Journal of Fluorescence, Vol. 9, No. 4, 1999

Temperature-Induced Changes in the Membrane Fluidity of *Zymomonas Mobilis*: Study Using a New Flourescent Probe, ABM

I. Kalnina,[1] M. M. Toma,[2] and I. Meirovics[1]

Received August 7, 1998; accepted April 10, 1999

The use of a newly synthesized fluorescent probe, ABM (conditional name), to determine changes of membrane composition (fluidity), biomass yield, ethanol productivity, and yield of *Zymomonas mobilis* under anaerobic conditions was investigated. A strong correlation between ABM spectral characteristics in cell suspension and all obtained parameters was observed. It was concluded that application of ABM is a useful tool to detect temperature-induced changes in the membrane composition of *Zymomonas mobilis*.

KEY WORDS: Membrane fluidity; *Zymomonas mobilis*; ABM.

INTRODUCTION

The bacterium *Zymomonas mobilis* appears to have tremendous potential for commercial ethanol production. *Z. mobilis* is an obligatory fermentative Gram-negative rod, capable of producing 1.9 mol of ethanol per mol of glucose [1]. In large-scale fermentations, one of the problems encountered is the increase in fermentor temperature owing to heat released during the metabolism of carbohydrate to ethanol. Such increases in temperature have been shown to decrease the efficiency of alcohol production [2,3].

Increases in growth temperature have also been shown to induce changes in the membrane composition of many eukaryotic organisms, eliciting an adaptive response [4,5]. It is very important to receive information about changes of physical chemical properties in the membranes of microorganisms with the express method. Fluorescent probes reflect the structural and functional transformation of the microenvironment by changing fluorescence parameters and they are not toxic to the cell [6]. Some properties of the benzanthrone aminoderivative ABM (conditional name) were described earlier.

The aim of this study was to characterize temperature-induced changes of the structural organization of *Z. mobilis* using the fluorescent method of analysis. In this study we have investigated the spectral characteristics of a new fluorescent probe, ABM, after binding to *Z. mobilis*. In this study the effects of incubation temperature on growth and ethanol production were determined.

MATERIALS AND METHODS

Zymomonas mobilis ATTC 29191 was used throughout this study. This organism was grown and maintained at 30°C on medium containing glucose, 50.0 g/L; yeast extract, 5.0 g/L; KH_2PO_4, 1.2 g/L; $(NH_4)_2SO_4$, 1.0 g/L; and $MgSO_4$. $7H_2O$, 0.5 g/L. The culture was renewed every 3 weeks and stored at 4°C.

Fermentations were carried out in 250-ml Erlenmeyer conical flasks containing 150 ml of fermentation medium at temperatures of 30, 32, 34, 36, 38, and 40°C

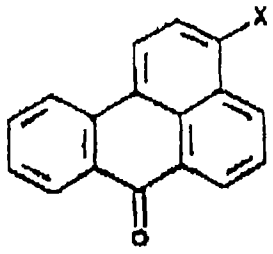
[1] Riga Technical University, Department of Organic Chemistry, Kalku str. 1, Riga LV-1048, Latvia.
[2] Institute of Microbiology and Biotechnology, University of Latvia, Kronvalda bulv. 4, Riga LV-1586, Latvia.

1053-0509/99/1200-0367$16.00/0

with 15 ml of previously grown inoculum. Biomass was determined by measuring the absorption at 550 nm and the corresponding dry weight was obtained from a standard curve. Glucose was determined using the dinitrosalicylic acid reagent [1].

The ethanol concentration was assayed by the gas chromatography method.

The fluorescent dye ABM (conditional name) was synthesized at the Riga Technical University, Department of Organic Chemistry.



Scheme 1

Synthesis was performed by means of substituting the bromide atom in 3-bromobenzanthrone with an appropriate amine.

The resulting suspension contained $0.5 \cdot 10^6$ cells/ml. The cell suspension was incubated with ABM (resulting concentration, 19.6 μM) at room temperature for 2 min. Fluorescence characteristics was registered on a Sigma 4M (Latvia) spectrofluorimeter at an excitation of wavelength 470 nm and an emission wavelength of 630 nm.

The anisotropy index "r" was calculated according to the equation

$$r = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + 2F_{\perp}}$$

where F is the fluorescence intensity parallel ($F_{\parallel}$) and perpendicular ($F_{\perp}$) to the light polarization [7].

