol*

Hexan-1-ol (mM)*	Fatty acid composition (% w/w)						U/S ratio
	14:0	16:0	16:1	18:0	18:1	20:1	
0.0 (8)	2.4 ± 1.2	35.2 ± 3.0	9.5 ± 1.9	6.9 ± 1.7	36.5 ± 2.7	9.7 ± 2.4	1.27 ± 0.17
1.0	1.8	32.7	7.3	7.7	37.5	12.9	1.37
2.5	2.1	39.9	6.9	8.0	32.5	10.6	1.00
5.0	2.6	43.5	8.3	7.3	28.7	9.7	0.88
7.5	2.8	45.2	8.6	8.5	26.5	8.4	0.77
10.0 (2)	4.6	48.4	8.2	8.2	23.3	7.5	0.64
12.5 (2)	5.0	50.0	7.6	9.0	21.8	6.7	0.57
15.0 (2)	5.5	53.9	6.6	9.1	19.4	5.5	0.46
17.5	5.1	58.2	5.4	10.0	19.3	2.1	0.37

*The numbers in parentheses indicate the number of repeat experiments.

Table 4. *GTF and FTF activities of S. salivarius grown in the presence of hexan-1-ol*

Hexan-1-ol (mM)	<i>t_d</i> (min)	GTF [mU (mg dry wt) ⁻¹]		FTF [mU (mg dry wt) ⁻¹]	
		Extracellular	Cell-associated	Extracellular	Cell-associated
0.0	29	61	0	194	1470
1.0	31	38	0	180	1377
2.5	30	19	0	158	1287
5.0	30	16	0	150	950
7.5	32	10	0	125	983
10.0	33	35	0	110	674
12.5	36	91	0	97	377
15.0	38	98	0	92	246
17.5	85	307	ND	ND	150

ND, not determined.

The addition of 17.5 mM-hexan-1-ol to the assay mixture resulted in a minor reduction (4%) in the measured extracellular GTF activity. Due to dilution of the sample in the assay mixture, this concentration of hexan-1-ol was at least 40 times that present during the assay of the enzyme, if one assumed a 100% carryover of the 17.5 mM-hexan-1-ol added to the culture medium. In contrast, measured cell-associated FTF activity was reduced by 14% by the same concentration of hexan-1-ol. The concentration of any *n*-alkanol transferred into the assay mixture as a contaminant of washed cells was, however, unknown. Similar results were obtained with all other *n*-alkanols studied.

Similar results to those obtained with hexan-1-ol were observed when cells were cultured with increasing concentrations of pentan-1-ol or heptan-1-ol: cell-associated FTF activity fell more rapidly than extracellular FTF activity (data not shown). Although the effects on extracellular GTF production were not as pronounced as those for growth in the presence of hexan-1-ol, extracellular GTF production increased fivefold in 50 mM-pentan-1-ol compared with the minimum value of 19 mU (mg dry wt)⁻¹ observed in 20 mM-pentan-1-ol. When cells were grown in the presence of 5 mM-heptan-1-ol a twofold increase in extracellular GTF production was observed, compared with the minimum value of 16 mU (mg dry wt)⁻¹ in 2 mM-heptan-1-ol. The average extracellular GTF activity of cells cultured in 1 l batch cultures in the absence of any added alcohol was 47 ± 14 mU (mg dry wt)⁻¹ (*n* = 10). Growth in the presence of either pentan-1-ol or heptan-1-ol resulted in fatty acid changes similar to those observed with hexan-1-ol (data not shown).

Influence of ethanol, propan-1-ol and propanoic acid on cultures of S. salivarius

The fatty acids of the membrane lipids of *S. salivarius* adapted to the presence of ethanol in the growth medium by increasing the content of hexadecanoic acid (16:0) essentially at the expense of the octadecenoic acid (18:1) and eicosenoic acid (20:1) moieties, a response similar to that seen with other n-alkanols (Table 5). As the concentration of ethanol in the medium was increased to 500 mM the U/S ratio of the cells decreased to 0.56 whilst the t_d rose steadily to a value of 55 min. Extracellular GTF production fell by 57% with 300 mM-ethanol, subsequently rising with 450 mM-ethanol to a maximum value which was 125% of that recorded in the absence of added alkanol. No cell-associated GTF activity was observed. Measured cell-associated FTF activity decreased with increasing concentrations of ethanol in the growth medium. With 500 mM-ethanol, the value was 68% less than that of similar cells grown in the absence of added alkanol, where the value was 1655 ± 151 mU (mg dry wt)⁻¹ ($n = 9$). The extracellular FTF activity remained constant at 250 ± 37 mU (mg dry wt)⁻¹ ($n = 16$) irrespective of the concentration of ethanol added to the growth medium.

