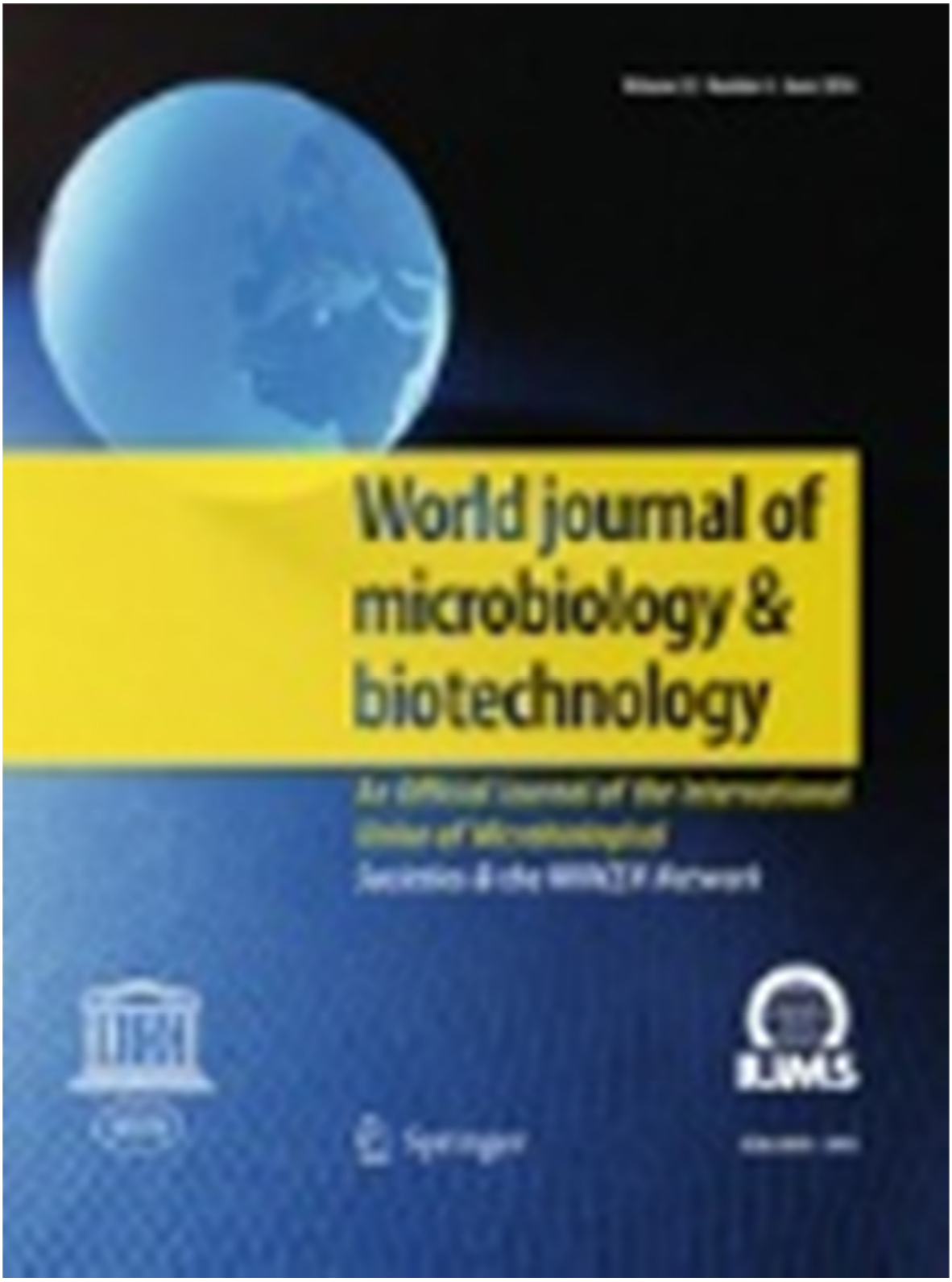
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ORIGINAL PAPER

Further investigation of relationships between membrane fluidity and ethanol tolerance in *Saccharomyces cerevisiae*

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Abstract
Membrane lipid unsaturation index and membrane fluidity have been related to yeast ethanol stress tolerance in published studies, however findings have been inconsistent. In this study, viability reduction on exposure to 18% (v/v) ethanol was compared to membrane fluidity determined by laurdan generalized polarization. Furthermore, in the determination of viability reduction, we examined the effectiveness of two methods, namely total plate count and methylene violet staining. We found a strong negative correlation between ethanol tolerance and membrane fluidity, indicated by negative Pearson correlation coefficients of −0.79, −0.65 and −0.69 for *Saccharomyces cerevisiae* strains A12, PDM and K7, respectively. We found that lower membrane fluidity leads to higher ethanol tolerance, as indicated by decreased viability reduction and higher laurdan generalized polarization in respiratory phase compared to respiro-fermentative phase cells. Total plate count better differentiated ethanol tolerance of yeast cells in different growth phases, while methylene violet staining was better to differentiate ethanol tolerance of the different yeast strains at a particular culture phase. Hence, both viability assessment methods have their own advantages and limitations, which should be considered when comparing stress tolerance in different situations.

Keywords Ethanol tolerance · Yeast · Generalized polarization · Membrane fluidity · Viability reduction · Spectrofluorometry

Introduction

Ethanol tolerance has been correlated with membrane fluidity in yeasts, although such correlations can differ from one strain to another, and depend upon intrinsic properties of the particular yeast strain investigated. In many studies, membrane fluidity has been inferred indirectly from measurement of the unsaturation index (UI) of the yeast membrane fatty acids; higher UI has been taken to represent higher membrane fluidity (Fajardo et al. 2011; Henderson and Block 2014). Such studies have indicated that increased UI relates to improved ethanol tolerance (Henderson and Block 2014). Introduction into *S. cerevisiae* of a gene for unsaturated fatty acid synthesis (*FAD2*) combined with overexpression of the mono-desaturase *OLE1* reportedly increased the UI and improved tolerance to exposure to 15% ethanol (Kajiwara et al. 2000; You et al. 2003).

However, membrane fluidity is not only influenced by fatty acid composition, but also other component(s) of the yeast plasma membrane, such as proteins and sterols (Alexandre et al. 1994b; Learmonth 2012). Therefore, the reports inferring fluidity from lipid composition need to be confirmed via more direct determination of membrane fluidity. Several studies have shown exposure of *S. cerevisiae* to high concentrations of ethanol results in increased membrane fluidity (Alexandre et al. 1994a; Learmonth 2012; Learmonth and Gratton 2002; Lloyd et al. 1993). Lloyd et al. (1993) reported that in *S. cerevisiae* grown in the presence of up to 9% (v/v) ethanol (as measured by EPR) mitochondrial

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