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Control of unsaturated fatty acid substituents in emulsans

A. Gorkovenko[a,1], J. Zhang[a,2], R.A. Gross[a,*], D.L. Kaplan[b]

[a]*Polytechnic University, Polymer Research Institute, Six Metrotech Center, Brooklyn, NY 11201, USA*
[b]*Tufts University, Chemical Engineering, 4 Colby Street, Medford, MA 02155, USA*

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Abstract

The ability to regulate the content of unsaturated fatty acids (FAs) of emulsans (EMs) formed by *Acinetobacter calcoaceticus* RAG-1 was studied. Studies of EM biosynthesis with $^{13}C_1$-labeled FAs demonstrated that 95 ± 7% of 16:1(9-*cis*) incorporated into EMs (EM-FAs) were formed by desaturation of the carbon source 16:0. An aerobic desaturation mechanism involving Δ-9 desaturase activity was proposed to explain these results. The direct incorporation of Δ-9-*cis* unsaturated acids occured concurrently with a decrease in the content of other 9-*cis* unsaturated EM-FAs. Important factors which ultimately determined the composition of unsaturated EM-FAs were the following: (i) feedback inhibition of Δ-9 desaturase activity, (ii) direct incorporation of FAs from a carbon source and (iii) two-carbon unit elongation or removal. The incorporation of polyunsaturated FAs into EMs was also accomplished by the selective feeding method. For example, by feeding RAG-1 with 18:2(9,12-*trans*), an EM was formed that contained almost 55 nmol/mg-EM (GC-MS). The surface activities of the new EMs from unsaturated FAs were evaluated.

Keywords: Emulsan; Control; Fatty acids

1. Introduction

The gram negative bacterium *Acinetobacter calcoaceticus* RAG-1 grows on a variety of different substrates as sole carbon sources, including crude oil, middle chain length alkanes, alcohols, fatty acids (FAs) and triglycerides. When grown under proper conditions the microorganism produces a family of extracellular anionic lipoheteropolysaccharides known as emulsans (EMs) (Fig. 1) (Rosenberg et al., 1979; Gorkovenko et al., 1995; Gorkovenko et al., 1997). EM stabilizes a wide variety of oil-in-water emulsions by forming a strong film at the oil–water interface (Rosenberg et al., 1979; Zosim et al., 1981). The polysaccharide backbone of emulsan was reported to contain the amino sugars D-galactosamine, D-galactosaminuronic acid, and diamino-6-deoxy-D-glucose (Gutnick, 1987). FAs ranging in chain length from 10 to 18 carbons are covalently linked by N-acyl and O-ester bonds to the EM backbone. These FAs constitute between 5% to 15% (w/w) (Gutnick and Shabtai, 1987; Belsky et al., 1979) or 300 to 700 nmole of FA per mg of product (nmol/mg-EM) (Gutnick et al., 1982).

Previously, we reported that selectively-feeding *A. calcoaceticus* RAG-1 with various saturated *n*-alkanoic acids specifically altered the side chain composition of EMs (Gorkovenko et al., 1995; Gorkovenko et al., 1997). High levels of direct carbon source incorporation were found for FAs with chain lengths from 15 to 17-carbons. For example, by culturing *A. calcoaceticus* RAG-1 on 15:0, of the total EM-FAs formed, 53 mol% were either 15:0 or 15:1. In contrast, for EMs produced on FA substrates with even carbon chain lengths, odd carbon chain length side groups were not detected (Gorkovenko et al., 1995; Gorkovenko et al., 1997). Direct incorporation of a given saturated FA was always accompanied by an increase in the content of unsaturated acids of that chain length. The degree of EM-FA desaturation increased for relatively longer side groups. For example, the percent desaturation increased from 11% for 14-carbon to 85% for 18-carbon EM-FA substituents (Gorkovenko et al., 1997). Aerobic desaturation as a result of Δ-9 desaturase activity was postulated (Gorkovenko et al., 1997). Later, our laboratories reported that the selective feeding strategy was also useful for the direct incorporation of various 2-hydroxy FAs into EMs (Zhang et al., 1997).

