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STUDIES ON FERMENTATION, ALCOHOL PRODUCTION AND VIABILITY IN INDUCED MUTANTS OF THE BREWING YEAST *SACCHAROMYCES CEREVISIAE*.

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ABSTRACT

Brewing yeast, *Saccharomyces. cerevisiae* cultured on malt extract agar was UV-irradiated. Eighteen mutant yeasts (SCM 01 – SCM 18) selected (after visual examination) were tested for fermentation, alcohol production and viability by acid and gas production, reduction in specific gravity and turbidity respectively, with sugars and delignified sugarcane bagasse. The yeasts had varied fermentation profiles in glucose, sucrose, galactose, dextrose and mannitol but did no ferment lactose. Glucose, sucrose and galactose were fermented strongly with acid and gas production. Dextrose was fermented weakly by SCM 01, SCM 06, SCM 09 and SCM 10 (with acid production only) but strongly by all other yeasts. The wild type yeast did not ferment mannitol but SCM 04 did so strongly and SCM 02, SCM 03, SCM 06, SCM 07, SCM 09, SCM 10 and SCM 13 did so weakly. Specific gravity fell sharply within 36h after inoculation and slightly thereafter. Alcohol production varied among the yeasts. Some mutants produced more alcohol than the wild type yeast. Viability was generally lower in the mutants than in the wild-type yeast in the sucrose medium. The reverse was true in the sugarcane bagasse medium. Yeasts with high viability tended to have high alcohol production ability in the sucrose medium and vice-versa.

KEYWORDS: Alcohol production; fermentation; induced mutants; *Saccharomyces cerevisiae*; viability.

INTRODUCTION

Saccharomyces cerevisiae is perhaps the most economically important yeast species and has been extensively studied (Stewart, 1981). The yeasts have been much exploited by man to produce foods, beverages and medicines. Over one million metric tones of yeast are produced annually and over two million metric tones of alcohol are produced from all-purpose fermentations involving yeasts (Sikyta *el al.*, 1986). Apart from alcohol production, yeasts also play an important role in the fermentation processes of many African foods (Faparusi et al., 1973; Okafor, 1977; Okagbue, 1988; Oyewole and Odunfa, 1988; Sanni, 1985).

As the need to develop the science of fermentation gains significance, there has been a call for the exploration of ways to improve the capabilities of the yeasts (Stewart, 1981). Classical genetic techniques include the isolation of spontaneous and induced mutants. Latter day techniques include recombinatinant DNA and protoplast fusion. In keeping with the objective of improving the capabilities of the yeasts, the brewing yeast, *Saccharomyces cerevisiae* was exposed to ultra-violet radiation and the resultant mutants were selected and tested for fermentation, alcohol production and viability.

MATERALS AND METHODS

Brewing yeast, *Saccharomyces cerevisiae* from the Champion Brewery. Plc Uyo, Nigeria was collected into a sterile bottle from the Champion Brewery. Plc Uyo, Nigeria and stored at 5°C.

One milliliter of the yeast was aseptically transferred into a test-tube containing 9ml distilled water and was serially diluted down to 10^{-5}. Exactly 0.1 ml of the aliquot from 10^{-3}, 10^{-4} and 10^{-5} dilutions were pipetted and inoculated into sterile plates of malt extract agar (Oxoid UK Ltd.) by the spread plate method. The plates were incubated at 28°C for 48h to form the primary culture. The yeasts were than propagated in test-tubes containing yeast extract glucose broth (Oxoid) and incubated at 28°C for 48h.

Serial dilutions of the culture in the yeast extract glucose broth medium were made down to 10^{-7} and then 0.1ml aliquot from 10^{-7} was pipetted into two sets of petri-dishes. One set was placed in an irradiation chamber and UV-irradiated for 10min following a modification of the method reported by Harm. (1968). Molten malt extract agar was immediately poured into the petri-dishes and these were incubated in the dark at 28°C for 48h. The un-irradiated set was the wild-type yeast, which served as the control. After the incubation period, the colonies were visually evaluated and 18 mutant colonies (SCM 01 – SCM 18) were randomly selected and their fermentation, alcohol producing abilities as well as viability were tested.

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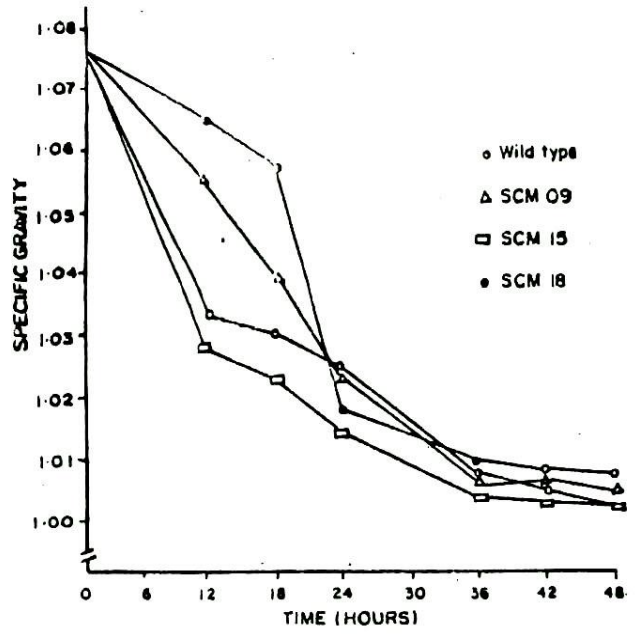

