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Influence of culture pH on freeze-drying viability of *Oenococcus oeni* and its relationship with fatty acid composition

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A B S T R A C T

To achieve successful malolactic fermentation in wine making, expanding interest in ready-to-use concentrated *Oenococcus oeni* starter culture has placed greater emphasis on developing starter production and preservation methods. The effect of growth factors on the survival of the *O. oeni* cells after lyophilization and its relationship with the membrane fatty acid composition were investigated. *O. oeni* cells in the early stationary phase survived better after freeze-drying than those in the mid-exponential phase, and those early stationary phase *O. oeni* cells cultured in designed ATB, FMATB and MATB growth media with different pH buffering capabilities exhibited different freeze-drying viabilities. Results concerning both membrane fatty acid composition and freeze-drying viability of *O. oeni* cells grown in those three media at initial culture pH 4.8, 4.0, 3.5, 3.2 were subjected to correlation analysis, indicating a decrease of the growth pH improved the freeze-drying survival of *O. oeni* cells, which was correlated with the enrichment of C19cyc11 and the decrease of C16:0 in the membrane lipids. A better understanding of the mechanisms of *O. oeni* resistance to lyophilization could rationalize their exploitation to prepare commercial starter cultures.

Keywords: *Oenococcus oeni*; Freeze-drying viability; Fatty acid composition; Culture pH

1. Introduction

Malolactic fermentation (MLF) has been recognized as an indispensable step in the elaboration of most wines, because this process decreases total acidity and improves the stability and quality of wine. The lactic acid bacterium *Oenococcus oeni* is the principal inducer of MLF (Lonvaud-Funel, 1999; Versari et al., 1999). As winemakers wish to exercise a greater degree of control over the MLF process, expanding interest in ready-to-use concentrated *O. oeni* starter culture, where the bacteria survive the direct inoculation into wine without any preceding adaptation or reactivation steps, has placed greater emphasis on developing starter production and preservation methods (Maicas et al., 2000).

Lyophilization offers stress conditions such as freezing, drying, and concentration stress, which diminish cell viability (Carvalho et al., 2004). Maicas et al. (2000) suggested that the use of freeze-dried cultures of *O. oeni* previously grown in MP (Medium of Preculture) were the best choice for industrial production, but the survival rate of freeze-dried suspensions of *O. oeni* cells grown in MP was less than 10% of the initial cells after one year storage. Zhao and Zhang (2005) obtained the highest viability (53.6%) after freeze-drying of *O. oeni*H-2 by using the best protectant (2.5% sodium glutamate) and rehydration (MGY medium) medium. Previous works (Garbay and Lonvaud-Funel, 1996; Guzzo et al., 1998; Beltramo et al., 2006) suggested that the acid stress adaptation treatment could improve *O. oeni* survival when directly inoculated in wine. Then a question was put forward that whether the acid stress adaptation treatment could take effect on the freeze-drying viability of *O. oeni* cells? Unfortunately, few works have been done on the effect of growth factors on the survival of *O.*

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oeni cells subsequent to freeze-drying process. Furthermore, to date no report is concerned with mechanism of *O. oeni* cells resistance to lyophilization.

Therefore, this work aims to investigate the effect of culture pH on the survival of the *O. oeni* cells subsequent to freeze-drying process. Then we looked for correlations among the culture pH, membrane fatty acid composition and the freeze-drying viability of *O. oeni* cells.

2. Materials and methods

2.1. Bacterial strain and culture conditions

O. oeni strain SD-2a (Li et al., 2006; Liu and Li, 2006) isolated from Chinese wines was studied. The culture media used were ATB, which contained (g L^{-1}): glucose, 10; yeast extract, 5; peptone, 10; $MgSO_4 \cdot 7H_2O$, 0.2; $MnSO_4 \cdot 4H_2O$, 0.05; Cysteine/HCl, 0.5; and tomato juice, 250 mL; FMATB, which contained 5 g L^{-1} glucose and 5 g L^{-1} DL-malate instead of 10 g L^{-1} glucose of ATB; MATB, which contained 10 g L^{-1} DL-malate instead of 10 g L^{-1} glucose of ATB. The medium pH was adjusted to 4.8. The stock culture was reactivated by three successive transfers in ATB medium. Then ATB, FMATB, MATB media with initial pH adjusted to 4.8, 4.0, 3.5 and 3.2 were inoculated from this pure culture and then statically incubated at 25 °C. Cell growth was monitored by absorbance readings at 600 nm using a spectrophotometer (Shimadzu, UV-1700, Japan).