The objectives of this study were the following: (i) to determine the major factors that influence the composition

* Corresponding author.
[1] Present affiliation: Moltech Corporation, 9000 South Rita Rd., Bld. 061, Tucson, AZ 85747-9108, USA
[2] Dept of Food Sci and Human Nutrition and Center for Crops Utilization Research, Iowa State University, USA

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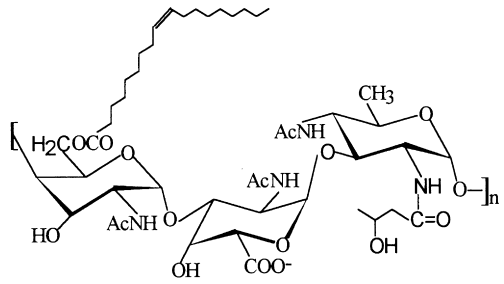

Fig. 1. A representitive structure of emulsan.

of unsaturated EM-FAs, (ii) to incorporate unusual unsaturated FAs into EMs and iii) to evaluate structure–property relationships of the resulting new EM-analogs.

2. Materials and methods

Chemicals. Oleic acid, 18:1(9-*cis*), 99%; *cis*-vaccenic acid, 18:1(11-*cis*), 99%; linoleic acid, 18:2(9,12-*cis*), 99%; linolelaidic acid 18:2(9,12-*trans*), 99%; linolenic acid 18:3(9,12,15-*cis*) 99%; and palmitoleic acid, 16:1(9-*cis*), 99%, were all purchased from Sigma and were used as received. Palmitic and stearic (95%) acids as well as the medium components were of reagent grade and were obtained from Aldrich. 13C1-labeled (99 atom% 13C) palmitate was acquired from Cambridge Isotope. Saturated FA methyl esters with chain lengths of C10 to C19 were obtained from Aldrich in purities of $\geq$ 99% and were used as received. Unsaturated methyl esters of the FAs listed above as well as methylpetroselinate, 16:1(6-*cis*), were obtained from Sigma in purities $>$ 99% and used as received.

Bacterial strain. The strain *A. calcoaceticus* RAG-1 (ATCC 31012) was obtained from the American Type Culture Collection (ATCC) (Reisfeld et al., 1972). For long term preservation, the cells were grown to the late logarithmic phase and stored at $-$ 196°C in cryovials.

2.1. Medium and culture conditions

Cells were grown in a defined (Shabatai and Wang, 1990) medium which contained (per liter of water): $K_2HPO_4.3H_2O$, 22.2 g; KH_2PO_4, 7.26 g; $MgSO_4.7H_2O$, 0.5 g; $(NH_4)_2SO_4$, 4 g; 1% w/v (unless otherwise specified) of the sole carbon source or cosubstrate mixture, and 3 ml of a trace metal solution which contained (per 100 ml): $CaCl_2.2H_2O$, 36.8 mg; $FeSO_4.H_2O$, 60.4 mg; $ZnSO_4.7H_2O$, 42.2; Na_2MoO_4, 69.6 mg; $CuSO_4.5H_2O$, 62.4 mg; $MnSO_4.4H_2O$, 59.4 mg; $CoCl_2.6H_2O$, 78.8 mg. The $MgSO_4.7H_2O$ stock solution (concentrated 100-fold) was sterilized separately and then added to the culture medium. The seed cultures and fermentations were carried out in 500 ml baffled shake flasks each containing 150 ml of the defined medium. The flasks were placed in a Lab-line incubator-shaker and maintained at 30°C (250 rpm). The cryovials of the frozen culture were rapidly thawed at 37°C and were used to innoculate liquid precultures containing ethanol (1% w/v) as the carbon source. For emulsan production, cells were cultured for 75 h. Additional details on cell preservation and innoculum preparation were described elsewhere (Gorkovenko et al., 1997).

