On the pH and Osmotic Stress Tolerance of High Ethanol Tolerant Palm Wine *Saccharomyces* Yeast Isolates

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Abstract

Saccharomyces yeast strains Y_{13}, Y_{522} and Y_{1189} isolated from fermenting palm wine juice showed marked differences in their optimum growth pH and possessed osmotolerance comparable to established industrial yeast strains. Shifts in medium pH beyond the growth optimum elicited obvious reductions in growth rate as depicted by the decrease in mean log growth rate (K) and influenced the response of the test yeasts to osmotic stress.

Key words: Osmotolerance, Ethanol tolerance, Osmotic stress, *Saccharomyces*

Introduction

Severe economic hardship in most developing countries of the tropics and abundance of yeasts in local palm wine juice informed the renewed interest in local palm wine *Saccharomyces* yeast strains for industrial ethanologenic fermentation (Ahmeh and Okagbue, 1987; Ameh *et al.* 1987; Okagbue and Ekejindu, 1988; Ogbonna and Obi, 1992; Sefa-Dedeh *et al*, 1999). These earlier studies depicted palm wine *Saccharomyces* strains as capable of high-level ethanol and sucrose tolerance (Ameh and Okagbue, 1987; Ezeogu and Emeruwa, 1993), good invertase activity (Desimone *et al*, 2002) as well as possessing high adaptability to molasses (Ezeogu and Okolo, 1994a; 1994b). Osmotolerance is a desirable trait for industrial yeasts and has been studied widely especially in relation to high gravity brewing, and industrial ethanol production with both brewers' and distillers' yeast strains (Benitez *et al.*, 1983; Okolo *et al.*, 1990; Desimone *et al*, 2002). In high gravity brewing, the yeast must overcome the osmotic stress imposed by the concentrated environment and also the toxic effects of ethanol and other metabolites (Okolo *et al.*, 1987; Ansanay-Galeote *et al*, 2001). Furthermore, the ability of yeast to withstand osmotic stress is related to both genetic and environmental factors including medium pH (O'Connor and Ingledew, 1990; Kajiwara *et al*, 2000; Ansanay-Galeote *et al*, 2001). In fact, Sefa-Dedeh *et al* (1999) demonstrated that medium pH played a particularly important rolé in the capacity of yeast cells to tolerate osmotic stress.

In this study, data are presented of the tolerance of palm wine *Saccharomyces* yeasts to osmotic shock and the influence of medium pH on the ability of the yeasts to withstand osmotic stress.

Materials and Methods

Yeast cultures: Strains of *Saccharomyces* yeasts designated Y_{13}, Y_{522} and Y_{1189} were isolated from fermenting palm wine juice as

described previously (Ezeogu and Emeruwa, 1993) and used for this study. The bottom brewers' yeast, *Saccharomyces cerevisiae* (var uvarum) designated *By,* was obtained from Premier Breweries PLC, Onitsha, Nigeria and used as reference yeast.

Media: Yeast cultures were routinely maintained on YPG medium. This medium contained per litre: yeast extract, 5g; glucose, 10g; peptone, 10g and agar 15g. The medium used for yeast propagation and batch fermentation comprised (g per litre): KH_2PO_4, 3; $(NH_4)SO_4$, 3; yeast extract, 4; $MgSO_4.7H_2O$, 0.25; $CaCl_2.2H_2O$, 0.25; $ZnSO_4.7H_2O$, 0.22 and glucose, 20. Sorbitol at concentrations ranging from 0 to 40% ($^w/_v$) was used to vary the osmotic pressure of the medium. pH of the basal medium was adjusted in the range 3 – 6 using 0.1M HC1 or NaOH solution where appropriate prior to sterilization by autoclaving at 121^{0}C and 15psi.

Batch fermentation: Portions of sterile fermentation medium (50ml) were inoculated with yeast cells to a concentration of 1 x 10^6 cells/ml, in media of lower pH values. Marked differences were however observed with respect to pH optima for growth of the various yeasts. For instance, isolates Y_{13} and Y_{522} grew best at pH 6.0 while Y_{1189} showed optimum growth at pH 4.5. The brewer's reference yeast, *By*, exhibited optimum growth at pH 5.0. At pH 3.0, cell growth was remarkably reduced for all the yeast isolates presumably due to stress induced by an environment of high hydrogen ion concentration. Similar effects have been noted for *Saccharomyces cerevisiae* strains 303, 507 and 624 as well as for *Khyveromyces* and *Candida* yeasts (Casey and Ingledew, 1986) and for a distilling yeast strain (Okolo, 1986). 150ml Erhlenmeyer flasks and incubated for 24h with shaking at 250rpm at 30°C. Cells used for inoculation had been grown for 24h in same medium. Samples were withdrawn immediately after inoculation and at the end of fermentation for optical density measurements using a spectrophotometer (Spectronic-20) at 660nm.

