Original Article
Life Science

Osmotolerance and Fermentative Pattern of Brewer's Yeast

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ABSTRACT [ENGLISH/ANGLAIS]

Yeast strains isolated from locally brewed beverages which comprised of different species were tested for physiological characteristics such as fermentative ability, growth on 50% $^{w}/_{v}$ glucose, nitrate assimilation and osmotolerance capacity using industrial strain as standard. Among the *Saccharomyces cerevisiae* and non-saccharomyces strains analysed, 83.93% were highly osmotolerant while 16.07% were moderately osmotolerant compared with the standard strain which was also moderately osmotolerant. All yeast strains fermented glucose and sucrose while none fermented lactose. Furthermore, both *S. cerevisiae* and non-saccharomyces strains grew on 50% glucose while only the non-saccharomyces strain assimilated nitrate. This implies that, yeasts are highly tolerant to osmotic shock and there is variation in their ability to assimilate nutrients.

Keywords: Fermentation, yeast, osmotolerance, cell density, concentration

RÉSUMÉ [FRANÇAIS/FRENCH]

Des souches de levure isolées de boissons brassées localement comprenant des espèces qui ont été testées pour les caractéristiques physiologiques tels que aptitude à la fermentation, la croissance à 50% p / v de glucose, de l'assimilation du nitrate et des capacités osmotolérance utilisant souche industrielle de série. Parmi les Saccharomyces cerevisiae et non-Saccharomyces souches analysées, 83,93% étaient très osmotolérante tout 16,07% étaient modérément osmotolérante rapport à la souche standard qui était aussi modérément osmotolérante. Toutes les souches de levure fermentée glucose et le saccharose alors qu'aucun fermenté au lactose. En outre, à la fois de S. cerevisiae et non-Saccharomyces souches a augmenté sur le glucose 50%, tandis que les non-souche Saccharomyces nitrate assimilé. Cela implique que, les levures sont très tolérants à un choc osmotique et il existe des variations dans leur capacité à assimiler les nutriments. . Alimentation à une conclusion brève ici

Mots-clés: Fermentation, de levure, osmotolérance, la densité des cellules, la concentration

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Accepted/Accepté: February, 2012

Full Citation: Jimoh SO, Ado SA, Ameh JB, Whong CMZ. Osmotolerance and Fermentative Pattern of Brewer's Yeast World Journal of Life Sciences and Medical Research 2012;2(2):59-64.

INTRODUCTION

The fundamental physiological characteristic of Brewer's yeast is their ability to degrade carbohydrates, usually six-carbon molecules and some disaccharides such as sucrose and maltose into two-carbon components such as ethanol without completely oxidizing them to carbondioxide, even in the presence of oxygen. Yeasts which produce or accumulate ethanol in the presence of oxygen are called Crabtree-positive yeast. The Crabtree effect is an alcoholic fermentation where the degradation of hexose sugars in the presence of oxygen is due to insufficient capacity, saturation, or repression of the respiratory metabolism, leading to pyruvate overflow. Thus during ethanol production, the energy for growth is provided by the glycolysis and fermentation pathways rather than by the respiration pathways. In the case of *S. cerevisiae* the Crabtree effect relies largely on a glucose-repression circuit, which in the presence of glucose or other C6 carbohydrates, represses respiration [1].

The monosaccharides are transported across the cellular membrane by common membrane transporters which facilitate diffusion and active transport process. Among the four putative identified for glucose uptake, the most important glucose uptake systems recognized are low affinity (constitutively expressed) and a high affinity transporter, which is repressed in the presence of high glucose concentrations [2]. Catabolite repression (the repression of the high affinity transporter) is a trait only found in fermentative yeast strains [3]. Furthermore, substrate inhibition is a common phenomenon in fermentations which occurs when substrate concentration exceeds a strain-dependent level. The kinetic investigations involving substrate inhibition in ethanol fermentations, especially in *S. cerevisiae*, are very limited,

possibly because the simultaneous saccharification and fermentation is widely used in the ethanol production from starch materials, in which substrate inhibition is not significant since sugar is consumed by yeast cells immediately after it is released and a low sugar concentration is maintained within the fermentation system. However, for the ethanol production from molasses, substrate inhibition is more likely, due to the high initial sugar concentration in the medium.