Biopolymer Isolation. The method used was adapted from a literature procedure (Rosenberg et al., 1979). The procedure which involves isolation of the cell-free supernatant by centrifugation, precipitation of the crude product by ammonium sulfate, dialysis against water to remove low molecular weight water solubles and Soxhlet extraction using ether to remove residual lipids, was described in detail elsewhere (Gorkovenko et al., 1997). Purified emulsan yields ranged from 250 to 580 mg/L.

Analysis of EM-FAs. The saponification protocol for hydrolysis of acylated EM-FAs was described elsewhere (Gorkovenko et al., 1997). The fatty acid mixture was esterified by diazomethane in ether and the volatiles were removed in vacuo. The remaining material was dissolved in 1 ml of $CHCl_3$, filtered through a 0.45 μm syringe filter, dried using an argon stream and taken up in 50 μL of $CHCl_3$. The fatty acid compositions of N- and O-acylated EM-FAs were determined by gas chromatography (GC) on a Perkin Elmer model 8500 GC calibrated by determining relative response factors with fatty acid methyl ester standards and nonadecanoic acid methyl ester as the internal standard (see above). The column was a cross-linked 5% phenyl methyl silicone (HP Ultra 2, 25 m $\times$ 0.2 mm, film thickness 0.33 μm). The typical column temperature profile was as follows: 1 min at 140°C, 5°C/min ramp to 240°C, and then 3 min at 240°C. A splitless injector (290°C) and an FID detector (310°C) were used. Further identification of GC peaks was carried out by GC-mass spectrometry (MS). GC-MS was performed on a Hewlett Packard 5890 Series II GC coupled to a mass selective detector (HP 5971 Series) using EI ionization and the identical GC-column, elution temperature profile and sample preparation method as above. Mass spectra of peaks were compared to those in the instrument data bank (NIST library) as well as to mass spectra collected for methyl esters of known structure.

Studies using $^{13}C_1$-labeled palmitic acid (16:0) as a carbon source Fermentations were performed in 50 ml of minimum salt medium (as above) containing potassium acetate (413 mg, 84 mM) and 16:0 (250 mg, 20 mM) as cosubstrates. The $^{13}C_1$ content of 16:0 was varied from 1.1 to 99% by preparing mixtures of $^{13}C_1$-16:0 and non-labeled 16:0. The mol% of $^{13}C_1$-labeled 16:0 and 16:1 (9-*cis*) EM substituents was determined by averaging the relative intensities of the isotopomer ions from the fatty acid methyl esters corresponding to the McLafferty rearrangement (m/z 74 and 75) and carbomethoxy ions (m/z 87 and 88) (Budzikiewicz et al., 1967). The percentage of ^{13}C was based on a calibration curve generated using fatty acid methyl esters containing from 1.1% to 99% $^{13}C_1$.

Emulsification test. Emulsification activity was evaluated

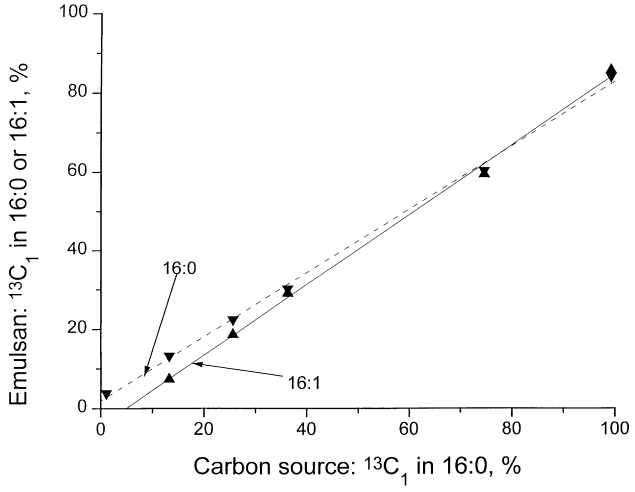

Fig. 2. The $^{13}C_1$ content of 16:0 and 16:1(9-cis) EM-FAs versus the $^{13}C_1$ enrichment of the 16:0 carbon source. In these experiments, potassium acetate (413 mg, 84 mM) and 16:0 (250 mg, 20 mM) were cosubstrates.

