Vibrational Spectroscopy 64 (2013) 51–57

Contents lists available at SciVerse ScienceDirect

Vibrational Spectroscopy

journal homepage: www.elsevier.com/locate/vibspec

FT-IR spectroscopic investigation of bacterial cell envelopes from *Zymomonas mobilis* which have different surface hydrophobicities

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ARTICLE INFO

Article history:
Received 22 February 2012
Received in revised form 11 October 2012
Accepted 16 October 2012
Available online 5 November 2012

Keywords:
Hydrophobicity
Zymomonas mobilis
FT-IR spectroscopy
Cell envelopes

ABSTRACT

Changes in the cell envelope composition of bacteria *Zymomonas mobilis* 113S which resulted in varied cell surface hydrophobicity (CSH) were examined using FT-IR-spectroscopy and conventional methods of biochemical analysis. Significant differences in all analyses were detected between hydrophilic and hydrophobic compounds in the envelopes of *Z. mobilis*. A significant positive linear relationship ($P < 0.01$) was observed between CSH values of *Z. mobilis* and the ratio of absorbance band intensities assigned to the CH_2 and CH_3 vibrations from lipids as well as the asymmetric and symmetric stretching vibrations of these methylene and methyl groups. Differences were also seen between the ratio of band absorbances arising from Amide I and Amide II. In addition, the ratio of absorbance intensities assigned to carbohydrates was found to correlate with the CSH of *Z. mobilis*. Furthermore, a significant inverse relationship ($P < 0.01$) was detected between CSH of *Z. mobilis* and the band position (frequency) shift of asymmetric and symmetric stretching of methylene groups, alkyl esters (>C═O *str*) and Amide I, as well as a positive linear relationship ($P < 0.05$) between the band position shift of Amide II and carbohydrates and CSH of *Z. mobilis*. Qualitative and quantitative composition of the cell envelopes was found to correlate with both the CSH of *Z. mobilis* and the spectral data. The observed multiple correlations between the concentration of proteins, carbohydrates and lipids of cell envelopes and selected indices of FT-IR spectra indicate the complex relationship of *Z. mobilis* cell envelope constituents. The results of present study suggest that FT-IR spectroscopy can be used as a convenient method to evaluate the properties of cell envelopes from Gram-negative bacteria.

1. Introduction

Gram-negative ethanologenic bacteria *Zymomonas mobilis* exhibit a considerable biosynthetic potential in regard to various metabolites (ethanol, sorbitol, gluconic acid, levan and fructose oligomers) which makes them attractive for industrial biotechnological applications [1–3].

Originally the strain *Z. mobilis* 113S was reported to demonstrate good productivity characteristics for levan, oligosaccharide and ethanol synthesis under appropriate fermentation conditions [4], including the systems of immobilized cells [5–7].

The majority of the vital activities in Gram-negative bacteria (sensory, protective, transport and energy generation functions) are dependent on structural and functional properties of cell envelope containing the cytoplasmic and the outer membrane as principal components [8]. The outer membrane contains considerable amounts of protein, phospholipids and most or all of the lipopolysaccharide of the cell envelope [9]. Essential features of the cell envelope can be considered from a variety of standpoints including macroscopic physico-chemical characteristics as well as an exact molecular composition. The cell surface hydrophobicity (CSH) has been acknowledged as a physical measurable macroscopic characteristic of bacteria which, in general, reflects the ratio between hydrophobic and hydrophilic constituents of the cell envelope [10]. In microbiological practice CSH appears to be concerned with diverse processes and interactions of physiological and technological importance [11–13]. CSH responds to a wide variety of environmental factors and, in turn, appears to be involved in the cell-to-cell interaction, adherence of bacteria to solid surfaces and host tissue, partitioning at liquid–liquid, solid–liquid or liquid–air interfaces, resistance of cells to specific treatments by organic solvents or antibiotics etc.

We have previously detected changes in the CSH of *Z. mobilis* 113S in response to varied environmental conditions, including temperature and phase of growth, concentration or type of carbon source etc. These have implication to the impaired barrier function, and hence the survival of more hydrophilic cells [14,15].

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http://dx.doi.org/10.1016/j.vibspec.2012.10.008

Infrared (IR) spectroscopy is a non-destructive technique which allows one to obtain information on the overall chemical composition of a sample [16–18]. Over the years this technique has been used for biochemical analysis and characterization of biological samples [17,19,20] as well as being applied for fermentation processes and product monitoring [21–24]. The macromolecular composition of microbial biomass is an integral characteristic of the organism's physiological state, reflecting the influence of cultivation conditions on cell regulatory mechanisms [18,19,25].