Results and Discussion

Effect of assay pH on yeast growth: Medium pH is widely believed to affect important yeast physiological properties including the growth kinetics, fermentative activity and cell viability (Sefa-Dedeh *et al*, 1999; Kajiwara *et al*, 2000; Ansanay-Galeote *et al*, 2001). The effects of initial medium pH in the range 3 – 6 on growth of palm wine *Saccharomyces* yeast isolates Y_{13}, Y_{522}, and Y_{1189} were therefore evaluated. Figure 1 summarizes the growth characteristics of these yeast isolates in media of varying pH values. Media pH in the range 4 – 6 were generally salutary to yeast cell growth in contrast to The yeast cells studied here however, differed in the extent to which lowering of medium pH to 3.0 suppressed their growth rates. For instance, more than 7-fold reduction in rates of yeast cell growth was observed for Y_{13} at pH 3.0 compared to the growth rate at pH 4.0. Similarly growth rates were reduced 5.2 and 4.9 folds for Y_{1189} and Y_{522} respectively at pH 3.0 compared to rates at pH 4.0. Reduction in growth rate of the reference yeast, *By*, was 5.2-fold at pH 3.0 compared to values at pH 4.0. These differences in yeast strain sensitivity to H^+ stress at pH 3.0 suggest important role for genetic factors in mediation of yeast tolerance to extremes of pH. These observations are in reasonable

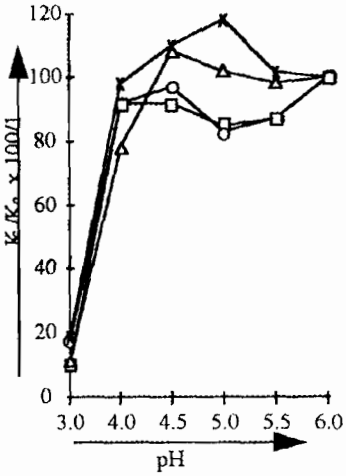

Fig. 1: Effect of medium pH on logarithmic growth rate constants of palm wine yeast isolates

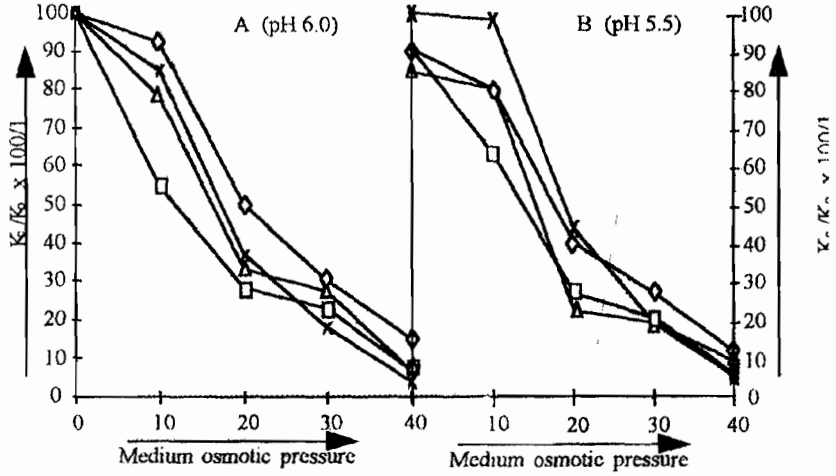

Fig. 2. Mean log growth rate (K_c) in % of rates in basal medium at pH 6.0 (K_o) versus medium osmotic pressure measured as % sorbitol