by a method described elsewhere (Rosenberg et al., 1979). Briefly, the procedure used 0.2 ml of hexadecane as the oil phase and 15 ml of EM solutions (83 mg/L, see below) in 0.02 M Tris-Mg buffer (pH 7.2) containing 0.01 M $MgSO_4$. The mixtures were agitated by shaking at 250 rpm (30°C) for 1 h in 250 ml baffled flasks, then transferred to Klett tubes (10 mm diameter) and left for 10 min unagitated. The turbidity was measured using a Klett-Summerson colorimeter fitted with a green filter.

Surface tension (ST) and interfacial tension (IT) measurements. ST and IT were measured following standard methods (ASTM D1331-89 and ASTM D971-91) using a Fisher Scientific Tensiometer 21. All ST and IT readings were recorded after 2 h equilibration of unagitated solutions containing 83 mg/L of an EM-analog. The selection of 83 mg/L was based on work carried out elsewhere (manuscript in preparation) which showed that the critical micelle concentration of EM-analogs in these solutions was $\leq$ 50 mg/L. Hexadecane was used as the oil phase for IT measurements.

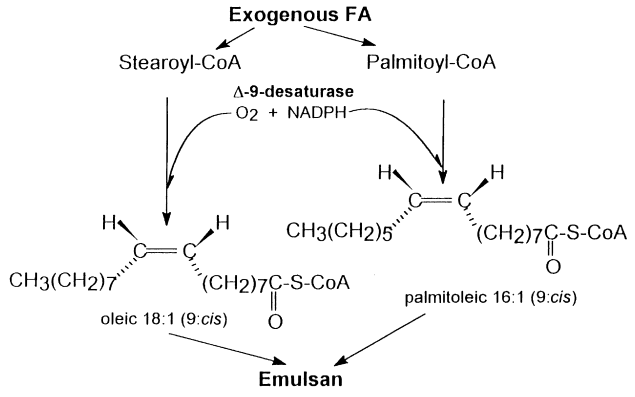

Fig. 3. The desaturation of preformed FAs by the aerobic desaturation pathway which involves the action of a Δ-9-desaturase.

3. Results and discussion

3.1. Aerobic desaturation mechanism for the formuation of unsaturated FAs

The most abundant unsaturated EM-FA substituents usually produced from even chain FA carbon sources are 16:1 (9-*cis*) and 18:1 (9-*cis*) (Belsky et al., 1979; Gorkovenko et al., 1995; Gorkovenko et al., 1997). To investigate biochemical pathways involved in the formation of unsaturated EM-FAs, cultivations were performed using acetate (84 mM) and palmitate (20 mM) as cosubstrates. In these fermentations, the mol% of $^{13}C_1$-labeled 16:0 was varied incrementally from 1.1 (natural abundance) to 99% so that values of $^{13}C_1$-EM FA incorporation could be generated from multiple data points. The resulting 16:0 and 16:1 (9-*cis*) EM-FAs were analyzed by GC-MS to determine the mol% of $^{13}C_1$-label. Plots of the $^{13}C_1$-mol% for both 16:0 and 16:1 (9-*cis*) substituents versus the carbon source $^{13}C_1$-mol% are shown in Fig. 2. Analysis by linear regression showed that both plots were linear (correlation coefficients of 0.9989 and 0.9987, respectively) and had similar slopes (0.81 and 0.89, respectively). Extrapolation by linear regression to 100% the ^{13}C1-labeled 16:0 carbon source showed that 84 $\pm$ 2% of 16:0 EM FAs were incorporated intact from the carbon source. Similarly, by extrapolating to 100% 13C1-labeled 16:0 carbon source, we determined that 85 $\pm$ 2% of 16:1 (9-*cis*) EM-FAs were 13C1-labeled. Comparison of these results suggests that 95 $\pm$ 7% of the 16:1 (9-*cis*) FA pool was formed from the 16:0 carbon source. Hence, it was concluded that double bonds were introduced directly into preformed saturated long-chain acyl-CoA substrates by an aerobic desaturation pathway. This biochemical route is considered almost universal for bacteria and generally involves a protein complex that has a Δ-9-desaturase (Gurr and Harwood, 1991). In all cases, Δ-9-desaturases introduce *cis* double bonds at carbon atoms 9 and 10 counting from the carboxyl end of the FA. The enzymes and cofactors involved in double bond formation by this pathway have been described elsewhere (Gurr and Harwood, 1991), and the general reaction scheme is shown in Fig. 3. As was mentioned above, the fraction of unsaturated EM-FAs increased for relatively longer side groups (Gorkovenko et al., 1997). This is consistent with previous studies on a Δ-9-desaturase wherein the FA chain length effectiveness of the enzyme was in the order 18 $>$ 17 $>$ 16 $>$ 15 $>$ 14 carbons (Gurr and Harwood, 1991).