FIG. 1:

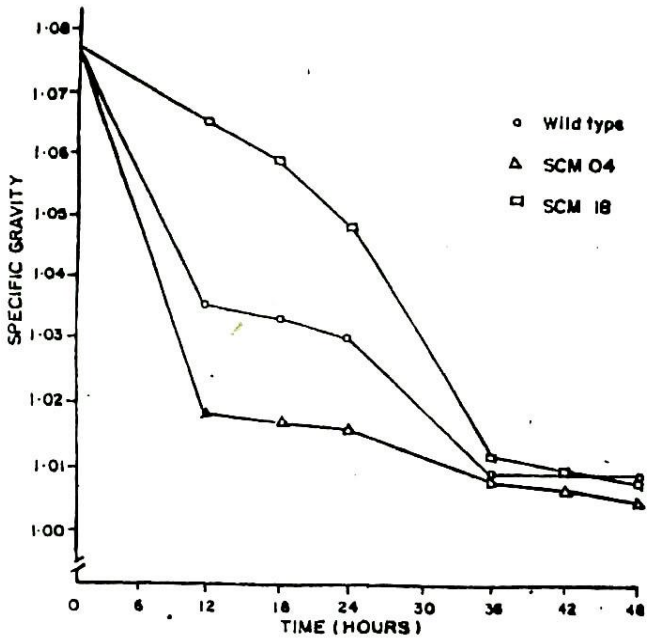

FIG. 2:

(a)). In the sugarcane bagasse medium the mean percentage reduction in specific gravity of the mutant yeasts, SCM 04 and SCM 18 was significantly greater than that of the wild-type yeast (Table 3 (b)).

Table 4 shows the mean turbidity at 0h and 48h after inoculation and the mean difference in turbidity of the yeasts in the sucrose and sugarcane bagasse media. The on-way analysis of variance for the mean difference in the turbidity of the medium 48h after inoculation indicated very highly significant differences between the yeasts ($p<0.001$). In the sucrose medium, the mean values for the mutant yeasts were significantly less than that of the wild-type yeast (Table 4(b) (i). In the

Table 1: Summary of the fermentation abilities of the wild-type and mutant yeasts in the different sugar media.

Sugar	Yeasts																
	Wild-type	SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08	SCM09	SCM10	SCM11	SCM12	SCM13	SCM14	SCM15	SCM16	SCM17	SCM18
Glucose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Sucrose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Galactose	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
Dextrose	AG	A	AG	AG	AG	AG	AG	AG	A	A	AG	AG	AG	AG	AG	AG	AG	AG	AG
Mannitol	O	A	A	AG	O	A	A	O	A	A	O	O	A	O	O	O	O	O	O
Lactose	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

AG = fermentation with the production of acid and gas
A = fermentation with the production of acid only
O= no fermentation.

Table 2: Fermentation profiles of the wild-type and mutant yeasts in the different sugar media.

Sugar	Time (hours) after inoculation 0 – 12	13 – 24	25 - 36	Over 36
Glucose	-	SCM 16, SCM 15, SCM 11, SCM 10, SCM 13, SCM 04, Wild-Type, SCM 06, SCM 18, SCM 02, SCM 01, SCM 14, SCM 05, SCM 17, SCM 08, SCM 07, SCM 12, SCM 03, SCM 09		
Sucrose	-	SCM 16, SCM 15, SCM 11, SCM 10, SCM 13, SCM 04, Wild-Type, SCM 06, SCM 18, SCM 02, SCM 01, SCM 14, SCM 05, SCM 17, SCM 08, SCM 07, SCM 12, SCM 03, SCM 09		
Galactose	-	SCM 03, SCM 06, SCM 07, SCM 08, SCM 10, SCM 12, SCM 18, Wild-Type, SCM 05, SCM 15, SCM 02, SCM 09	SCM 16, SCM 04, SCM 01, SCM 11, SCM 14, SCM 17	SCM 13
Dextrose	-	SCM 14, SCM 16, SCM 18, SCM 09, SCM 07, SCM 04, Wild-Type, SCM 03, SCM 11, SCM 08, SCM 02, SCM 17, SCM 06, SCM 12, SCM 15, SCM 13, SCM 10, SCM 01, SCM 05.		
Mannitol	SCM02	SCM 04	*SCM 03, SCM 06, SCM 07, SCM 09, SCM 10	*SCM01, SCM13
Lactose	-	-		

*The first recorded yeast in each time grouping was the fastest in starting fermentation followed consecutively by the others

sugarcane bagasse medium, both mutant yeasts, SCM 04 and SCM 18, had a greater mean difference in turbidity than the wild-type yeast. (Table 4(b) (ii)).