We have reported that the changes in the whole cell biochemical composition are reflected in FT-IR spectra by several indices [14,15]. Namely in the region between 1200 cm^{-1} and 900 cm^{-1} [14], which is mainly dominated by a sequence of bands due to C—O, C—C, C—O—C and C—O—P stretching vibrations of polysaccharides [20] and these groups mainly occur in carbohydrates. We have also observed changes in the region between 3000 cm^{-1} and 2800 cm^{-1} [15], which is dominated by the absorption modes of lipids aliphatic chains [20]. The present study examines the changes in *Z. mobilis* 113S cell envelope composition in relation to varied CSH. Conventional methods of biochemical analysis and FT-IR spectroscopy were used to benchmark the principal cell component data.

2. Materials and methods

2.1. Strain and culture conditions

Zymomonas mobilis strain 113S [4] was maintained and grown in batch culture without aeration as described previously [14]. In order to modify the CSH of *Z. mobilis* the cultivations were performed at different temperatures (20–25 °C to obtain the hydrophilic cells and 37–40 °C to obtain the hydrophobic cells) and/or at varied concentrations of glucose as a sole carbon source (0.14–0.28 M to obtain the hydrophilic cells and 0.56–1.11 M to obtain the hydrophobic cells) as reported previously [14].

2.2. Preparation of cells and measurements of the cells surface hydrophobicity

Cells from the appropriate growth medium were recovered by centrifugation (7000 × *g*, 10 min, 20 °C), washed twice with 20 mM phosphate-buffer (pH 7.0) and resuspended in the same buffer at the standardized concentration of cells (OD_{660} = 0.5).

CSH was measured by a modified microbial adherence to hydrocarbon (MATH) method [26] using o-xylene (*Sigma*) as described previously [14]. The respective cell suspension (3 ml; $OD_{660} = 0.5 \pm 0.01$) was mixed with 0.75 ml of o-xylene by vortexing for 2 min. After phase separation (40 min) the aqueous layer was extracted and contaminating xylene removed by bubbling air (2 ml s^{-1}) for 8 min and the optical density of the aqueous phase was determined at 660 nm (OD_{660}). The results were expressed as percentage reductions in absorbance of the test suspensions (with o-xylene) as compared to the control suspensions (without o-xylene).

2.3. Cell envelope extraction

The cell suspension was sonicated for 20 min at 0.5 kHz in an ice–water bath. Unbroken cells were removed by centrifugation (6000 × *g*, 10 min, 4 °C). The envelopes were pelleted by ultracentrifugation (100,000 × *g*, 60 min, 4 °C), washed once with the phosphate buffer, and kept at −20 °C [26]. The quality of the cell envelope extraction was monitored by electron microscope (Olympus BX51).

2.4. Analytical measurements

The content of total membrane proteins was assayed by dye-binding Lowry (Folin-Ciocalteu's reagent, Fluka Chem. AG, Switzerland) procedure [27], using bovine serum albumin (*Sigma*) as a standard. The amount of phosphate was determined by the modified method of Bartlett [28]. The content of phospholipids in lipopolysaccharide (LPS) and membranes was calculated from the estimates of phosphorus, assuming that the LPS content is about 6% of phosphorus [29] and the total lipid content of LPS is about 30% [30,31]. Total neutral carbohydrates of membranes were determined by means of Dreywood's Anthrone reagent [32], using glucose as a standard.

2.5. FT-IR spectroscopy

FT-IR absorption spectra of *Z. mobilis* cell envelopes were registered on a microplate reader HTS-XT (BRUKER) [33]. 10–30 μl of each sample were dried on a 96 place silicon plate at ~50 °C, spectra collected over the wavenumber range of 4000–600 cm^{-1}, with 64 scans, and a resolution of 4 cm^{-1}. In total 84 spectra were collected and averaged over the series of three separate experiments. The data were processed with OPUS 6.5 (BRUKER) software; baseline corrected by rubber-band method, second derivative spectra evaluated. The intensity of each band was obtained by measuring the peak height defined as absorbance value difference between the band maximum and the baseline.

2.6. Data processing and analysis

Hydrophilic and hydrophobic cell envelopes were obtained from 21 independent cultivations. All analytical measurements for each sample were performed at least in triplicate.

The data were processed by correlation analysis using the software Statgraphics ®Plus (Manugistics, Inc., US) and SPSS 11.0 for Windows (SPSS Inc., Il, US) and included Pearson (parametric) correlation as well as non-parametric Spearman correlation testing via. The data were also subjected to the multiple linear regression analysis using the same software. The Fisher's *F*-test for analysis of variance (ANOVA) was performed to evaluate the statistical significance of regression models and the Student's *t*-test was employed to check the significance of regression coefficients. The *P* values <0.05 were considered to be statistically significant for both parametric and non-parametric tests.