2.2. Lyophilization of O. oeniSD-2a

Cells at early stationary stage were harvested under aseptic conditions by centrifugation (6000 g, 10 min) in a centrifuge (5804 R, Eppendorf, Germany). The growth medium was decanted and the harvested cells were washed once in aseptic distilled water. Cell pellet was resuspended in sterile protective agents containing 10% (w/v) skim milk solids to make a cell suspension containing approximately 1.0×10^{10} CFU/mL, allowed to equilibrate for 15 min at room temperature. Aliquots (2 mL) of each resuspension were transferred into sterilized 7 mL vials and were frozen at −20 °C overnight. Then the samples were immediately freeze-dried for 33 h in a freeze-dryer (Alpha 1–2, Christ, Germany) at a condenser temperature −45 °C, and at a chamber pressure <0.06 mbar. The end temperature of samples is 28 °C. After freeze-drying, the vials were sealed under vacuum.

2.3. Determination of cell viability

The number of viable cells before and after freeze-drying was determined as colony forming units (CFU). Decimal dilutions were prepared from the suspension before freezing and were plated on ATB agar. Freeze-dried samples were rehydrated to the original volume with sterile deionized water, incubated at room temperature for 15 min and subsequently plated on ATB agar. Plates were incubated at 25 °C for at least 7 days before the colonies were counted. Viability after freeze-drying is defined as the percentage of CFU/mL remained culturable immediately after lyophilization and rehydration, calculated on the initial CFU/mL determined before lyophilization.

2.4. Analysis of fatty acid composition

The membrane fatty acid composition of the bacteria was determined using gas chromatography as described by Rozes et al. (1993). Concentrated cells were washed twice in sterile distilled water. Methylation and extraction were performed by adding 2.0 mL of sodium methoxide (1 mol L^{-1} in methanol) (Sigma–Aldrich, Steinheim, Germany) and shaking for 1 min. Fatty acid methyl esters were extracted with 1 mL of hexane. After decanting for 2 min, the upper phase was removed and stored at −80 °C in an airtight glass bottle until analysis.

The analyses were performed on a gas chromatographer (HP 6890, Hewlett Packard, Avondale, PA) equipped with a mass selective detector (Agilent 5973, Hewlett Packard). A capillary column (BPX 70, 60 m × 0.25 mm, SGE, Victoria, Australia) was used. Helium was used as carrier gas (1.2 mL/min), and the injection volume was 1 μL. Injection was done in splitless mode for 2 min. The oven temperature was increased from 65 to 230 °C at 5 °C/min, and maintained for 10 min at 230 °C. Injection and detection temperatures were 230 °C.

Results were expressed as relative percentages of each fatty acid, which were calculated as the ratio of the surface area of the considered peak to the total area of all peaks. Analyses were made in triplicate. All chemicals were analytical-grade reagents.

2.5. Statistical analysis

To relate the resistance to freeze-drying to the fatty acid composition of *O. oeni*SD-2a, Pearson correlation analysis (DPS software) was performed for all the fermentation conditions studied. The freeze-drying viability, final medium pH values and five composition variables (C14:0, C16:0, C16:1cis9, C18:1cis11, and C19cyc11 relative concentrations) were considered.

3. Results

3.1. Effect of growth phase on the freeze-drying viability

As *O. oeni*SD-2a cultures were grown in ATB medium (Fig. 1), medium pH values decreased from 4.8 to 3.6 in cell growth cycle for production of organic acids mainly caused by glucose metabolism. Those mid-exponential phase (OD_{600} = 0.9) and early stationary phase (OD_{600} = 1.5) cells grown in ATB were harvested by centrifugation and freeze-dried. Before and after lyophilization the number of viable cells was determined. Results (Table 1) showed cells in early stationary phase notably survived well than that in mid-exponential phase.

To understand the physiological modifications induced by the growth phase, the membrane fatty acid composition of

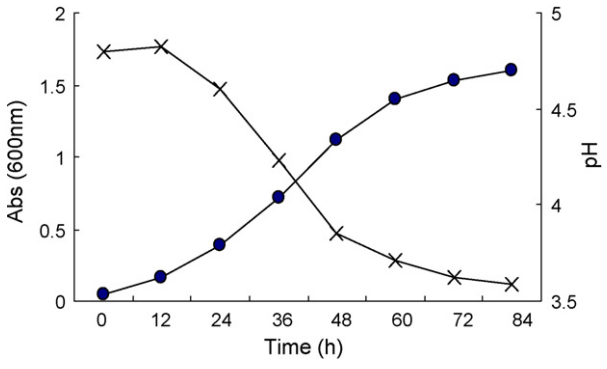

Fig. 1 – The growth curves (circles) and change in pH (crosses) in ATB medium.