Correlative relationships among the spectral characteristics of ABM [fluorescence maximum wavelength, fluorescence intensity in cell suspension, membrane microviscosity (anisotropy index)], biomass, and ethanol production of *Z. mobilis* were determined.

Statistical differences and correlation of independent variables were determined using programs for the Student t test and Whitney–Mann U test [8,9].

RESULTS

Investigations were performed by means of the newly synthesized fluorescent probe ABM [10–13]. Previous studies by other investigators have reported that the optimal temperature for grown and ethanol production was between 30 and 35°C. A plot of the specific growth rates for *Z. mobilis* CP4 at different temperatures indicated that the optimal growth temperature was approximately 30°C, with 37 and 41°C failing to cause an increase in growth rate as predicted by kinetic considerations [14]. Based on these results, six growth temperatures were chosen for investigation: 30, 32, 34, 36, 38, and 40°C (Table I). Results show the effect of the growth temperature on the biomass of the microorganism, efficiency of ethanol production, fluorescence intensity (F) of ABM in cell suspension, membrane fluidity (Table I), and yield characteristics of the microorganism. (Fig. 1). The results indicated that the optimal growth temperature of *Z. mobilis* was approximately 30–34°C.

Cell growth and ethanol production are reduced at elevated temperatures. A fermentation temperature from 34 to 40°C considerably decreases cell growth, ethanol production, and yield characteristics (Fig. 1). In suspensions of the microorganism grown at different temperatures, the ABM emission spectra maximum was not changed (630 nm) (Fig. 2). The decrease in growth was accompanied by a decreasing ABM fluorescence intensity in the cell suspension and an increasing membrane microviscosity (Table I).

Fluorescence depolarization studies indicate that the membranes of *Z. mobilis* with increasing growth temperatures were progressively more rigid as indicated by the higher polarization values observed at the common assay temperature of 20°C (Table I).

DISCUSSION

Investigations were performed by means of the newly synthesized fluorescent probe ABM [10–13]. Flu-

Table I. The Effect of Growth Temperature on Biomass, Ethanol Production, and Membrane Fluidity of *Zymomonas mobilis*[a]

Growth temperature (°C)	Biomass ($g \cdot L^{-1}$)	Ethanol ($g \cdot L^{-1}$)	ABM (F; rel. U)	Anisotropy index r*
30	1.14	22.6	1.14	0.323
32	1.10	19.6	1.11	0.324
34	1.08	18.9	1.04	0.328
36	1.05	8.7	0.75	0.341
38	0.73	5.0	0.38	0.354
40	0.26	2.2	0.21	0.367

[a] Polarization assay temperature, +20°C.

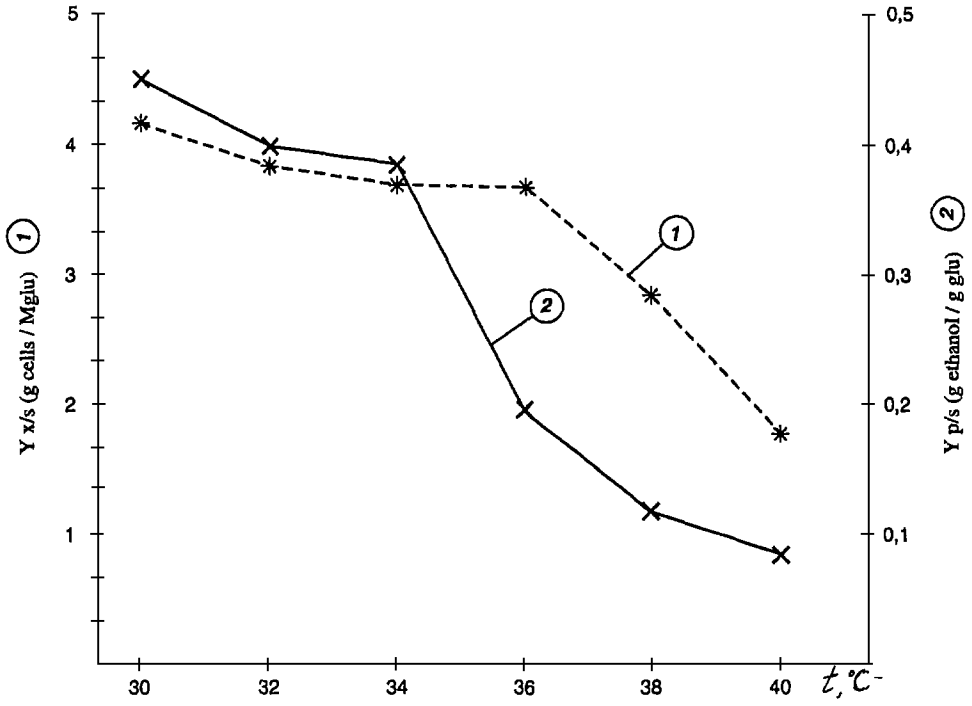


Fig. 1. Effect of growth temperature on yield characteristics of *Zymomonas mobilis*.