3. Results

Changes in the cell envelope composition of bacteria *Zymomonas mobilis* 113S with varied (12–63.3%) CSH were examined by FT-IR spectroscopy and conventional analytical chemistry methods. Cell envelopes of *Z. mobilis* 113S with different CSH exhibited noticeable variation of band absorbance and wavenumber at the 3000–900 cm^{-1} region (Fig. 1). Vector normalized FT-IR spectra of hydrophobic cell envelopes showed more intensive absorption peaks assigned to lipids (3000–2800 cm^{-1}), proteins (1700–1500 cm^{-1}) and carbohydrates (1200–900 cm^{-1}). At the same time, variation of differences in absorbance intensities and vibration wavenumbers was detected between the fractions of hydrophilic and hydrophobic cells (Table 1).

In the region between 3000 cm^{-1} and 2800 cm^{-1}, representing the absorption modes of fatty acid chains (e.g., membrane phospholipids) [20,25], significant direct relationships (0.554 ($P < 0.01$) and 0.510 ($P < 0.01$)) were found between the CSH of *Z. mobilis* 113S,

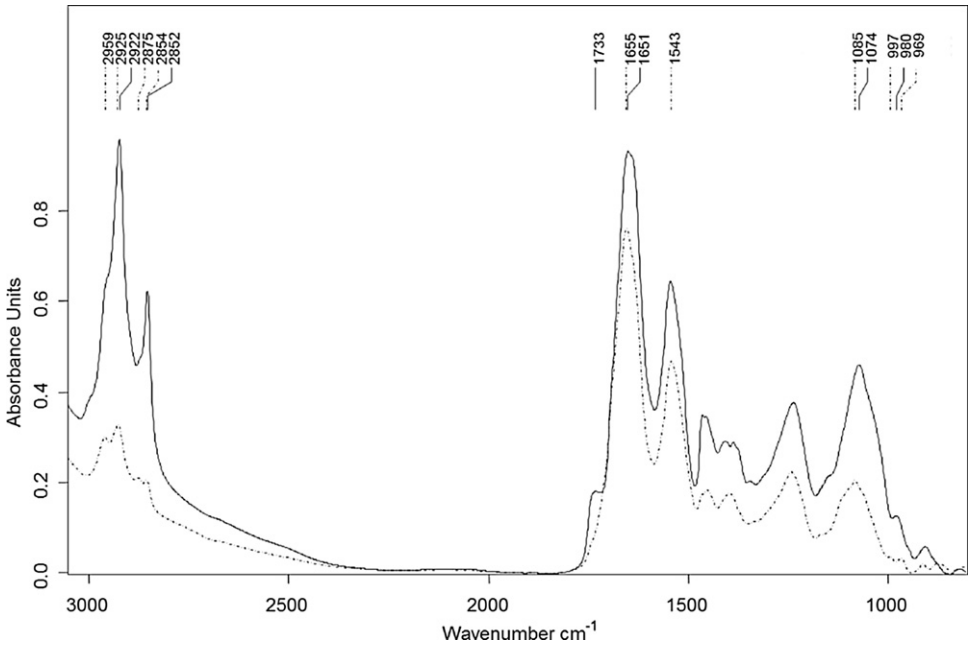

Fig. 1. Vector normalized FT-IR spectra of *Z. mobilis* 113S cell envelopes with different CSH: (—) CSH = 63.32%; (- - -) CSH = 17.23%.

Table 1
The differences of FT-IR indices between the hydrophilic and hydrophobic cells of *Z. mobilis* 113S.

FT-IR indices (raw spectra)	Cell surface properties Hydrophilic, $n = 10$	Hydrophobic, $n = 11$	Significance of differences (P)
Absorbance			
ν_{as} (CH_3)	62.75 ± 3.25	79.18 ± 5.55	0.0220*
ν_{as} (CH_2)	34.80 ± 2.39	47,82 ± 4.53	0.0232*
ν_{sym} (CH_3)	97.55 ± 7.89	145.14 ± 15.56	0.0265**
ν_{sym} (CH_2)	45.45 ± 4.58	72.45 ± 9.10	0.0017**
>C═O *str* (esters)	27.25 ± 1.30	35.41 ± 2.47	0.0066**
Vibrational frequencies (cm^{-1})			
ν_{as} (CH_2)	2924.68 ± 0.21	2923.61 ± 0.27	0.0067**
ν_{sym} (CH_3)	2873.93 ± 0.25	2872.48 ± 0.54	0.0373**
ν_{sym} (CH_2)	2853.91 ± 0.13	2853.28 ± 0.18	0.0151**
>C═O *str* (esters)	1734.19 ± 0.24	1741.84 ± 0.81	0.0430**
Amide I	1654.93 ± 0.14	1653.33 ± 0.87	0.0410**
Amide II	1542.89 ± 0.19	1543.41 ± 0.16	0.0480*
C—O—C, C—O *str* (in carbohydrates)	970.19 ± 0.67	975.57 ± 1.47	0.0046*