Table 1 – Medium pH when harvested and freeze-drying viability of *O. oeni*SD-2a cells grown in ATB medium at different growth phase

Growth phase	Medium pH when harvested	Viability (%)
Mid-exponential phase	4.1	8.2 ± 2.0
Early stationary phase	3.6	67.5 ± 5.2

*O. oeni*SD-2a was characterized (Fig. 2). Eleven fatty acids were found in *O. oeni*SD-2a grown in ATB medium at 25 °C. However, the predominant fatty acids were: myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1cis9), cisvaccenic (C18:1cis11) and lactobacillic acids (C19cyc11), which represented more than 90% of the total fatty acids identified. These results agreed with those of Schmitt et al. (1989) and Drici-Cachon et al. (1996). Minor fatty acids C14:1trans11 had never been detected previously, may be related to the different strains and culture media used. C18:0, C16:1cis11, C17cyc9, C16:2cis9,12 and C18:2cis9,12 were also detected but in very low quantities.

As *O. oeni* cultures progress from the mid-exponential phase to early stationary growth phase, the main change occurred that the levels of C19cyc11 distinctly increased at the expense of C18:lcis11, and the amount of C16:0 decreased from 30.5% to 26.6%. We assumed that modification of the fatty acids composition should include the effect of environmental pH in addition to age of culture, which might be involved in the resistance to lyophilization. To test this potential correlation, the following series of assays were performed by changing culture pH in three kinds of culture media, and by harvesting cells at early stationary stage.

3.2. *Effect of growth media and culture pH on the freeze-drying viability and fatty acids composition*

To test our assumption, we designed three kinds of growth media with different pH buffering capability. When early stationary phase *O. oeni* cells cultured in ATB, FMATB, MATB with the same initial pH 4.8 were harvested, the final medium pH were 3.6, 4.1, 4.9, respectively, which increased along with an increase of DL-malate content in the medium. The experimental results (Table 2) showed under initial pH 4.8 conditions, the highest freeze-drying survival rate occurred in ATB medium, which corresponded with the lowest final medium pH. In this case, the fatty acid profiles were altered according to the culture media (Fig. 3). Interestingly, the relative amount of C16:1cis9 and C18:1cis11 nearly remained constant. The major

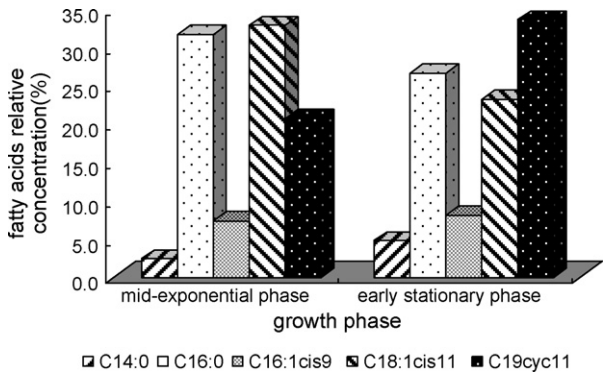

Fig. 2 – Changes in relative concentration of five major fatty acids of *O. oeni*SD-2a cells grown in ATB medium at different growth phase.

Table 2 – Fatty acid profiles, final medium pH and freeze-drying viability of *O. oeni*SD-2a cells in early stationary phase grown in different media and at different initial pH