MATERIALS AND METHODS

Isolation and Characterisation of Yeast Strain

The yeast strains were isolated from locally fermented beverages and characterized using API AUX 20C kit.

Growth at High Concentration of Glucose

A basal medium composed of 50% ($^{w}/_{v}$) glucose (Oxoid), 1% ($^{w}/_{v}$) yeast extract (Oxoid), 1.5% ($^{w}/_{v}$) peptone (Oxoid) and 2% ($^{w}/_{v}$)) agar (Oxoid) was inoculated with the selected yeast strains and incubated at room temperature (28 – 30^{0}C) for 72 hours and observed for growth.

Fermentative Potential of Yeast Strains

Sugar fermentative ability of the yeast strains was determined using the following sugars: glucose, sucrose, lactose, galactose, maltose and rafinose. The basal medium contained 0.5% ($^{w}/_{v}$) yeast extract (Oxoid), 0.75% ($^{w}/_{v}$) peptone (Oxoid) and bromothymol blue indicator. The medium was dispensed into test tubes containing inverted durham tubes, cotton-plugged, sterilized at 121 ^{o}C for 15 minutes and allowed to cool to room temperature. An aliquot of 2.0 ml filter sterilized sugar solution (6% $^{w}/_{v}$) was added aseptically to the test tubes and inoculated with 0.1 ml of 24 hours yeast suspension. The uninoculated tube was used as control for each sugar substrate and the growth medium tubes were incubated at room temperature (28 – 30 ^{o}C) for 48 hours. Thus, gas production and medium colour change indicated the fermentative activity of the yeast strains.

Nitrate Assimilation

The nitrate assimilation test was analysed using basal medium comprising of yeast carbon base (11.7 %$^{w}/_{v}$) and potassium nitrate (0.78 %$^{w}/_{v}$).Nitrate assimilation medium which comprised of 0.5 ml of the basal medium and 4.5 ml of sterile distilled water was mixed thoroughly, inoculated with 0.1 ml aliquot (1.2 x 10^{2} cells/ml) of 24 hours old washed yeast culture and incubated at room temperature (26-28 ^{o}C) for 7 days. Turbidity or presence of sediment in the medium after 3 to 7 days indicated the ability of the yeast to utilize nitrate as its nitrogen source.

Osmotolerance Test

Osmotolerance activity of the yeast strains were analysed according to the methodology of [4] with slight modification. Yeast-peptone-dextrose broth containing yeast extract, 10 g/l; peptone, 20g/l and varying concentrations of glucose (80, 120, 160, 180 and 200 g/l) was utilized. The medium was inoculated with 0.1 ml of 1.2 x 10^{2} cells/ml suspension of yeast isolate and the turbidity was determined at 0 hour and 12 hours interval for 60 hours using CECIL CE 1020 spectrophotometer at 540 nm.

RESULTS

Fermentative Activities and Nitrate Assimilation Test

The physiological characteristics with respect to the fermentative activities and assimilation rate of yeast strains isolated are recorded in table 1.

Osmotolerance Pattern of Yeast Isolates

The initial optical density (turbidity) at 0 hour was 0.392 OD thus, yeast isolates that possessed optical density greater than 0.392 OD at all glucose concentrations were categorized as highly osmotolerant strains, moderately osmotolerant (greater than 0.392 OD at 8% to 16% w/v), slightly tolerant (greater than 0.392 OD at 8% to 12% w/v) and non-tolerant (less than 0.392 OD at 8% w/v) (table 2). Among the 56 isolates, 47 (83.93%) strains were highly osmotolerant, moderately osmotolerant (9 strains, 16.07%), while none were slightly tolerant or non-tolerant respectively (figure 1). The industrial brewing yeast (standard strain) also tolerated 8 -16% glucose concentration, thus was categorized as moderately osmotolerant strain. Statistically, there was significant difference in the biomass yield obtained at different glucose concentrations using ANOVA test (P=0.05).