3.2. Feedback inhibition of desturase activity

Based on the above mechanism of desaturation, use of unsaturated FAs as carbon sources might result in product feedback inhibition of desaturase activity. This possibility was investigated by analysis of unsaturated 16- and 18-carbon chain length FAs in EMs formed on the following carbon sources: 16:0, 16:1(9-*cis*), 18:0 and 18:1(9-*cis*).

Table 1
Effects of the carbon source on 16- and 18-carbon chain length unsaturated emulsan fatty acids

Carbon source	Emulsan fatty acids nmol/mg-EM (mole%)[a] 16:1 (9-*cis*)	16:1 (7-*cis*)	18:1 (9-*cis*)	18:1 (11-*cis*)	18:2 (9,12-*cis*)	18:2 (9,12-*trans*)	total fatty acids (nmole/mg)
16:0	148 (22.7)		15 (2.3)	< 1	nd[b]	nd	651
16:1(9-*cis*)	98 (48.6)	nd	2 (0.9)	11 (5.5)	nd	nd	201
18:0	40 (6.4)	nd	131(21.1)	nd	nd	nd	622
18:1(9-*cis*)	< 1	12 (5.7)	39 (19.3)	nd	nd	nd	201
18:1(9-*cis*) methyl ester	10 (1.7)	37(6.6)	139 (24.9)	nd	nd	nd	558
18:1(11-*cis*)	23 (8.9)	nd	nd	34 (12.9)	nd	nd	261
18:2(9,12-*cis*)	3 (1.4)	nd	3.4 (1.7)	nd	2 (0.9)	nd	200
18:2(9,12-*trans*)	8 (1.6)	nd	nd	nd	nd	55 (10.6)[d]	517
18:3(9,12,15-*cis*)	no growth						
18:3(9,12,15-*cis*) + ethanol[c]	18 (6.5)	nd	43 (15.5)	nd	nd	nd	280

[a] The fatty acid composition in nmol/mg-emulsan and mol% was determined by GC analysis (see Materials and Methods);
[b] nd is not detected;
[c] The cosubstrate mixture consisted of (by wt.) 0.2% 18:3(9,12,15-*cis*) and 1% ethanol;
[d] Since 18:1(9-*cis*) and 18:2(9,12-*trans*) EM FAs were not resolved by GC, the incorporation of the latter may be < 55 nmol/mg-EM (see Results and discussion).