* Parametric *t*-test
** Non-parametric *W*-test

and the ratio of absorbance intensities assigned to the asymmetric and symmetric stretching of methylene groups (ν_{as} (CH_2) and ν_{sym} (CH_2), respectively) (Table 2). Furthermore, significant inverse relationships were detected between CSH of *Z. mobilis* and the band position variation of asymmetric and symmetric stretching of methylene groups (ν_{as} (CH_2) and ν_{sym} (CH_2)), respectively (Table 3). The band position of ν_{as} (CH_2) (2922.35–2925.43 cm^{-1}) was found to vary within a range of 3.08 cm^{-1} (coefficient of variation 44.4%)

Table 2
The correlation between the cell surface hydrophobicity of *Z. mobilis* 113S and FT-IR absorbance intensities.

Parameter to be correlated	Relationship		
	FT-IR indices	Correlation coefficient, $n = 21$	
		Parametric(r)	Nonparametric(ρ)
Absorbance intensities			
CSH	ν_{as} (CH_3)	0.515[a]	0.505[a]
CSH	ν_{as} (CH_2)	0.554[b]	0.576[a]
CSH	ν_{sym} (CH_3)	0.502[a]	0.555[b]
CSH	ν_{sym} (CH_2)	0.510[a]	0.580[b]
CSH	C—O—C, C—O str (in carbohydrates)	−0.532[a]	−0.445[a]
ν_{as} (CH_3)/ν_{sym} (CH_3) ratio	Amide I/Amide II ratio	0.451[b]	
ν_{as} (CH2)/ν_{sym} (CH_2) ratio	Amide I/Amide II ratio	0.634[b]	0.492[a]

[a] Correlation is significant at the 0.05 level.
[b] Correlation is significant at the 0.01 level.

Table 3
The correlation between *Z. mobilis* 113S cell surface hydrophobicity, FT-IR band position shifts and ratio of protein (P), carbohydrate (C) and lipid (L) concentrations.

Parameter to be correlated	Relationship		
	FT-IR indices	Correlation coefficient, $n = 21$	
		Parametric (r)	Nonparametric (ρ)
Band position shift (raw spectra)			
CSH	ν_{as} (CH_2)	−0.567[b]	−0.649[b]
CSH	ν_{sym} (CH_2)	−0.621[b]	−0.651[b]
CSH	>C═O *str* (esters)	−0.639[b]	−0.585[b]
CSH	Amide I	−0.503[a]	−0.434[a]
CSH	Amide II	0.513[a]	0.469[a]
CSH	C—O—C, C—O *str* (in carbohydrates)	0.480[a]	0.645[b]
Concentration ratio (determined by biochemical analysis)			
CSH	P/C	−0.836[b]	−0.905[b]
CSH	C/L	−0.814[b]	−0.830[b]

[a] Correlation is significant at the 0.05 level.
[b] Correlation is significant at the 0.01 level.

likewise ν_{sym} (CH_2) (2852.4–2854.4 cm^{-1}) varied within a range of 2 cm^{-1} (coefficient of variation 37.2%), both of these were inversely proportional to increasing CSH values.

Moreover, a significant direct relationship was found between CSH of *Z. mobilis* with the ratio of absorbance intensities most likely arising from asymmetric and symmetric stretching vibrations of methyl groups (ν_{as} (CH_3) and ν_{sym} (CH_3), at about 2960 cm^{-1} and 2870 cm^{-1}, correspondingly (Table 2).

In the region between 1745 cm^{-1} and 1735 cm^{-1}, which represents the absorption modes of alkyl esters and fatty acids [20,25] a significant inverse relationship was detected between the CSH values of *Z. mobilis* 113S and the band position variation of alkyl esters (>C═O *str*) (Table 3). The band positions of >C═O *str* (1735.55–1745.13 cm^{-1}) of esters were found to vary within a range of 9.58 cm^{-1} (coefficient of variation 26.7%) and these were inversely proportional to increasing CSH values.

For vibrations in the region between 1700 cm^{-1} and 1500 cm^{-1}, which represents the absorption modes of Amide I and Amide II, C—N, C═O and N—H *str* of protein and peptides amide [20,25], a significant inverse relationship was detected between CSH of *Z. mobilis* 113S and the band position variation of Amide I, and direct relationship between the band position variation of Amide II and CSH of *Z. mobilis* (Table 3). The band position of Amide I (1645.42–1655.45 cm^{-1}) was found to vary within a range of 10.03 cm^{-1} (coefficient of variation 24.4%) and was inversely proportional to increasing CSH values, whereas the band position of Amide II (1541.76–1544.44 cm^{-1}) varied within a range of 2.68 cm^{-1} (coefficient of variation 28.2%) and was directly proportional to increasing CSH values.