DISCUSSION

Yeast metabolic processes are mediated by enzymic reactions and regulations of the underlying pathways have been studied by several researchers [5]. The fermentation of glucose to ethanol represents a series of coordinated enzymatic reactions and it is internally balancing and thermodynamically favorable provided that cellular enzymes consume the net ATP generated from substrate-level phosphorylation [6]. The requirements for this process include glucose, functional

enzymes, coenzymes (NAD^+, thiamine pyrophosphate, ADP, ATP), cofactors (Mg^{2+}, Zn^{2+}), appropriate internal pH, a functional membrane to maintain the concentration of reactants and enzymes and a glucose uptake system.

Table 1: This table shows physiological characteristics of yeast strains

Isolate	Fermentative Pattern						Growth on 50% glucose	Nitrate assimilation
	Glucose	Galactose	Maltose	Sucrose	Lactose	Rafinose		
Candida magnolia	+	-	-	+	-	-	+	+
Candida pelliculosa	+	+	+	+	-	-	+	+
Saccharomyces cerevisiae 1	+	+/-	+	+	-	+	+	-
Saccharomyces cerevisiae 2	+	+/-	+	+	-	+	+	-
Rhodotorula mucilaginosa 2	+	+	+	+	-	-	+	-
Rhodotorula mucilaginosa 1	+	+	+	+	-	-	+	-
Candida utilis	+	+	+	+	-	-	+	+
Candida colliculosa	+	+/-	+	+	-	+	+	+
Rhodotorula glutinis	+	-	+	+	-	-	+	-
Trichosporon asahii	+	-	+	+	-	-	+	-
Cryptococcus albidus	+	+	+	+	-	-	+	-

+ = Sugar fermented with gas production; - = No fermentation; V=Variation (+/-)

Table 2: This table shows biomass yield of yeast isolates with respect to osmotic tension