Comparison of fermentations on 16:0 and 16:1(9-*cis*) showed that the latter resulted in substantially lower quantities of EM-18:1(9-*cis*) side chains (Table 1). Similarly, comparison of fermentations on 18:0 and 18:1(9-*cis*) showed that the latter carbon source gave an EM from which EM-16:1(9-*cis*) side chains were barely detected (Table 1). In contrast, incubation of *A. calcoaceticus* RAG-1 on 18:0 gave 40 nmol/mg-EM of 16:1(9-*cis*) side chains. When *A. calcoaceticus* RAG-1 was cultured on saturated FA carbon sources, the fractions of side groups with unsaturated 16- and 18-carbon chain lengths were normally about 0.35–0.50 and 0.85–0.90, respectively (Gorkovenko et al., 1997). Similar cultivations on 18:1(9-*cis*) and 16:1(9-*cis*) gave mole fractions of side groups with unsaturated 16- and 18-carbon chain lengths of 0.00 and 0.44, respectively. These results accord with the conclusion that desaturase feedback inhibition strongly influences the composition of EM-FAs.

3.3. Formation of EMs with unusual unsaturated FAs

In some cases, analyses of positional isomers of unsaturated EM-FA methyl esters by GC-MS revealed electron ionization mass spectra which could not be differentiated. Difficulties were encountered for the following isomeric pairs: 16:1(7-*cis*)/16:1(9-*cis*) and 18:1(9-*cis*)/18:1(11-*cis*). To identify these isomers, differences in GC retention times of standard methyl esters were used. For example, the standard methyl esters of 18:1(9-*cis*) and 18:1(11-*cis*) were baseline-resolved and had retention times of 18.40 ± 0.2 and 18.48 ± 0.2 min, respectively. The GC peak eluted at 14.48 ± 0.2 min had a molecular ion of 268 m/z and was identified by the NIST library search as a 16:1 methyl ester with 98%–99% correlation. However, the commercially available 16:1(9-*cis*) and 16:1(6-*cis*) methyl ester standards were not eluted at this position in GC runs. This peak at 14.48 ± 0.2 min was assigned to the FA 16:1(7-*cis*), which was not available commercially, based on the following: (i) exclusion from consideration of 16:1(9-*cis*) and 16:1(6-*cis*) FA methyl esters based on retention times, (ii) the decrease in GC retention times for *cis*-monounsaturated FAs as the double bond position is closer to the carboxylic acid chain end, (iii) that the 14.48 ± 0.2 min GC peak had a mass spectrum which corresponded with that of a monounsaturated 16-carbon FA and (iv) this acid was observed only in EMs obtained from cultures grown on 18:1(9-*cis*). The latter argument will be expanded upon below.

When *A. calcoaceticus* RAG-1 was grown on either saturated *n*-alkanoic acids with chain lengths from 12 to 18 carbons or on non-FA carbon sources such as acetate or ethanol, 16:1(9-*cis*) was the only 16-carbon unsaturated EM-FA detected (Gorkovenko et al., 1997; Zhang et al., 1997). However, when 18:1(9-*cis*) or its methyl ester were fed, large changes in the 16:1 EM-FA composition resulted (Table 1). Specifically, 16:1(7-*cis*) instead of 16:1(9-*cis*) was found to be the dominant unsaturated 16-carbon EM-FA. For example, when 18:1(9-*cis*) was fed instead of 18:0, 16:1(9-*cis*) side chains decreased from 40 to < 1 nmol/mg while 16:1(7-*cis*) increased from 0 to 12 nmol/mg. These results are explained by inhibition of Δ-9-desaturase activity by 18:1(9-*cis*) and β-oxidation of 18:1(9-*cis*)-CoA to form the 16:1(7-*cis*) EM-FA. When 18:1(11-*cis*) was fed, 18:1(9-*cis*) EM-FA side chains were not detected. Instead, large quantities of 18:1(11-*cis*) (34 nmol/mg, 12.9 mol%) and 16:1(9-*cis*) (23 nmol/mg, 8.9 mol%) EM-FA side chains were found. As Δ-9-desaturase activity should not be inhibited by 18:1(11-*cis*), the formation of 16:1(9-*cis*) side groups may occur by catabolism of the carbon source, subsequent FA-biosynthesis and desaturation. Also, considering the above results for the carbon source 18:1(9-*cis*), 16:1(9-*cis*) side groups may in part be formed by β-oxidation of 18:1(11-*cis*). Formation of EM 18:1(11-*cis*)