Moreover, a strong positive correlation was observed between the ratio of absorbances A_{1654}/A_{1543} and A_{2924}/A_{2853}, most probably assigned to Amide I and Amide II and asymmetric and symmetric stretching vibrations of methylene groups (ν_{as} (CH_2) and ν_{sym} (CH_2), respectively (Table 2). At the same time, a significant direct relationship was detected between the ratio of absorbances A_{1654}/A_{1543} and A_{2956}/A_{2873}, which could be assigned to Amide I and Amide II, and asymmetric and symmetric stretching vibrations of methyl groups (ν_{as} (CH_3) and ν_{sym} (CH_3), correspondingly [20,25] (Table 2).

The region between 1200 cm^{-1} and 900 cm^{-1}, assigned to carbohydrates [20,25] showed a significant inverse relationship between the CSH of *Z. mobilis* 113S and the ratio of absorbance intensities was detected (Table 2). In addition, a significant positive correlation was found between CSH of *Z. mobilis* and the band position of carbohydrate (Table 3). The position of band 968.82–982 cm^{-1} varied within a range of 13.18 cm^{-1} (coefficient of variation 54.9%), in a directly proportional fashion to increasing CSH values.

To determine whether the observed band position shifts can be attributed to changes in the conformation of compounds, or arise from actual differences in their concentrations the second derivative spectra were analyzed. In both the lipid (3000–2800 cm^{-1}) and ester (1745–1735 cm^{-1}) regions of the second derivative spectra a sharp reduction (2920.75–2921.79 cm^{-1}; 2851.38–2852.36 cm^{-1}, 1746.32–1747.20 cm^{-1}) of band position shifts was observed, while appreciable shifts persisted in the protein (1700–1500 cm^{-1}) and carbohydrate (1200 cm^{-1} and 900 cm^{-1}) regions. Consequently, the position of the Amide I band varied from 1655.48 to 1659.48 cm^{-1} (4 cm^{-1} shift) and the band position of carbohydrates shifted within a range of 9.95 cm^{-1} (966.23–976.18 cm^{-1}).

In addition to the above mentioned correlations, multiple linear regression (MLR) models were also developed to evaluate the complex relationships between the CSH of *Z. mobilis* cell envelopes and the FT-IR spectral data (Table 4). The absorbance intensities (or ratio of absorbance intensities) at fixed wavenumbers were considered as the independent variables together with CSH values as the depend variables (Table 4). Only two variables were needed to form the MLR model (I), which was confirmed by significant rank correlation coefficients (Kendall's $\tau = 0.450$, $P < 0.01$, Spearman's $\rho = 0.662$, $P < 0.01$). Furthermore, the absorbance intensities at 2875 cm^{-1}, 1088 cm^{-1} and ratio of absorbance at 1088/985 cm^{-1} formed a statistically robust MLR model ($R^2 = 0.81$) linking the CSH values again directly with the FT-IR spectral data (Table 4). The highly significant adjusted R^2 value also indicates that the model adequately represents the actual relationships between the FT-IR absorbance intensities and CSH since 22.11% of the total variance remains unexplained (Table 4).

Concentrations of Z. *mobilis* cell envelope components estimated by quantitative biochemical analysis were found to correlate with the CSH values (Fig. 2). Thus, the content of proteins and lipids in cell envelopes increases in a direct proportion to the increasing CSH. At the same time, the carbohydrate concentration in cell envelope fractions decreases with the increasing CSH values (Fig. 2).

Corresponding MLR models (II–IV) were developed to examine the relationships between the concentration of proteins, carbohydrates and lipids of *Z. mobilis* cell envelopes and FT-IR spectral data (Table 4). The concentrations of principal cell envelope components were found to correlate with the spectral data (the absorbance intensities and the ratio of absorbance intensities). Two variables turned out to be enough to form MLR models with significant rank correlation coefficients: model II (Kendall's $\tau = 0.320$, $P < 0.025$, Spearman's $\rho = 0.498$, $P < 0.025$), model III (Kendall's $\tau = 0.446$,

Table 4
Elements and the statistical indices for multiple linear regression models which link the concentration of lipids, proteins and carbohydrates and hydrophobicity of *Z. mobilis* 113S cell envelopes and FT-IR absorbance intensities at fixed wavenumbers.