Increasing the concentration of propan-1-ol in the growth medium from 0 to 25 mM gave rise to increasing proportions of odd-chain-length fatty acids in the membrane lipids. Addition of up to 100 mM-propan-1-ol resulted in little further increase in the odd-chain-length fatty acids (Table 6). However, unlike growth in the presence of any other n-alkanol, the results obtained from separate experiments gave different quantitative patterns for the fatty acid profiles. Major quantitative differences were observed in the levels of pentadecanoic (15:0), hexadecanoic (16:0), heptadecanoic (17:0), heptadecenoic (17:1), and octadecenoic acids (18:1) between experiments (data not shown).

In contrast to cells grown in the presence of propan-1-ol, the presence of up to 100 mM-propanoic acid in the growth medium caused a small rise in the proportion of odd-chain-length to even-chain-length fatty acids. For comparison, the fatty acid profile of *S. salivarius* grown in the presence of 25 mM-propanoic acid is given in Table 6. Growth of *S. salivarius* in the presence of 25 mM-propan-1-ol (the concentration required for near maximum accumulation of odd-chain-length fatty acids) produced a higher proportion of odd-chain-length fatty acids in the membrane lipids than did growth in the presence of 25 mM-propanoic acid.

Extracellular GTF production appeared to be inversely related to the concentration of propan-1-ol present in the growth medium despite an increase in the t_d of the culture from 28 min to 37 min as the concentration of propan-1-ol in the medium was increased to 100 mM. A 40% reduction in the extracellular enzyme activity was observed in cells grown in the presence of 100 mM-propan-1-ol. No cell-associated GTF activity was detected in the presence or absence of propan-1-ol.

Extracellular FTF activity remained constant at 235 ± 32 mU (mg dry wt)⁻¹ ($n = 7$) throughout the range of concentrations of propan-1-ol added to the growth medium, in a manner similar to that observed for growth in the presence of ethanol. However, unlike the extracellular FTF activity, the cell-associated activity decreased by 38% as the concentration of propan-1-ol in the growth medium was increased to 100 mM.

Ethanol as a source of carbon for S. salivarius

The preferred incorporation of odd-chain-length fatty acids into the membrane lipids of *S. salivarius* grown in the presence of propan-1-ol compared with propanoic acid suggested that propan-1-ol could be metabolized via ADH and aldehyde dehydrogenase to propanoyl-CoA, which could subsequently be utilized for catabolic processes including fatty acid biosynthesis. If this were the case, then ethanol should also be metabolized to form acetyl-CoA by the same pathway. This possibility was examined by growing cells in the presence of 400 mM-[¹⁴C]ethanol. Analysis of the fatty acids of the membrane lipids at the stationary phase of growth indicated that 17.6% of the carbon of the fatty acids was derived from the ethanol added to the growth medium.

Table 5. Influence of ethanol on the fatty acid profile of *S. salivarius*

Ethanol (mM)	Fatty acid composition (% w/w)*						U/S ratio
	14:0	16:0	16:1	18:0	18:1	20:1	
0	1.8	37.7	10.9	6.0	36.1	7.6	1.21
100	2.6	42.3	11.2	6.5	32.5	4.9	0.96
200	3.9	44.9	10.6	6.5	29.3	5.0	0.82
300	3.0	48.5	9.0	6.4	29.6	3.7	0.74
350	4.2	49.2	11.0	6.8	25.6	3.4	0.67
400	3.5	51.8	10.5	5.8	25.9	2.6	0.64
450	4.4	53.1	10.1	6.7	23.2	2.5	0.57
500	4.5	52.7	10.7	6.9	24.2	1.2	0.56

* Mean values from duplicate experiments.

Table 6. Fatty acid profiles of *S. salivarius* strains

Strain	Additive	Concn (mM)	Fatty acid composition (% w/w)										U/S ratio
			14:0	15:0*	16:0	16:1	17:0*	17:1*	18:0	18:1	19:1*	20:1	
ATCC 25975	Propan-1-ol	0	1.7	—	36.8	7.3	—	—	6.2	37.3	—	10.6	1.24
		25†	4.3	20.7	22.6	1.1	18.0	12.6	6.0	7.9	1.0	4.0	0.40
		100†	3.1	17.3	21.4	1.5	17.0	18.0	4.6	9.5	1.8	4.4	0.58
MJ 37501	Propanoic acid	25‡	3.6	2.7	33.1	5.7	7.5	6.0	6.9	24.7	4.1	5.9	0.86
		0†	3.5	—	34.1	11.7	—	—	8.6	33.6	—	8.6	1.17
		25‡	3.2	—	37.4	10.4	—	—	8.3	30.6	—	7.3	0.99
Prop-2-en-1-ol		100	3.5	4.8	29.7	7.3	11.6	5.7	9.9	19.1	3.5	3.7	0.65
		100	2.6	—	38.4	11.1	—	—	7.9	32.6	—	5.7	0.98

• Identification based on relative retention time at a constant temperature of 180 °C

† Different quantitative patterns were observed between experiments (see text).

‡ Mean values from duplicate experiments.