Table 2
Determinations of surface tension (ST), interfacial tension (IT) and emulsification activity for selected EMs from unsaturated carbon sources[a]

Carbon source(s)	Emulsification activity (KU)[c]	Surface tension (dyn/cm)	Interfacial tension[d] (dyn/cm)
18:1(11-cis)	129 ± 19	47.8 ± 0.9	19.3 ± 0.1
16:1(9-cis)	214 ± 18	48.8 ± 0.8	19.1 ± 0.1
18:3 (9,12,15cis), + ethanol[b]	221 ± 10	42.9 ± 0.9	18.3 ± 0.4
18:2 (9,12trans)	260 ± 37	48.4 ± 0.4	17.9 ± 0.1
18:2(9,12cis)	345 ± 28	49.2 ± 0.5	17.1 ± 0.1

[a] The mean and standard deviations reported were from n = 3;
[b] Cosubstrate mixture of 18:3(9,12,15-*cis*) (0.2% w/v) and ethanol (1.0% w/v);
[c] KU is Klett Units from turbidity measurements;
[d] Oil phase was hexadecane.

substituents when *A. calcoaceticus* RAG-1 was fed with the corresponding FA was explained by the direct incorporation of this FA. Examination of Table 1 reveals that substantial quantities of 18:1(11-*cis*) resulted from growth on 16:1 (9-*cis*), which indicates elongation of 16:1(9-*cis*)-CoA by a 2-carbon unit. These products of 2-carbon metabolism were apparently suitable substrates for an emulsan acyl transferase.

Most bacteria do not have the appropriate enzymes for the synthesis of polyunsaturated acids (Gurr and Harwood, 1991). Furthermore, polyunsaturated acids were not found in EMs (Gorkovenko et al., 1997). Therefore, the selective feeding of these acids was investigated as a strategy to incorporate them into EMs. Initially, *A. calcoaceticus* RAG-1 was fed 18:2(9,12-*trans*) and the EM-analog formed was analyzed. Unfortunately, we were unable to resolve 18:1(9-*cis*) and 18:2(9,12-*trans*) methyl esters by GC while running standards. However, comparison of the mass spectra over the entire width of the relevant GC peak showed a high correlation (95%) to the standard spectrum of methyl 18:2(9,12-*trans)* and a poor correlation (23%) to the spectrum of methyl 18:1(9-*cis*). Thus, it was concluded that the GC peak consisted primarily of methyl 18:2(9,12-*trans*) (about 55 nmol/mg-EM) with possible low level contamination by methyl 18:1(9-*cis*). Incorporation into EM of 18:2(9,12-*trans*) suggests that this acid was a suitable substrate for an EM acyl transferase. In contrast, when *A. calcoaceticus* RAG-1 was fed the *cis*-structural isomer 18:2(9,12-*cis*), the EM formed had only 2 nmol/mg-EM (0.9 mol%) of 18:2(9,12-*cis*) side chains. Thus, differences between the *cis* or trans isomeric forms of 18:2(9,12) resulted in large variations in the ability of the EM-acyl transferase to incorporate these substrates. Incubation of *A. calcoaceticus* RAG-1 on 18:3(9,12,15-*cis*) as the sole carbon source did not support cell growth. We then incubated the bacterium on a cosubstrate mixture of 18:3(9,12,15-*cis*) (0.2% w/v) and ethanol (1.0% w/v) that supported cell growth. However, the EM formed had no unusual FAs and the direct incorporation of 18:3(9,12,15-*cis*) was not observed.

Inspection of Table 1 suggests that the polyunsaturated acids 18:2(9,12-*trans*) and 18:2(9,12-*cis*) may also inhibit Δ-9-desaturase activity. Incubations of the microorganism on 18:2(9,12-*trans*) and 18:2(9,12-*cis*), respectively, gave only 8 and 3 nmol/mg-EM of the 16:1(9-*cis*) substituent. These results provide preliminary insights into product feedback inhibition of the RAG-1 Δ-9-desaturase system by more complex polyunsaturated FA feedstocks.