Fatty acids (%[a] of total fatty acids)	pH 4.8			pH 4.0			pH 3.5			pH 3.2		
	ATB	FMATB	MATB	ATB	FMATB	MATB	ATB	FMATB	MATB	ATB	FMATB	MATB
C14:0	4.88 ± 0.38	3.25 ± 1.01	4.20 ± 0.20	5.51 ± 1.23	4.36 ± 0.46	4.24 ± 0.72	3.50 ± 0.53	4.60 ± 0.25	1.85 ± 0.33	4.44 ± 0.32	5.15 ± 0.60	2.67 ± 0.21
C14:1trans11	0.35 ± 0.11	0.28 ± 0.02	0.29 ± 0.02	0.40 ± 0.15	0.31 ± 0.09	0.29 ± 0.23	0.10 ± 0.01	0.43 ± 0.03	0.24 ± 0.02	0.37 ± 0.09	0.42 ± 0/03	0.13 ± 0.01
C16:0	29.63 ± 1.73	34.64 ± 1.22	38.44 ± 1.59	31.54 ± 2.59	33.95 ± 3.18	39.73 ± 2.01	26.10 ± 1.19	25.72 ± 1.42	27.03 ± 3.08	26.15 ± 2.04	24.52 ± 1.85	24.13 ± 1.36
C16:1cis9	9.38 ± 0.73	8.01 ± 1.04	8.71 ± 0.59	10.05 ± 1.72	9.00 ± 0.85	8.57 ± 1.23	7.21 ± 0.82	9.03 ± 1.02	6.98 ± 0.43	7.92 ± 0.80	8.32 ± 0.69	6.74 ± 0.58
C16:1cis11	0.84 ± 0.15	0.89 ± 0.23	0.91 ± 0.12	0.68 ± 0.33	0.59 ± 0.20	0.71 ± 0.14	0.44 ± 0.06	0.53 ± 0.03	0.46 ± 0.15	0.49 ± 0.03	0.51 ± 0.10	0.46 ± 0.13
C17cyc9	0.31 ± 0.06	0.30 ± 0.03	0.47 ± 0.11	0.78 ± 0.16	0.54 ± 0.21	0.64 ± 0.18	1.12 ± 0.17	1.05 ± 0.08	0.37 ± 0.02	0.94 ± 0.10	0.67 ± 0.04	0.72 ± 0.06
C16:2cis9,12	1.18 ± 0.20	0.81 ± 0.15	0.75 ± 0.08	1.12 ± 0.35	0.84 ± 0.21	0.74 ± 0.13	0.74 ± 0.04	1.23 ± 0.21	2.23 ± 0.08	1.60 ± 0.12	0.94 ± 0.13	1.03 ± 0.08
C18:0	0.81 ± 0.04	1.81 ± 0.02	1.61 ± 0.09	1.74 ± 0.37	1.79 ± 0.19	1.40 ± 0.07	1.58 ± 0.21	2.03 ± 0.13	2.55 ± 0.14	1.61 ± 0.27	1.28 ± 0.07	2.03 ± 0.17
C18:1cis11	20.98 ± 2.13	21.25 ± 1.83	21.37 ± 1.68	13.15 ± 2.73	14.23 ± 1.22	9.79 ± 1.04	10.67 ± 0.94	9.66 ± 1.13	12.86 ± 1.08	10.64 ± 0.66	8.83 ± 0.45	11.24 ± 1.11
C18:2cis9,12	0.49 ± 0.13	0.44 ± 0.05	0.48 ± 0.04	0.37 ± 0.11	0.35 ± 0.08	0.57 ± 0.07	0.49 ± 0.04	0.87 ± 0.05	1.24 ± 0.08	0.68 ± 0.05	0.44 ± 0.03	0.92 ± 0.10
C19cyc11	31.16 ± 2.86	28.31 ± 2.29	22.77 ± 2.31	34.66 ± 1.05	34.04 ± 2.76	33.31 ± 3.01	48.05 ± 3.67	44.84 ± 2.05	43.18 ± 3.16	45.17 ± 4.17	48.91 ± 4.51	49.94 ± 2.19
Viability[b]	67.5 ± 5.2	48.8 ± 6.7	41.3 ± 4.7	54.6 ± 6.2	87.6 ± 8.9	62.6 ± 5.1	90.7 ± 3.9	117.6 ± 10.1	85.0 ± 4.8	76.8 ± 5.1	97.3 ± 7.6	64.9 ± 8.3
Final medium pH[c]	3.6	4.1	4.9	3.5	3.7	4.1	3.4	3.5	3.7	3.3	3.4	3.5

[a] The reported relative percentages were calculated with respect to the total fatty acid methyl esters.
[b] The values are means ± standard deviations for three independent cultures.
[c] The medium pH when cells in early stationary phase were harvested.

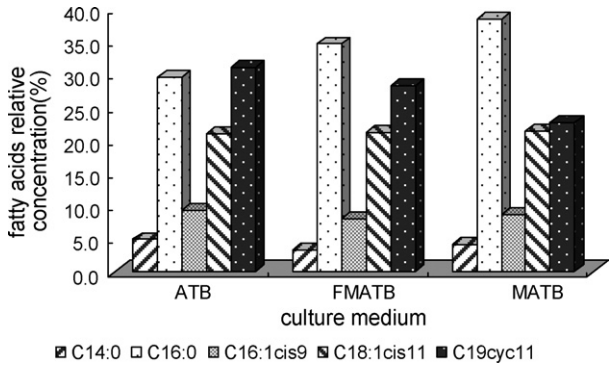

Fig. 3 – Change in relative concentration of five major fatty acids of *O. oeni*SD-2a cells grown under different growth media with initial pH 4.8.

changes occurred in the proportions of C16:0 and C19cyc11. We found the lower medium pH when harvested, the higher the proportions of C19cyc11 and the lower that of C16:0, which indicated medium pH could take effect on cellular fatty acids profiles of cells, consequently leading to different tolerance to freeze-drying.