Regression model	Dependent variable	Parameters[a]	Regression coefficient	S.E.	*t* value	*P* value	R^2%	$R^2_{adjusted}$ %	VIF[b]
I	Hydrophobicity	*Constant*	−0.0000195	0.1026	−0.00019	0.0000	81.21	77.89	
		1088	−0.6859	0.1085	−6.3210	0.0000	95.29[c]		1.065
		2875	0.7407	0.1183	6.2589	0.0000			1.267
		1088/985	0.4718	0.1217	3.8781	0.0012			1.339
II	Proteins	*Constant*	−0.0000287	0.1211	−0.00024	0.0001	75.35	69.19	
		1088	−0.6417	0.1315	−4.8799	0.0002	94.84[c]		1.122
		1088/985	0.4011	0.1604	2.5001	0.0237			1.671
		2927/2854	−0.5805	0.1756	−3.3050	0.0045			2.002
		1600	0.4309	0.2050	2.1023	0.0517			2.727
III	Carbohydrates	*Constant*	0.0000195	0.1319	0.00015	0.0004	70.77	63.46	
		1088	0.5958	0.1475	4.0399	0.0009	83.62[c]		1.191
		2960/2875	0.4549	0.1754	2.5926	0.0196			1.685
		1088/985	−0.4367	0.1580	−2.7440	0.0138			1.366
		2960/2927	0.3738	0.1681	2.2235	0.0409			1.549
IV	Lipids	*Constant*	−0.0000136	0.1321	−0.00001	0.0004	70.68	63.36	
		2875	0.6898	0.2930	2.3540	0.0317	80.36[c]		3.686
		1455/1400	−1.2173	0.2385	−5.1042	0.0001			3.104
		1660/1455	0.8418	0.1978	4.2558	0.0006			2.135
		2960/2854	−1.0447	0.3626	−2.8814	0.0109			3.488

[a] Elements of multiple linear regression which represent the absorbance intensities at fixed wavenumbers cm^{-1} (absorbance intensities at 1700–1500 cm^{-1} represent the absorption modes of Amide I and Amide II, C—N *str* of protein and peptides amide; at 1200–900 cm^{-1} assigned to carbohydrates; between 3000 cm^{-1} and 2800 cm^{-1} representing the absorption modes of fatty acid chains (e.g., membrane phospholipids); at 1470–1350 cm^{-1} provide information about membrane fatty acids) and the constant (intercept) of equation.
[b] The variance inflation factor which indicates the impact of multicollinearity between the independent variables [34].
[c] LOCCV (leave-one-out cross-validation).

$P<0.01$, Spearman's $\rho=0.663$, $P<0.01$) and model IV (Kendall's $\tau=0.345$, $P<0.025$, Spearman's $\rho=0.581$, $P<0.01$). Furthermore, MLR models with 4 variables were able to predict the concentration of proteins, carbohydrates and lipids with good accuracy (75%, 71% and 71% R^2, correspondingly) (Table 4). These results indicate statistically robust multivariate relationships between the FT-IR spectral data and the concentration of proteins, carbohydrates and lipids of *Z. mobilis* cell envelopes. The plots of the estimates predicted from the MLR models versus the actual concentrations of proteins, lipids and carbohydrates are shown in Fig. 3. It should be noted that rather small or moderate VIF [34] values (Table 4) also indicate that the observed multivariate relationships are not formed due to the multicollinearity of independent variables.

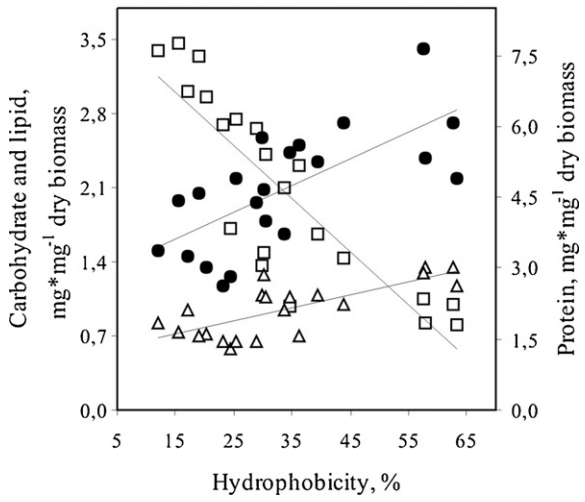

Fig. 2. The relationship between the cell surface hydrophobicity and the concentrations of proteins (●) $r=0.693$, $\rho=0.728$, $P<0.01$, lipids (△) $r=0.744$, $\rho=0.691$, $P<0.01$ and carbohydrates (□) $r=-0.855$, $\rho=-0.895$, $P<0.01$ of *Z. mobilis* 113S cell envelopes determined by biochemical analysis.