Metabolism of ethanol and propan-1-ol by a constitutive ADH in S. salivarius

A spontaneous mutant, *S. salivarius* MJ 37501, resistant to 200 mM-prop-2-en-1-ol was isolated. This mutant possessed 3 % of the ethanol dehydrogenase and 1 % of the propan-1-ol dehydrogenase activity of the parent strain, in which specific activities of 4.17 and 3.08 $\mu\text{mol min}^{-1} (\text{mg protein})^{-1}$ respectively were obtained. The mutant was unable to form odd-chain-length fatty acids when grown in the presence of 25 mM-propan-1-ol, although odd-chain-length fatty acids were detected as the concentration of propan-1-ol in the growth medium was increased above this value. At 100 mM-propan-1-ol, odd-chain-length fatty acids represented 26 % of the total fatty acids, confirming the leaky nature of the *adh* mutation in this strain (Table 6). Changes in GTF and FTF expression were similar to those observed by adding ethanol to the growth medium (data not shown).

Influence of prop-2-en-1-ol on S. salivarius MJ 37501

The resistance of *S. salivarius* MJ 37501 to prop-2-en-1-ol afforded the opportunity to observe any physiological changes that might occur in cells grown in the presence of an unsaturated linear alkanol. However, growth of the strain in increasing concentrations of prop-2-en-1-ol up to 100 mM failed to significantly influence the t_d , cellular or extracellular GTF or FTF expression, or the fatty acid profile of the membrane lipids (Table 6).

DISCUSSION

When *S. salivarius* was grown in the presence of a series of n-alkanols of C_1 – C_{10} chain length, the concentration of a given n-alkanol required to cause an alteration in the fatty acid composition of the membrane lipids appeared to be inversely related to the chain length, as was found for *E. coli* (Ingram, 1976). Compared with hexan-1-ol for instance, a 40-fold greater concentration of ethanol was required to reduce the U/S ratio to 0.64 – half the value observed in the absence of any added alkanol (Tables 3 and 5). Irrespective of the alkanol added to the growth medium, the membrane fatty acids became more saturated when compared with cells grown in the absence of added alkanol. The changes were in sharp contrast to those reported for *E. coli* K12, where addition of the short-chain alkanols, methanol to butan-1-ol, caused the degree of saturation to decrease rather than increase (Ingram, 1976).

While incorporation of propanoic acid has been reported to produce odd-chain-length fatty acids in *E. coli* (Ingram *et al.*, 1977), growth of *S. salivarius* in the presence of 25 mM-propanoic acid elicited a 70 % lesser incorporation of odd-chain-length fatty acids than did growth in the presence of the corresponding n-alkanol at the same concentration. Use of the leaky *adh* strain, MJ 37501, indicated that high extracellular concentrations of propan-1-ol and ethanol could be metabolized via a constitutive ADH activity present in the parent strain. By inference, both ethanol and propan-1-ol crossed the plasma membrane of *S. salivarius* and so, in theory at least, could affect lipid packing at various depths within or on either side of the membrane. For propan-1-ol this transfer was significant at a concentration of 25 mM.

The effects of any set of amphiphilic compounds depend not only on the localization of each member of that set within the membrane but also on the specific depth each member of the set penetrates the bilayer (Brasseur *et al.*, 1985). In the case of *S. salivarius* ATCC 25975, reduced cell-associated FTF activity was observed with increasing concentrations of each n-alkanol studied. In contrast, each member of the set of n-alkanols which was examined over a range of concentrations possessed a point at which extracellular GTF production was minimal. Increasing the concentration of the n-alkanol past this point stimulated GTF production. This effect was greatest for hexan-1-ol, since the degree of stimulation of GTF production following the minimum was not as great for the preceding n-alkanol, pentan-1-ol, nor for the following n-alkanols heptan-1-ol and octan-1-ol (data not shown). It may be more than coincidental that hexan-1-ol is the n-alkanol which has been reported to have a number of unique physiological effects: for instance it theoretically forms the most stable n-alkanol clusters within a dipalmitoylphosphatidylcholine bilayer (Brasseur *et al.*, 1985), it is the n-alkanol at which a levelling off of inhibition constants (K_i) for luciferase activity occurs (Franks & Lieb, 1985) and it is the n-alkanol which causes the greatest changes in the fatty acids and fluidity of *E. coli*

membranes (Sullivan *et al.*, 1979; Ingram & Vreeland, 1980). In fact, growth of *S. salivarius* in the presence of 17.5 mM-hexan-1-ol stimulated extracellular GTF production fivefold while suppressing cell-associated FTF expression tenfold. Similar changes of the same order of magnitude for both glycosyltransferase activities occur when cells are grown in the presence of 3–5 mM-octyl β -D-glucopyranoside (Jacques, 1985). Such a result may imply a similar orientation and perturbation by the surfactant and the n-alkanol relative to the protein and lipid components of the membrane.

The high degree of specificity and control of lipid composition in bacterial membranes provides significant circumstantial evidence that lipid composition is critical to membrane function. The present study of the effects of n-alkanols on the fatty acid composition and the mode of expression of the glycosyltransferase enzymes in *S. salivarius* supports such a view.

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