orescent microscopy revealed the distribution of ABM in such cell membranes as plasma, mitochondrial, and nuclear, but there were no signs of localization of probe inside the nucleus. ABM is located in the depth of the phospholipid bilayer of the cell membrane and the environment of the fluorescent probe is quite polar, similar to that of methanol [10,12].

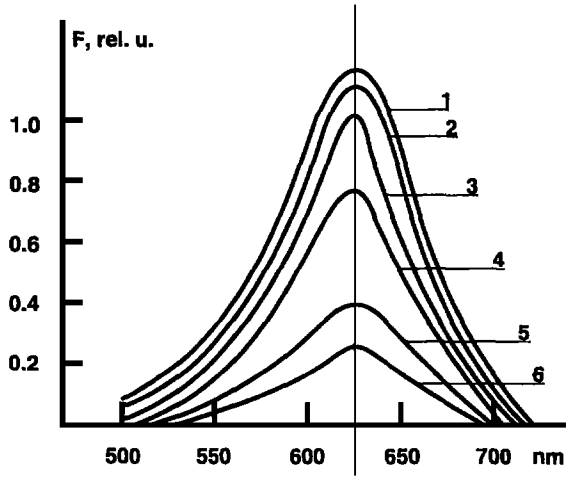


Fig. 2. Emisson spectrum of ABM fluoresence (λ_{ex}, 470 nm) in a *Zymomonas mobilis* suspension. Microorganism grown at (1) 30°C; (2) 32°C; (3) 34°C; (4) 36°C; (5) 38°C; (6) 40°C. ABM concentration in sample, 19.6 μ*M*.

Information exists on changes in the fatty acid composition of human lymphocytes during the period of blastic transformation and different pathologies [5,15–18]. Studies on lymphoid cells have demonstrated an enrichment of polyunsaturated acids in the early steps of activation. This fact has a significant positive influence on membrane microviscosity. In the case of active lung tuberculosis the degree of fatty acid unsaturation lessens and ends up with a rising membrane microviscosity [5,11–13].

Changes in membrane microviscosity have been shown to correlate not only with the plasma lipid level, but also with a decline in *in vitro* mitogen responsiveness [10,19]. Structural changes obviously influence incorporation of ABM in the cell membrane [10–13,16]. These results are in agreement with flow cytometric experiments: in the case of the investigated pathology a strong bimodal distribution with high and low ABM disappeared due to lymphocytes with intermediate F occurrence [11–13].

It was established that the spectral characteristics of ABM in cell suspension qualitatively characterize the structural and functional alterations of cells during pathological phenomena and are in correlation with the severity of the disease [10–13].

Increases in growth temperature have also been shown to induce changes in the membrane composition

of many microorganisms, eliciting an adaptive response [4,14,20]. In *Z. mobilis* with increasing temperature, the proportion of vaccenic acid declined, with an increase in myristic acid, and the proportion of phosphatidylcholine and cardiolipid increased, with decreases in phosphatidylethanolamine and phosphatidylglycerol. This increase in saturated fatty acid has been well documented in *Escherichia coli* [20,21] and also occurs in *Z. mobilis* CP4 [14]. Thus, the membrane composition of *Z. mobilis* was progressively shifted toward the ultimate products of phospholipid biosynthesis with increasing growth temperature. The phospholipid/protein ratio declined [14,22]. This index of membranes from cells grown at 20°C was approximately twice that of membranes from cells grown at 41°C [14]. Though such changes in other microorganisms have received little attention and are generally smaller than those found in *Z. mobilis*, they appear to be widespread. De Siervo [23] has shown that the phospholipid content (percentage cell dry weight) of *E. coli* is increased upon shifting cultures from 37 to 27°C.