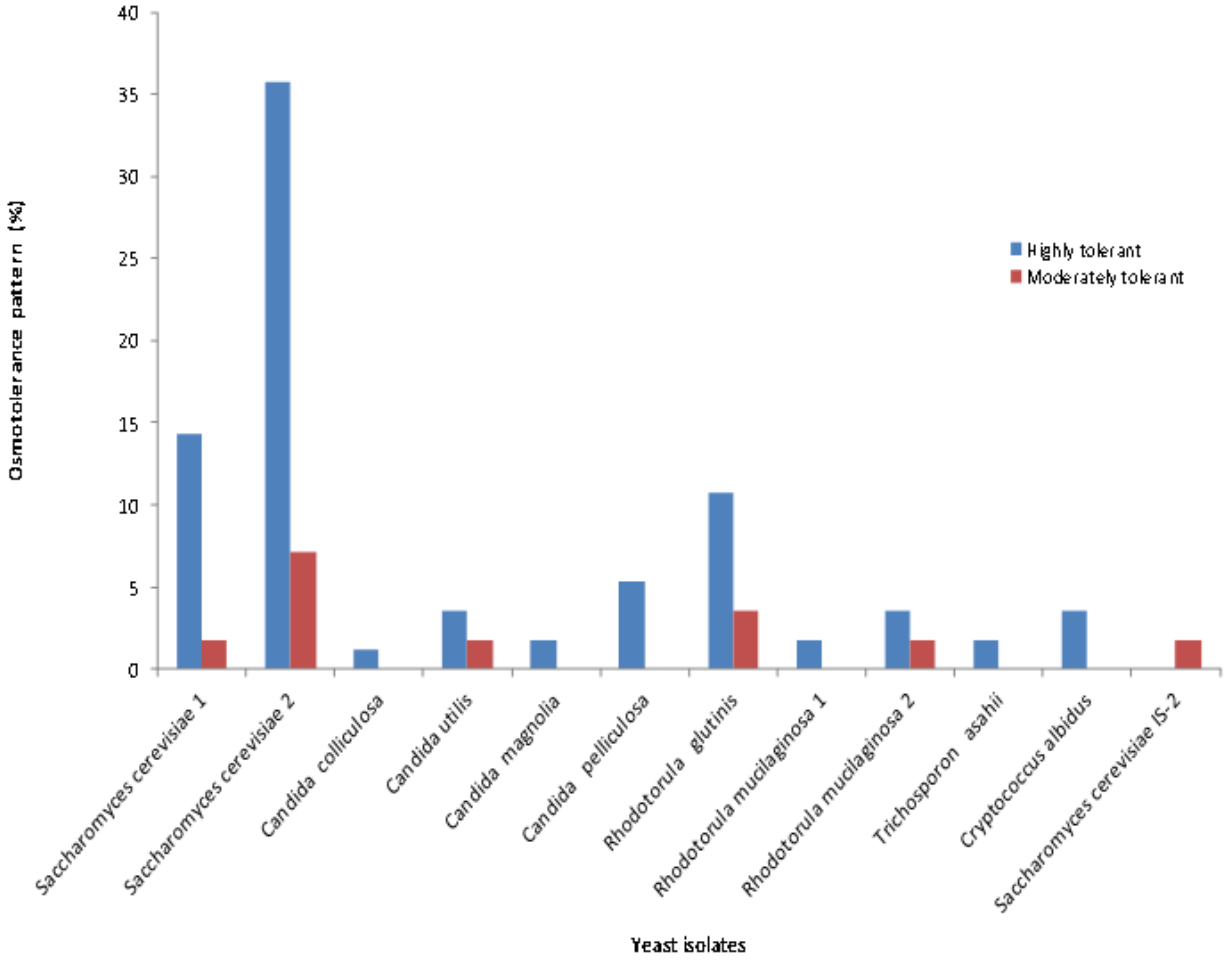
Isolate code	Biomass yield (optical density; OD) at different glucose concentration ($^w/_v$)			
	8%	12%	16%	20%
PB11	1.278 ±0.001	1.221 ± 0.011	1.139 ±0.003	0.538 ±0.002
PB12	1.818 ±0.001	1.381 ±0.003	1.022 ±0.012	0.472 ±0.001
PF18	1.247 ±0.001	1.153 ±0.001	1.141 ±0.016	0.845 ±0.011
PH19	1.654 ±0.002	1.402 ±0.016	1.134 ±0.001	0.279 ±0.011
PH20	1.303 ±0.001	0.935 ±0.002	0.704 ±0.011	0.663 ±0.012
PL23	1.754 ±0.002	1.268 ±0.001	0.604 ±0.013	0.532 ±0.001
PL24	1.419 ±0.014	1.033 ±0.015	0.642 ±0.009	0.376 ±0.002
PN26	1.988 ±0.012	1.194 ±0.003	0.855 ±0.001	0.539 ±0.012
PQ28	1.670 ±0.001	1.274 ± 0.002	0.842 ±0.014	0.795 ±0.011
PC13	1.466 ±0.003	1.342 ± 0.001	1.138 ±0.013	0.602 ±0.015
PC14	1.494 ±0.013	1.116 ±0.014	1.026 ±0.002	0.543 ±0.013
PF17	1.543 ±0.015	1.338 ±0.012	0.847 ±0.001	0.627 ±0.003
PJ21	1.502 ±0.021	1.436 ±0.011	1.095 ±0.003	0.955 ±0.001
PQ27	1.611 ± 0.002	1.566 ±0.001	1.235 ±0.012	0.792 ±0.004
PJ22	1.776 ±0.012	1.284 ±0.011	1.266 ±0.002	0.950 ±0.015
BK32	1.842 ±0.013	1.748 ±0.014	1.147 ±0.004	0.242 ±0.011
BL35	1.852 ±0.015	1.548 ±0.003	1.227 ±0.001	0.757 ±0.019