Previous studies have indicated the adapted *O. oeni* cells grown at acidic pH increased the survival in wine (Garbay and Lonvaud-Funel, 1996; Guzzo et al., 1998; Beltramo et al., 2006), and regulation of membrane fluidity might be one of those adaptive response mechanisms (Tourdot-Marechal et al., 2000; Chu-Ky et al., 2005). In our study, further investigations were carried out to clarify the effect of acid stress adaptation treatment on freeze-drying viability of *O. oeni* cells. For the following series of assays we subjected cells at early stationary phase grown under different acid stress conditions to freeze-drying process, and the freeze-drying viability and their fatty acids composition were determined.

Results (Table 2) showed that a decrease of initial medium pH resulted in a decrease of final medium pH, regardless of medium composition. By and large, a decrease of medium pH could increase the freeze-drying viability of *O. oeni*SD-2a cells cultured in the same media. Although the freeze-drying viability of pH 3.2 treatments slightly declined, it was still higher than that of pH 4.8 and pH 4.0 treatments. The fatty acid profiles altered in response to the acid stress, and the major changes occurred in the proportions of C16:0, C18:1cis11 and C19cyc11. *O. oeni*SD-2a cells subjected to pH 3.2 and 3.5 treatments had distinctly higher proportion of C19cyc11. Therefore, we assumed that acid stress treatment could induce cross-protection against freeze-drying and this response mechanism might closely be related with the changes in membrane fatty acid composition. However, there are some exceptions, for *O. oeni*SD-2a cells cultured in ATB medium with the initial pH 4.0, its freeze-drying viability was lower than that of the initial pH 4.8, but we found the saturated fatty acid relative concentration increased in those cell membrane lipids.

On the other hand, medium composition also could affect the bacterial freeze-drying viability. From the Table 2, we found FMATB medium generally offered better survival rate than two other media under acid stress conditions. Especially for *O. oeni*SD-2a cells cultured under conditions of FMATB medium with initial pH 3.5, they exhibited the highest freeze-drying viability, which might be involved in other mechanism such as accumulation of more osmoprotective compounds by a certain metabolic pathway in addition to enhancement of C19cyc11 relative concentration in the membrane lipids. G-Alegria et al. (2004) also reported that the freeze-drying recovery rate of *O. oeni* cells is over 100%, and they revealed *O. oeni* cells acquired a larger morphology and the increased growth rate after lyophilisation. In a word, pH conditions and culture composition should be well managed to insure higher freeze-drying viability of *O. oeni* cells.

3.3. *Relationship between cellular fatty acid composition and freeze-drying viability of O. oeni*SD-2a

In order to seek a possible relationship among media pH, fatty acid composition and freeze-drying viability, all these results were subjected to Pearson correlation analysis with seven variables (C14:0; C16:0; C16:1cis9; C18:lcis11; C19cyc11; freeze-drying viability; final medium pH). The correlation matrix (Table 3) calculated by DPS software showed the significant negative correlations between C19cyc11 and C16:0, C18:lcis11. It indicated that the levels of C19cyc11 increased at expense of C18:lcis11 for C18:lcis11 was precursor fatty acid, and the role of C16:0 could be replaced by C19cyc11. The freeze-drying viability exhibited a significant positive correlation with the levels of C19cyc11, and had a significant negative correlation with the levels of C16:0, which indicated C19cyc11, rather than C16:0, could offer more tolerance ability of *O. oeni* cells to freeze-drying. However, fatty acid composition and freeze-drying viability of *O. oeni* cells were determined by the medium pH when cells harvested. There were significant negative correlations between the medium pH and the levels of C19cyc11, and between the medium pH and freeze-drying viability, suggesting that low growth pH stimulated the synthesis of C19cyc11, which might play an important role in acid stress adaptive response, however, which also took effect on the resistance of *O. oeni* cells to freeze-drying.

4. Discussion

Freeze-drying is the method often used for the preservation and long-term storage of bacterial cells. However, it has negative effects on the viability of many cell types. Although many factors are concerned with microbial cell survival throughout freeze-drying and storage, the growth factors is also a critical parameter, which determine the physiological state of bacteria (Carvalho et al., 2004). Moreover, the cytoplasmic membrane is a key target for freeze- or desiccation-induced damage (Broadbent and Lin, 1999).