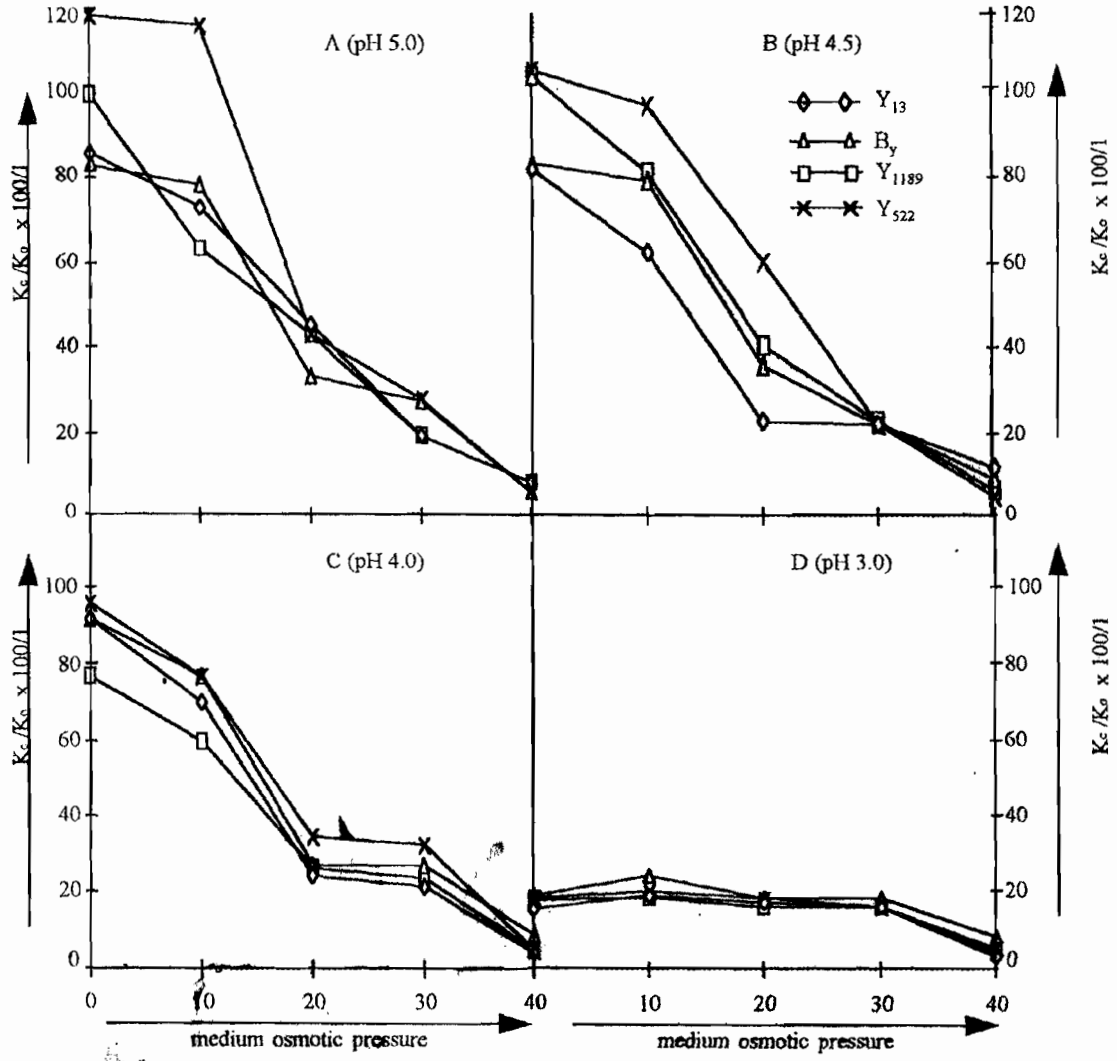

Fig. 3: Mean log growth rate (K_c) in % of rates in basal medium at pH 6.0 K_o versus medium osmotic pressure measured as % sorbitol

agreement with Casey and Ingledew (1986) and Ansanay-Galeote *et al* (2001) who established strain-dependent differences in yeast growth characteristics under different environmental conditions.

Effect of osmotic pressure on yeast growth: The effects of high medium osmotic pressure on yeast cell growth and fermentative characteristics have been widely studied for traditional industrial yeast strains such as wine, brewers', bakers', sake and distillers' yeasts (Casey and Ingledew, 1986; Sefa-Dedeh *et al*, 1999; Kajiwara *et al*, 2000; Desimone *et al*, 2002). Sugar concentrations ≥15% ($^{w}/_{v}$) have been reported to elicit obvious changes on the physiological characteristics of these industrial yeasts including alterations in cell volume (Atkinson *et al.*, 1977), prolonged lag phase and highly reduced cell viability (Nishino *et al.*, 1985, Okolo, 1986; Kajiwara *et al*, 2000). As an additional step in characterization of our palm wine *Saccharomyces* yeasts for potential industrial use, the effect of pH on the ability of these yeasts to tolerate osmotic stress was evaluated. Data presented as mean log growth rate (Kc), in % of rates in the basal medium at pH 6.0 are shown in figures 2 and 3. The data are based on mean values of triplicate tests performed on two occasions. Generally, a reduction in K value was observed as the concentration of medium sorbitol was increased. Furthermore, the extent to which each of the palm wine yeasts was sensitive to increased osmotic pressure was dependent on both the assay pH and yeast strain. For instance, at 10% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure, yeast growth rate dropped 11 - 44% below control values (Figures 2A). At pH 6.0, Y_{13} exhibited highest osmotolerance of all the palm wine yeast isolates and also showed the smallest decrease in K value at all the osmotic pressure evaluated. Strain Y_{522} gave moderate osmotolerance values at all osmotic pressure levels examined except 40% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure where values of K for this yeast were the lowest of all the test palm wine yeasts. Yeast isolate Y_{1189} was most sensitive of al the test yeasts showing the greatest dip in K value upon exposure to successive increases in medium osmotic pressure. At 10% and 20% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure, the brewers' reference yeast, B_y, also showed osmotolerance comparable to the palm wine yeasts. However, at 30% and 40% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure, B_y was less tolerant of osmotic stress than the palm wine yeast isolates. Changes in medium pH beyond the optimum growth pH caused variations in the capacity of the yeasts to tolerate increased osmotic stress. At pH 5.5, improvements in mean log growth rate (K) were observed for all the yeasts at 10% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure relative to basal medium of similar pH (Figure 2B). Higher osmotic pressures i.e. ≥ 20% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure however, elicited depression of yeast K-values. These observations are consistent with the reports of Atkinson *et al* (1977) on the effect of glucose and sucrose on the multiplication rates of selected brewers' yeasts. Remarkable improvements in yeast K-values were also observed for all the yeasts at pH 5.0 and at all levels of medium osmotic pressure evaluated relative to values obtained at pH 6.0. As depicted in Figure 3A, improvements in K-values apparently due to changes in pH was most dramatic for the reference yeast, B_y, at 10% and 20% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure

compared to all the test palm wine yeasts. Nevertheless, at 30% and 40% ($^{w}/_{v}$) sorbitol concentration, B_y continued to exhibit highest k-values of all the yeasts studied presumably indicating that at pH 5.0, which is the optimum growth pH for the brewers' reference yeast, this yeast was the most osmotolerant of all the yeasts studied. Improvements in the K-values of B_y was also observed at pH 4.5, where 10% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure continued to elicit enhancements in K-values of the yeasts compared to values obtained at pH 6.0 in the basal medium.

Shifts in medium pH were also observed to cause variations in the response of the test palm wine *Saccharomyces* isolates to osmotic stress. Mean log growth rate for Y_{1189} peaked at pH 4.5 in the 10% and 20% ($^{w}/_{v}$) sorbitol supplemented media. Similar results were also noted for Y_{13} and Y_{522} at these pH values but to varying extents (Figures 3A and 3B). Significantly better growth rates were observed for all the palm wine yeast isolates at pH 4.5 and 5.0 compared to rates at pH 4.0 and 3.0 (Figure 3C and 3D). At these lower pH values, increase in medium osmotic pressure was observed to elicit progressively stronger inhibitory effects on growth rate of all the yeasts. Occurrence of higher osmotic pressure effects on the yeasts at lower pH values might be attributable to additional effects of environmental stress due primarily to medium H^+ concentration. Similar observations have been reported by several other workers (Casey and Ingledew, 1986; Okolo, 1986; Sefa-Dedeh *et al*, 1999; Ansanay-Galeote *et al*, 2001) investigating the effects of pH on ethanol toxicity in yeasts. At pH 4.0, K-values for the yeasts were depressed 25, 67, 73 and 94% upon incubation in media of 10, 20, 30, and 40% ($^{w}/_{v}$) sorbitol equivalent of osmotic pressure respectively compared to values obtained in the basal control medium. At pH 3.0, highest K-values were recorded at 10% ($^{w}/_{v}$) medium sorbitol level indicating presumably an enhancement of yeast growth rate as osmotic pressure was increased up to 10% ($^{w}/_{v}$) sorbitol; equivalent before recording a progressive decline (Figure 3D). The palm wine *Saccharomyces* yeast isolates exhibited similar osmotolerance as the brewers' reference yeast. However, osmotolerance values obtained for our yeast isolates appeared to be higher than values reported previously (Benitez *et al.*, 1983). This might be due, at least in part, to the use of non-fermentable sorbitol rather than sucrose or glucose used by these workers to raise medium osmotic pressure. Growth of yeast on high concentrations of fermentable sugars has been associated with massive intracellular build-up of ethanol leading to a potentiation to the inhibitory effects of osmotic stress on yeast cells during ethanologenic fermentation (Casey and Ingledew, 1986; Ansanay-Galeote *et al*, 2001; Desimone *et al*, 2002). Moreover, equal concentrations of different sugars differ in their ability to inhibit yeast growth (Okolo, 1986).

In conclusion, data presented in this study revealed that the tolerance of palm wine *Saccharomyces* yeasts to osmotic stress was to a very significant extent modulated by medium pH with most of the yeasts showing highest osmotolerance indices at or close to their optimum pH for growth. It is also advanced that palm wine *Saccharomyces* yeast isolates possess pH and osmotolerance which are at least comparable to established industrial yeast strains.

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