Table 2 continued:

Isolate code	Biomass yield (optical density; OD) at different glucose concentration (w/v)			
	8%	12%	16%	20%
BM36	2.036 ±0.011	1.116 ±0.001	0.681 ±0.013	0.611 ±0.017
BN38	2.035 ±0.014	1.794 ±0.011	1.602 ±0.001	0.564 ±0.012
BP41	1.396 ±0.013	1.416 ±0.001	1.911 ±0.012	0.799 ±0.019
BP42	1.252 ±0.003	1.697 ±0.013	1.791 ±0.011	0.525 ±0.012
BQ43	0.979 ±0.001	1.451 ±0.002	1.841 ±0.014	0.500 ±0.023
BQ44	0.678 ±0.002	1.572 ±0.001	1.768 ±0.012	0.656 ±0.016
BR46	1.346 ±0.001	1.461 ±0.015	1.824 ±0.002	0.845 ±0.012
BS47	1.348 ±0.001	1.705 ±0.013	1.732 ±0.011	0.464 ±0.022
BS48	1.311 ±0.012	1.412 ±0.001	1.752 ±0.013	0.802 ±0.016
BT49	1.281 ±0.012	1.292 ±0.001	1.862 ±0.016	0.517 ±0.019
BU53	1.553 ±0.014	1.583 ±0.043	1.622 ±0.011	0.519 ±0.012
BU54	1.718 ±0.001	1.654 ±0.013	1.242 ±0.021	0.828 ±0.011
BV55	1.928 ±0.012	1.056 ±0.001	0.886 ±0.019	0.630 ±0.031
BV56	1.882 ±0.014	1.811 ±0.011	1.668 ±0.013	0.715 ±0.016
BW57	1.067 ±0.011	1.432 ±0.002	1.926 ±0.015	0.911±0.001
BX60	1.098 ±0.012	1.426 ±0.001	1.678 ±0.013	0.826 ±0.018
BY62	1.170 ±0.011	1.241 ±0.001	1.604 ±0.012	0.462 ±0.012
BW58	1.281 ±0.013	1.312 ±0.003	1.832 ±0.021	0.670 ±0.023
BY63	1.079 ±0.001	1.372 ±0.001	1.819 ±0.018	0.301 ±0.024
BK33	1.264 ±0.014	1.109 ±0.001	1.045 ±0.013	0.477 ±0.017
TA62	1.234 ±0.002	1.405 ±0.012	1.546 ±0.011	0.312 ±0.019
TC63	1.132 ±0.001	1.575 ± 0.011	1.856 ±0.014	1.217 ±0.021
TC64	1.081 ±0.001	1.445 ±0.001	1.802 ±0.022	0.479 ±0.012
TE72	1.157 ±0.001	1.184 ±0.002	1.628 ±0.009	0.805 ±0.026
TI85	1.287 ±0.002	1.354 ±0.001	1.532 ±0.007	0.659 ±0.025
TI86	1.359 ±0.004	1.378 ±0.003	1.578 ±0.002	0.286 ±0.012
TM94	1.448 ±0.006	1.592 ±0.002	1.689 ±0.001	0.623 ±0.021
TP95	1.194 ±0.012	1.268 ±0.001	1.586 ±0.015	0.929 ±0.016
TP96	1.293 ±0.013	1.784 ±0.018	0.873 ±0.002	0.298 ±0.012
TV97	1.272 ±0.011	1.458 ±0.012	1.772 ±0.017	0.535 ±0.008
TV98	2.055 ±0.015	1.594 ±0.001	1.184 ±0.023	0.612±0.003
TW99	1.517 ±0.011	1.557 ±0.021	1.586 ±0.025	0.721 ±0.019
TX91	0.724 ±0.009	1.166 ±0.004	1.284±0.017	0.656 ±0.016
TX92	0.658 ±0.007	1.047 ±0.021	1.205±0.015	0.267 ±0.012
TY89	1.324 ±0.013	1.752 ±0.018	1.994 ±0.021	1.062 ±0.025
TA61	0.435±0.014	0.624 ±0.022	1.761 ±0.013	0.581 ±0.016
TY90	1.487±0.022	1.641 ±0.014	1.711 ±0.011	0.632±0.011
TK87	1.971±0.016	1.514 ±0.019	1.167 ±0.006	0.463 ±0.021
TK88	1.426 ±0.012	1.696 ±0.023	1.770 ±0.027	0.061 ±0.008
IS-2	1.335 ±0.015	1.561 ±0.011	1.756 ±0.013	0.305 ±0.001