We observed *O. oeni* cells in the early stationary phase survived better after freeze-drying than those in the mid-exponential phase. Previous reports have been shown that *O. oeni* cells changed membrane fatty acids profile (Garbay et al., 1995; Drici-Cachon et al., 1996) and produced stress proteins (Guzzo et al., 1997) in the stationary phase. In our studies, it is the first time to show such changes were involved in the enhanced its resistance to lyophilization. This finding was in agreement with observations reported in *Pseudomonas putida* (Muñoz-Rojas et al., 2006) and *Lactobacillus bulgaricus* (Teixeira et al., 1995).

The mechanisms underlying the protection afforded by the various media tested are rather complex, and one kind of explanation is the modification on the fatty acid profile (Carvalho et al., 2003). It has previously been described that cultivation in the presence of Tween 80 brings about changes of the fatty acid composition of lactic acid bacteria cells, and that these changes influence their subsequent resistance to

Table 3 – Correlation values between fatty acids composition, final medium pH and the freeze-drying viability of *O. oeni*SD-2a cells grown in different medium and at different initial pH

	C14:0	C16:0	C16:1cis9	C18:1cis11	C19cyc11	Viability	Final medium pH[a]
C14:0	1.00						
C16:0	0.39	1.00					
C16:1cis9	0.85**	0.53	1.00				
C18:1cis11	−0.38	0.10	−0.01	1.00			
C19cyc11	−0.21	−0.81**	−0.54	−0.64*	1.00		
Viability	0.05	−0.60*	−0.11	−0.50	0.70**	1.00	
Final medium pH[a]	−0.11	0.70*	0.13	0.55	−0.81**	−0.64*	1.00

[a] The medium pH when cells in early stationary phase were harvested.
* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

freezing (Goldberg and Eschar, 1977). When *O. oeni* cells were cultured in designed growth media with different pH buffering capability, it was confirmed that a distinct increase in the levels of C19cyc11 should include the effect of environmental pH in addition to growth, and the fatty acid composition of *O. oeni* cells had a close relationship with its freeze-drying viability. Fozo and Quivery (2004) testified the fatty acid composition of *Streptococcus mutans* changed with self-acidification. Silva et al. (2005) also showed *Lactobacillus delbrueckii* cells grown under noncontrolled pH (final pH is about 3.5) were more resistant to drying than cells grown under controlled pH (pH 6.5).

Winery practices recommend commercial freeze-dried bacterial cultures of *O. oeni* for direct inoculation into wines to improve the control of MLF, adaptation processes have been shown to enhance the survival of *O. oeni* cells to stress conditions in wine, which is linked to stress response mechanisms (Guzzo et al., 1994; Garbay and Lonvaud-Funel, 1996; Guzzo et al., 1998; Beltramo et al., 2006). It is known that cross-protection to other stress often was induced by the expression of a given adaptive response (Guchte et al., 2002). In our studies, we found *O. oeni* cells cultured under acid stress conditions promoted lyophilization resistance, especially for pH 3.5 treatment, which resulted in the highest freeze-drying survival rate. The similar observations were also obtained in other microorganisms. Gilliland and Rich (1990) found significantly higher populations of *Lactobacillus acidophilus* cultures were obtained after freezing by growth at lower pH than at higher pH. Palmfeldt and Hahn-Hägerdal (2000) showed *Lactobacillus reuteri* cells were grown at pH 5 yielded higher freeze-drying survival rate than at pH 6. Béal et al. (2001) also proposed that the decrease of the fermentation pH improved the recovery of the acidification activity of *Streptococcus thermophilus* after frozen storage. In the case of *O. oeni* strains, Beltramo et al. (2006) confirmed the existence of a cross-protection phenomenon induced by acid adaptation.