3.4. Surface activity of EM-analogs from unsaturated carbon sources

Solutions of EM-analogs (83 mg/L) were analyzed to determine surface tensions (ST) at the air–water interface, interfacial tensions (IT) at the water–hexadecane interface and emulsification activities by turbidity measurements using hexadecane as the oil phase (see Materials and Methods). Values of ST, IT and emulsification activity are listed in Table 2. ST and IT values ranged from 42.9 to 48.8 dyn/cm and 17.9 to 19.3, respectively. Comparison of IT and emulsifying activity values showed no apparent correlations. Similarly, there were no regular trends observed when comparing ST and emulsifying activity. This inability to correlate emulsification activity with ST and IT values was also observed elsewhere for emulsan analogs prepared from incubations of *A. calcoaceticus* RAG-1 on hydroxyacids (Zhang et al., 1997). In general, the emulsification activities of EMs produced on unsaturated FAs were higher than for the EMs formed from *n*-alkanoic acids (Gorkovenko et al., 1997) and hydroxyacids (Zhang et al., 1997). The relationships between emulsification activity and product structure is a complex multivariable problem where sites of chain acylation, main chain sugar composition, molecular weight, FA composition, degree of heteropolysaccharide FA substitution and non-covalent interactions with proteins would need to be considered. Our laboratories have completed a study which showed that to some extent, EM emulsification activity can be correlated with the substituent FA composition and degree of substitution (manuscript in preparation). Further work to probe the effects of other EM structural features on its surface activity is underway.

4. Summary of results

$^{13}C_1$-labeling studies demonstrated that 95 ± 7% of the 16:1 (9-*cis*) FA pool was formed from the 16:0 carbon source. Therefore, we concluded that double bonds were introduced directly into preformed saturated long-chain acyl-CoA substrates by an aerobic desaturation pathway. Growth of *A. calcoaceticus* RAG-1 on 16:1(9-*cis*) and 18:1(9-*cis*) gave fractions of 18- and 16-carbon chain length unsaturated EM-FAs, respectively, that were well below those given by saturated FAs used as carbon sources. This result and others in this study showed that Δ-9 desaturase feedback inhibition strongly influences the composition of EM-FAs. Indeed, it appears that the use of controlled amounts of unsaturated FAs in *A. calcoaceticus* RAG-1 incubations may be an important mechanism by which EM substituent unsaturation can be regulated. When RAG-1 was fed with 18:1(9-*cis*) or its methyl ester, 16:1(7-*cis*) instead of 16:1(9-*cis*) was found to be the dominant unsaturated 16-carbon EM-FA. Furthermore, substantial quantities of 18:1(11-*cis*) resulted from incubations on 16:1 (9-*cis*). Thus, 2-carbon metabolism of unsaturated carbon sources resulted in the formation of EMs containing unusual monounsaturated FAs. Furthermore, cultivations of *A. calcoaceticus* RAG-1 on 18:1(11-*cis*) resulted in an EM with large quantities of 18:1(11-*cis*) (34 nmol/mg, 12.9 mol%), which was attributed to the direct incorporation of 18:1(11-*cis*). The selective feeding strategy was also used to prepare EMs with polyunsaturated FAs. For example, by feeding *A. calcoaceticus* RAG-1 with 18:2(9,12-*trans*), an EM was formed that by GC-MS analysis contained almost 55 nmol/mg-EM of 18:2(9,12-*trans*) side groups. Comparison of the *cis* and trans isomeric forms of 18:2(9,12) as carbon sources suggested that the *trans* form was preferred by the *A. calcoaceticus* RAG-1 EM-acyl transferase(s). Evaluations of the emulsification activity for selected EM-analogs produced from unsaturated FAs showed that, in general, the values were higher than those for EMs formed from *n*-alkanoic acids (Gorkovenko et al., 1997) and hydroxyacids (Zhang et al., 1997).

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