**Results are means and standard deviation of triplicate analysis; Biomass yield at 0hour = 0.392 OD*

Figure 1: This figure shows osmotolerance pattern of yeast strains



NOTE : *The industrial strain Saccharomyces cerevisiae 2 IS-2 was moderately tolerant*

The ability of *R. mucilaginosa 1*, *R. mucilaginosa 2*, *R. glutinis*, *S. cerevisiae 1* and *S. cerevisiae 2* to ferment maltose (disaccharide) shows that the yeast strains possess uptake mechanism that involves two systems and these include an energy-dependent maltose permease which transports the maltose intact across the cellular membrane and a maltase (alpha- glucosidase) which hydrolyses maltose internally to yield two glucose units [7] thus, the mechanism is mediated genetically by three maltose utilization genes (MAL genes) which are involved in the operation of the high- affinity maltose transporter [3].

Yeast deal with an osmotically challenging environment via at least two separate mechanisms, depending on whether the challenge is chronic or acute[8], which eventually leads to variation in the biomass yield and osmotolerance pattern of the yeast subjected to high glucose concentrations in this research (table 2). The osmotolerant rate of 83.93% and 16.07% (Figure 1 obtained for highly osmotolerant and moderately osmotolerant strains may be as a result of the chronic response or acquired osmotolerance which occurs following long-term exposure to the hypertonic medium which is primarily due to the activation of the high-osmolarity glycerol mitogen-activated protein (HOG-MAP) kinase cascade with increased production and specific activity of NADH-dependent glycerol-3-phosphate dehydrogenase (GPDH) [9]. In this research, biomass yield was determined at 12 hours interval for 72hours in other to eradicate the acute response which occurs during sudden exposure of nonosmotically challenged cells to an osmotically stressful environment [8].

The property possessed by the highly osmotolerant *S. cerevisiae* and the non-saccharomyces strains to tolerate hyperosmotic stress is as a result of their ability to increase the rate of production and retention of glycerol [10]. The result also agrees with the report of Rios [11] which stated that the activity and induction of the glycerol-producing enzyme GPDH is highest when cells utilizes glucose as the carbon source which eventually

leads to carbon catabolite repression conditions when cells grown in glucose produce the greatest amounts of glycerol [9]. Furthermore, tolerance to osmotic shock by the yeast strains obtained in this research is also dependent on the physiological state of the cells according to Mager [12] which also reported that the exponential-phase cells are much more sensitive to osmotic shock relative to the slow-growing postdiauxic-phase cells or non-growing stationary-phase cells.

CONCLUSION

It is concluded that this research shows that yeasts are highly tolerant to osmotic shock and their metabolic processes comprise of biochemical assimilation (in anabolic pathway) and dissimilation (in catabolic pathway or fermentation) of nutrients.

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ACKNOWLEDGEMENT / SOURCE(S) OF SUPPORT

Nil

CONFLICT OF INTEREST

No conflict of interests was declared by authors