Our study showed the freeze-drying survival of *O. oeni*SD-2a, which was correlated with the enrichment of C19cyc11 and the decrease of C16:0 in the membrane lipids of those cells. Cyclopropane fatty acids are formed by addition of a methylene group, derived from the methyl group of S-adenosyl methionine, across the carbon–carbon double bond of unsaturated fatty acids (Goldberg and Eschar, 1977). Smittle et al. (1974) suggested that the cyclopropane fatty acids prevent close packing of lipids in cell membranes, making them more elastic and flexible during exposure to low temperature. In addition, cyclopropane fatty acids, once formed, appear to be stable membrane components. Rather than being labile, it appeared to be considerably less reactive than the corresponding unsaturated fatty acids toward certain forms of oxidation (Grogan and Cronan, 1997). High resistance of *L. acidophilus* cells during frozen storage was related to high cyclopropane fatty acids concentrations (Wang et al., 2005). Muñoz-Rojas et al. (2006) testified the importance of cyclopropane fatty acids in response of *P. putida* stains to freeze-drying. Several other reports (Chang and Cronan, 1999; Quivey et al., 2000; Zhao et al., 2003) have indicated that cyclopropane fatty acid are involved in the resistance to other stresses. In our studies, we cannot rule out the role of stress protein and compatible solutes played in protecting the cells against damage during lyophilization. Nevertheless, we suggest that the shift to membranes with a higher percentage of cyclopropane fatty acids might serve as a survival mechanism of *O. oeni* cells against freeze-drying. To our knowledge, it is the first time to clarify the mechanisms of high survival after lyophilisation in *O. oeni*. In addition, a decrease of final medium pH was recommended in *O. oeni* starter culture preservation. To gain further insight into the potential role of C19cyc11 in freeze-drying, detailed transcriptional studies of cyclopropane fatty acids synthesis in *O. oeni* cells will be performed in the future.

References

Béal, C., Fonseca, F. and Corrieu, G., 2001, Resistance to freezing and frozen storage of *Streptococcus thermophilus* is related to membrane fatty acid composition. J Dairy Sci, 84: 2347–2356.

Beltramo, C., Desroche, N. and Tourdot-Maréchal, R., 2006, Real-time PCR for characterizing the stress response of *Oenococcus oeni* in a wine-like medium. Res Microbiol, 157: 267–274.

Broadbent, J.R. and Lin, C., 1999, Effect of heat shock or cold shock treatment on the resistance of *Lactococcus lactis* to freezing and lyophilization. Cryobiology, 39: 88–102.

Carvalho, A.S., Silva, J., Ho, P., Teixeira, P. and Malcata, F.X., 2004, Relevant factors for the preparation of freeze-dried lactic acid bacteria. Int Dairy J, 14: 835–847.

Carvalho, A.S., Silva, J., Ho, P., Teixeira, P., Malcata, F.X. and Gibbs, P., 2003, Effect of various growth media upon survival during storage of freeze-dried *Enterococcus faecalis* and *Enterococcus durans*. J Appl Microbiol, 94: 947–952.

Chang, Y.Y. and Cronan, J.E., 1999, Membrane cyclopropane fatty acid content is a major factor in acid resistance of *Escherichia coli*. Mol Microbiol, 33: 249–259.

Chu-Ky, S., Tourdot-Marechal, R., Marechal, P.A. and Guzzo, J., 2005, Combined cold, acid, ethanol shocks in *Oenococcus oeni*: effects on membrane fluidity and cell viability. Biochim Biophys Acta, 1717: 118–124.

Drici-Cachon, Z., Guzzo, J., Cavin, J.F. and Diviès, C., 1996, Effect of pH and age of culture on cellular fatty acid composition of Leuconostoc oenos. Lett Appl Microbiol, 44: 785–789.

Fozo, E.M. and Quivery, R.G., 2004, Shifts in the membrane fatty acid profile of *Streptococcus mutans* enhance survival in acidic environments. Appl Environ Microbiol, 70: 929–936.

G-Alegria, E., Lopez, Isabel, Ruiz, J.I., Saenz, J., Fernandez, E., Zarazaga, M., Dizy, M., Torres, C. and Ruiz-Larrea, F., 2004, High tolerance of wild Lactobacillus plantarum and *Oenococcus oeni* strains to lyophilisation and stress environmental conditions of acid pH and ethanol. FEMS Microbiol Lett, 230: 53–61.

Garbay, S. and Lonvaud-Funel, A., 1996, Response of *Leuconostoc oenos* to environmental changes. J Appl Bacteriol, 81: 619–625.

Garbay, S., Rozes, N. and Lonvaud-Funel, A., 1995, Fatty acid composition of *Leuconostoc oenos*, incidence of growth conditions and relationship with malolactic efficiency. Food Microbiol, 12: 387–395.

Gilliland, S.E. and Rich, C.N., 1990, Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. J Dairy Sci, 73: 1187–1192.

Goldberg, I. and Eschar, L., 1977, Stability of lactic acid bacteria to freezing as related to their fatty acid composition. Appl Environ Microbiol, 33: 489–496.

Grogan, D.W. and Cronan, J.E., 1997, Cyclopropane ring formation in membrane lipids of bacteria. Microbiol Molecul Biol Rev, 61: 429–441.

Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S. and Maguin, E., 2002, Stress responses in lactic acid bacteria. Antonie Van Leeuwenhoek, 82: 187–216.