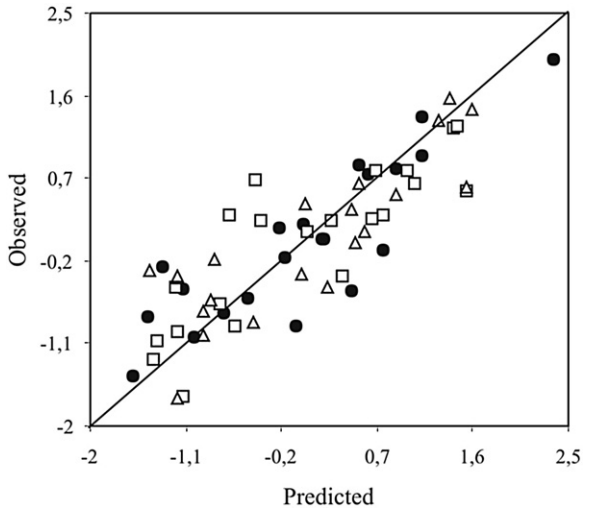

Fig. 3. The estimates from the multiple linear regression models (predicted) versus the known concentrations of proteins (○) $r=0.868$, $\rho=0.827$, $P<0.01$, lipids (△) $r=0.841$, $\rho=0.817$, $P<0.01$ and carbohydrates (■) $r=0.841$, $\rho=0.844$, $P<0.01$ (observed).

4. Discussion

Z. mobilis possess a normal Gram-negative cell wall consisting of a peptidoglycan monolayer with lipoprotein covalently linked to peptide units of peptidoglycan and outer membrane LPS layer [3]. Observed relationships between the CSH of *Z. mobilis* and the content of principal cell envelope components (Fig. 2, Table 3) appear in agreement with a common view on the hydrophobic character of constituents in the polymorphic composition of cell envelopes from proteobacteria [8,46,47]. It is known that the CSH, in general, reflects the ratio between hydrophobic and hydrophilic constituents of the cell envelope [10] and the spectral region from 3100 cm^{-1} to 2800 cm^{-1} is

dominated by the absorption modes of lipid aliphatic chains and the region from 1200 cm^{-1} to 900 cm^{-1} represent a stretching vibrations of various oligo- and polysaccharides [20]. The outer membrane of Gram-negative bacteria (including *Z. mobilis*) have a lipopolysaccharide (LPS) layer, which consists of hydrophilic closely packed oligosaccharide core and hydrophobic highly ordered hydrocarbon chain region [8]. Thus, the observed multiple regressions linking the CSH values of *Z. mobilis* cell envelopes and selected indices of FT-IR spectra (absorbance intensities at 2875 cm^{-1}, 1088 cm^{-1} and ratio of absorbance at 1088/985 cm^{-1}) (Table 4, model I) could reflect relations between the hydrophobic (lipid chains) and hydrophilic (carbohydrates) components of cell envelopes.

The observed multiple correlations between the concentration of proteins, carbohydrates and lipids of cell envelopes and selected indices of FT-IR spectra (Table 4, Fig. 3) could indicate the complex relationship of cell envelope constituents. Thus, changes in protein concentration, which appear as correlated with selected indices of FT-IR spectra (absorbance intensities at 1660 cm^{-1}, 1088 cm^{-1} and ratio of absorbance at 2927/2854 cm^{-1} and 1088/985 cm^{-1}) (Table 4, model II) could be associated with changes of lipoprotein or glycoprotein concentration in cell envelope. The multiple correlation of carbohydrate concentration and selected indices of FT-IR spectra (absorbance intensities at 1088 cm^{-1} and ratio of absorbance at 2960/2875 cm^{-1}, 1088/985 cm^{-1} and 2960/2927 cm^{-1}) (Table 4, model III) likely are associated with changes in outer membrane LPS concentration. The changes in lipid concentration, which were found to correlate with selected indices of FT-IR spectra (absorbance intensities at 2875 cm^{-1} and ratio of absorbance at 2960/2854 cm^{-1} 1455/1400 cm^{-1} and 1660/1455 cm^{-1}) (Table 4, model IV) could be associated with changes of phospholipids, lipoprotein or glycoprotein concentration in cell envelope of *Z. mobilis*.

A variety of structural rearrangements in bacterial envelopes caused by changes of the H-bonding, charge/dipole, hydrophobic and van der Waals interactions amongst others have been reported to impair the permeability barrier and therefore, the vital functions of cells [8,35]. The spectral region from 3100 cm^{-1} to 2800 cm^{-1} is dominated by the absorption modes of lipid aliphatic chains [20,25]. There are IR absorption bands due to antisymmetric and symmetric modes of the methyl chain at about 2960 cm^{-1} and 2870 cm^{-1}, correspondingly. The observed changes of FT-IR spectral indices assigned to ν_{as} (CH_3) and ν_{sym} (CH_3) vibrations could indicate alternations in the orientation of methyl groups and in the chain packing mode of aliphatic residues [38] with the growth of *Z. mobilis* 113S CSH values (Table 2) since a sensitive orientation information utilizing vibrational spectroscopy can be gained from the observation of methyl vibrations [39]. At the region near 2920 cm^{-1} and 2850 cm^{-1}, there are IR absorption bands, which are due to antisymmetric and symmetric modes of the methylene chain at about 2960 cm^{-1} and 2870 cm^{-1}, respectively. The wavenumber of the absorption maximum of these bands could be conformation-sensitive and respond to the temperature induced changes of the *trans-gauche* ratio in acyl chains [20,36]. However, the second derivative spectra showed that the shifts of the band position in this region are significantly reduced in comparison with the shifts observed in raw spectra (Table 3) therefore most likely reflecting the component concentration changes, rather than *trans-gauche* conformational changes.