Fluorescence depolarization studies indicate that the membranes of *Z. mobilis*, unlike those of *E. coli* [16,18], do not maintain a constant membrane fluidity during growth at different temperatures. With increasing growth temperature, the membranes from *Z. mobilis* were progressively more rigid as indicated by the higher polarization values observed at the common assay temperature of 20°C. However, temperature-induced changes in composition were insufficient to compensate for the increase in fluidity that accompanied an increase in growth temperature. The observed changes in fluidity are consistent with the decrease in the proportion of unsaturated fatty acids during growth at the higher temperatures and with the reduction in the phospholipid/protein ratio [24]. These changes in the fatty acid composition of *Z. mobilis* were relatively small in comparison with those in other organisms. The shifts from phosphatidylethanolamine to phosphatidylcholine and from phosphatidylglycerol to cardiolipin observed in *Z. mobilis* would not be expected to cause a major increase in rigidity [5]. Thus, the decrease in phospholipid/protein ratios with increasing temperature appears to be the dominant event leading to an increase in membrane rigidity with increasing growth temperature.

There is a direct correlation between ABM fluorescence intensity and microorganism biomass ($r = +0.968$), ethanol productivity ($r = +0.971$), yield characteristics ($Y_{x/s}$, $r = +0.897$; $Y_{p/s}$, $r = +0.996$), and increasing growth temperature. A reverse correlation was obtained between ABM fluorescence intensity and membrane microviscosity ($r = -0.979$). The fluorescent probe ABM is useful for studying changes of membrane composition and fluidity, especially under different growth conditions of microorganisms.

REFERENCES

1. I. Swing and I. DeLay (1997) *Bacteriol. Rev.* **4**, 1–46.
2. I. Laudrin and G. Goma (1982) *Biotechnol. Lett.* **4**, 537–542.
3. K. Ohta, K. Supanwong, and S. Hayashida (1981) *J. Ferment. Technol.* **59**, 435–439.
4. W. R. Finnerty and R. A. Makula (1975) *Crit. Rev. Microbiol.* **4**, 1–40.
5. P. J. Qulinn and D. Chapmann (1980) *Crit. Rev. Biochem* **8**, 1–117.
6. J. R. Lakowicz (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.
7. M. Shinitzky (1978) *Biochim. Biophys. Acta* **515**, 367–394.
8. H. S. Mann and D. R. Whitney (1974) *Ann. Math. Stat.* **18**, 50.
9. D. B. Duncan (1970) *Biometrics* **26**, 141.
10. I. Kalnina, I. Meirovics, and E. Rashkina (1996) *Functional Materials*, Institute for Single Crystals, Kharkov, Ukraine, Vol. 3(4), pp. 551–555.
11. I. Kalnina, I. Meirovics, and J. Rashkina (1997) in *NATO Advanced Workshop on Synthesis, Optical Properties, and Applications of Near-Infrared (NIR) Dyes in High Technology Fields*, Trest, Czech Republic, P. 20.
12. I. Kalnina and I. Meirovics (1999) *J. Fluoresc.* **9**, 27–32.
13. H. Kruckova, M. Pilmane, I. Kalnina, I. Meirovics, and V. Scripchanova (1996) *Eur. Respir. J.* **9**(Suppl. 23):335.
14. A. S. Benschoter and L. O. Ingram (1986) *Appl. Environ. Microbiol.* **51**(6), 1278–1284.
15. G. E. Dobretsov (1983) *Fluorescent Probes in Investigation of Cells, Membranes and Lipoproteins*, Moscow, Nauka.
16. L. G. Kokina, G. E. Dobretsov, G. Walzel, E. M. Kogan, Y. I. Zimin, and A. Vladimirov (1981) *J. Immunol. Methods* **45**(2), 227–237.
17. M. Shinitzky (1984) *Physiology of Membrane Fluidity*, CRC Press, Boca Raton, Vol. 1, p. 1–52.
18. A. V. Izvekova (1991) *Sucess Contemp. Biol.* **111**, 577–591.
19. I. A. Huber, G. B. Xu, G. Juergens, E. Buhler, K. F. Gey, D. Schonitzer, K. N. Trail, and G. Wick (1991) *Eur. J. Immunol.* **21**, 2761–2765.
20. M. Sinensky (1974) *Proc. Natl. Acad. Sci. USA* **71**, 522–525.
21. J. E. Cronan and P. R. Vagelos (1972) *Biochim. Biophys. Acta* **286**, 5–60.
22. C. R. Raetz (1978) *Microbiol. Rev.* **3**, 614–659.
23. A. J. De Siervo (1969) *J. Bacteriol.* **100**, 1347–1349.
24. K. M. Dombek and L. O. Ingram (1984) *J. Bacteriol.* **157**, 233–239.