Guzzo, J., Cavin, J.F. and Divies, C., 1994, Induction of stress proteins in *Leuconostoc oenos* to perform direct inoculation of wine. Biotech Lett, 16: 1189–1194.

Guzzo, J., Jobin, M.P. and Divieés, C., 1998, Increase of sulfite tolerance in *Oenococcus oeni* by means of acidic adaptation. FEMS Microbiol Lett, 160: 43–47.

Guzzo, J., Pierre, F., Jobin, M.P., Samyn, B., Van Beeumen, J., Delmas, F., Cavin, J.F. and Divies, C., 1997, A small heat shock protein induced by multiple stresses and during stationary growth phase from *Leuconostoc oenos*. Lett Appl Microbiol, 24: 393–396.

Li, H., Zhang, C.H. and Liu, Y.L., 2006, Species attribution and distinguishing strains of *Oenococcus oeni* isolated from Chinese wines. World J Microbiol Biotechnol, 22: 515–518.

Liu, Y.L. and Li, H., 2006, Rapid specificity identification of *Oenocoouus oeni* with PCR. J Microbiol, 26: 26–29.

Lonvaud-Funel, A., 1999, Lactic acid bacteria in the quality improvement and depreciation of wine. Antonie Van Leeuwenhoek, 76: 317–331.

Maicas, S., Pardo, I. and Ferrerm, S., 2000, The effects of freezing and freeze-drying of *Oenococcus oeni* upon induction of malolactic fermentation in red wine. Int J Food Sci Technol, 35: 75–79.

Muñoz-Rojas, J., Bernal, P., Duque, E., Godoy, P. and Segura, A., 2006, Involvement of cyclopropane fatty acids in the response of *Pseudomonas putida* KT2440 to freeze-drying. Appl Environ Microbiol, 72: 472–477.

Palmfeldt, J. and Hahn-Hägerdal, B., 2000, Influence of culture pH on survival of *Lactobacillus reuteri* subjected to freeze-drying. Int J Food Microbiol, 55: 235–238.

Quivey, R.G., Faustoferri, R., Monahan, K. and Marquis, R., 2000, Shifts in membrane fatty acid profiles associated with acid adaptation of *Streptococcus mutants*. FEMS Microbiol Lett, 189: 89–92.

Rozes, N., Garbay, S., Denayrolles, M. and Lonvaud-Funel, A., 1993, A rapid method for the determination of bacterial fatty acid composition. Lett Appl Microbiol, 17: 126–131.

Schmitt, P., Mathot, A.G. and Divies, C., 1989, Fatty acid composition of the genus *Leuconostoc oenos*. Milchwissenschafl, 44: 556–559.

Silva, J., Carvalho, A.S., Ferreira, R., Vitorino, R., Amado, F., Domingues, P., Teixeira, P. and Gibbs, P.A., 2005, Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. *bulgaricus* to stress conditions during spray-drying. J Appl Microbiol, 98: 775–782.

Smittle, R.B., Gilliland, S.E., Speck, M.L. and Walter, W.M., 1974, Relationship of cellular fatty acid composition to survival of *Lactobacillus bulgaricus* in liquid nitrogen. Appl Mcriobiol, 27: 738–743.

Teixeira, P., Castro, H. and Kirby, R., 1995, Spray drying as a method for preparing concentrated cultures of *Lactobacillus bulgaricus*. J Appl Microbiol, 78: 456–462.

Tourdot-Marechal, R., Gaboriau, D., Beney, L. and Divies, C., 2000, Membrane fluidity of stressed cells of *Oenococcus oeni*. Int J Food Microbiol, 55: 269–273.

Versari, A., Parpinello, G.P. and Cattaneo, M., 1999, *Leuconostoc oenos* and MLF in wine: a review. J Ind Microbiol Biotechnol, 23: 447–455.

Wang, Y., Corrieu, G. and Béal, C., 2005, Fermentation pH and temperature influence the cryotolerance of *Lactobacillus acidophilus* RD758. J Dairy Sci, 88: 21–29.

Zhao, G. and Zhang, G., 2005, Effect of protective agents, freezing temperature, rehydration media on viability of malolactic bacteria subjected to freeze-drying. J Appl Microbiol, 99: 333–338.

Zhao, Y., Hindorff, L.A., Chuang, A., Monroe-Augustus, M., Lyristis, M., Harrison, M.L., Rudolph, F.B. and Bennet, G.N., 2003, Expression of a cloned cyclopropane fatty acid synthase gene reduces solvent formation in *Clostridium acetobutylicum* ATCC 824. Appl Environ Microbiol, 69: 2831–2928.