IR bands at 1740 and 1725 cm^{-1} due to the stretching of the C—O bond of 1,2-diacylglycerolipid bi-layers are also sensitive to the physical state of lipids [37,40]. The shift of C—O stretching wavenumber is considered as indicative for different hydrogen bonding interactions of the carbonyl oxygen with its immediate protein environment [41] since the ratio between the 1740 cm^{-1} and 1725 cm^{-1} band absorbances is larger in the liquid than in the gel phase [37]. However, observed wavenumber shift of alkyl esters (>C=O) from 1745 cm^{-1} to 1735 cm^{-1} with the increase of *Z. mobilis* CSH values in raw spectra (Table 3) was not confirmed by second derivative spectra and most likely are attributed to the component concentration changes.

Amide I and Amide II bands are two major bands of protein in the IR spectrum. The Amide I band (between 1700 cm^{-1} and 1600 cm^{-1}) is mainly associated with the C=O stretching vibration and is directly related to the backbone conformation. Amide II results from the N—H bending vibration and from the C—N stretching vibration. Amide bands are conformationally sensitive [42]. It is known that an increase in amide hydrogen-bonding causes the Amide I and Amide II band wavenumber to decrease and increase, respectively [43,44]. Hence, the observed changes of FT-IR spectra indices assigned to Amide I and Amide II vibrations in raw spectra (Table 3) which can also be observed as well remained in the second derivative spectra, Amide I band position shift could be attributed, at least in part, to the conformational changes of the protein as along with the changes of its concentration with increasing CSH values of *Z. mobilis*.

The correlation between the ratio of absorbances of the Amide I and Amide II, that results from the formation of intermolecular hydrogen-bonding insoluble protein [45], and antisymmentric and symmetric stretching vibrations of methylene groups [to ν_{as} (CH_2) and ν_{sym} (CH_2), correspondingly (Table 2), that respond to the changes of the *trans-gauche* ratio in the polymethylene chains, and thus to the chain order [46], could indicate the changes in interaction of membrane proteins and lipids (phospholipids) with the increase of *Z. mobilis* CSH values.

The region from 1200 cm^{-1} to 900 cm^{-1} is mostly dominated by a sequence of bands due to C—O, C—C, C—O—C and C—O—H stretching vibrations of various oligo- and polysaccharides [20] and these groups mainly occur in carbohydrates. In cell envelopes the observed decrease of absorbance intensities and significant wavenumber shift of bands assigned to carbohydrate vibrations (Tables 2 and 3) with the increase of *Z. mobilis* 113S CSH values could arise from the intramolecular H-bonding between carboxylic and hydroxyl groups, therefore indicating the decrease of carbohydrate concentration [16,20].

Therefore, the observed correlations between CSH values and band position shifts in lipid, protein and carbohydrate regions together with correlations between concentrations of proteins, carbohydrates and lipids as well as their ratio (Fig. 2, Table 3) most likely reflect the changes in the composition of the cell surface components of *Z. mobilis* including LPS, phospholipids, lipoproteins and glycoproteins, thus confirming the observed multiple correlations between the concentration of cell envelope constituents and selected indices of FT-IR spectra (Table 3, Fig. 3).

The results of this present study suggest that FT-IR spectroscopy could be considered as a convenient approach in cell envelope composition studies since it provides several advantages as compared with biochemical analyses. Namely, advantages of FT-IR spectroscopy: simple preparation, small quantity of sample, no need to perform laborious chemical extractions, provide quick reliable results in respect of the cell envelope structural properties. This investigation demonstrates that the chemical composition of *Z. mobilis* 113S cell envelopes is closely linked to the CSH values and reflects the relations between their hydrophilic and hydrophobic constituents.

Acknowledgements

This work was supported by the European Social Fund within the project “Support for Doctoral Studies at University of Latvia” - PhD L. Shakirova.

Mara Grube, Marita Gavare & Peteris Zikmanis thanks the project Nr. 2009/0207/1DP/1.1.1.2.0/09/APIA/VIAA/128 Establishment of Latvian interdisciplinary interuniversity scientific group of systems biology for financial support; http://www.sysbio.lv. RG thanks the UK BBSRC for financial support.

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