The correlation between the mean percentage reduction in specific gravity and the mean difference in turbidity of the yeast in the sucrose medium was positive and not significant (r = 0.0158 at 17 degree of freedom).

DISCUSSION

The wild-type and mutant yeasts fermented glucose, sucrose, dextrose and galactose with acid and gas production with varied fermentation profiles but did not ferment lactose (Table 1 and Table 2). The wild-type yeast and the yeast mutants, except SCM 01, SCM 02, SCM 03, SCM 04, SCM 07, SCM 09 and SCM 13 did not ferment mannitol: SCM 04 fermented it with acid and gas production. Acid production alone indicates weak fermentation while acid and gas production together indicate strong fermentation. It is probable that the effect of UV-irradiation created a new pathway or enzyme system that enabled these mutant yeasts to ferment mannitol. The observed differences in the fermentation profile of the mutants is probably due to genetic differences among them.

The observation that the wild-type yeast had a steep gradient of fall in specific gravity soon after inoculation (Fig. 1 and Fig. 2) suggests that the behaviour of those yeast mutants which had such a fall in specific gravity within 12h after inoculation was normal. Those yeast mutants in which the steep gradient of fall in specific gravity was delayed beyond 24h after inoculation exhibited abnormal behaviour. Mutant yeast SCM 04 behaved normally in both the sucrose and sugarcane bagasse media while mutant yeast SCM 18 behaved abnormally in both media.

The slight fall in specific gravity of the media 36h after inoculation (Fig. 1 and Fig. 2) was probably due to loss of viability of yeast cells as the alcohol content of the media increased.

UV radiation like the other ionizing radiations has similar effects as the chemical mutagenic compounds (Amer and Ali, 1968; Crocker 1953; Darlington and Mcleish, 1951; George and George 1976; Sach and Lang, 1960). Chromatin assembly is changed in some way in yeast mutants and causes increased levels of DNA damage. Such cells do not complete mitosis until the damage is repaired (Turner 1995). In the cells carrying such damage, check points or mechanisms for making sure that all the necessary

Table 3: Fall/reduction in specific gravity at various times during 48h after inoculation with the wild-type and mutant yeasts in (a) sucrose medium (b) Sugarcane bagasse medium.

		Yeast																	
Hours after inoculation	Wild-type	SCM01	SCM02	SCM03	SCM04	SCM05	SCM06	SCM07	SCM08	SCM09	SCM10	SCM11	SCM12	SCM13	SCM14	SCM15	SCM16	SCM17	SCM18	LSD (P=0.05)
(a) 0	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	1.073	
12	1.033	1.042	1.047	1.025	1.025	1.032	1.043	1.038	1.052	1.055	1.052	1.058	1.062	1.054	1.028	1.028	1.027	1.043	1.055	
18	1.030	1.038	1.044	1.017	1.017	1.028	1.024	1.033	1.047	1.039	1.050	1.049	1.057	1.043	1.027	1.023	1.022	1.037	1.054	
24	1.025	1.034	1.033	1.016	1.015	1.027	1.022	1.027	1.024	1.023	1.047	1.047	1.055	1.035	1.026	1.014	1.016	1.013	1.019	
36	1.008	1.009	1.010	1.010	1.10	1.011	1.008	1.010	1.008	1.007	1.007	1.010	1.010	1.013	1.010	1.005	1.005	1.012	1.010	
42	1.005	1.009	1.010	1.007	1.007	1.009	1.007	1.007	1.006	1.006	1.006	1.008	1.010	1.005	1.010	1.004	1.004	1.011	1.008	
48	1.005	1.005	1.004	1.007	1.004	1.0007	1.007	1.006	1.005	1.006	1.006	1.003	1.010	1.006	1.009	1.004	1.004	1.010	1.009	
% Reduction	6.34	6.24	6.43	6.15	6.43	6.15	6.15	6.24	6.34	6.24	6.24	6.52	5.87	6.24	5.96	6.43	6.43	5.87	5.95	0.03
(b) 0	1.075				1.075														1.075	
12	1.035				1.018														1.055	
18	1.033				1.017														1.059	
24	1.030				1.016														1.049	
36	1.009				1.008														1.012	
42	1.009				1.00[illegible]														1.009	
48	1.009				1.005														1.008	
% Reduction	6.14				6.42														6.23	

reduction in specific gravity and the mean difference in turbidity in the sucrose medium was positive and non-significant. This suggests that there was a tendency for the yeasts with a high difference in turbidity to have greater percent reduction in specific gravity. Carlson and Bolstein (1983) showed that yeasts contain up to six mutant sucrase genes, and Stewart et al., (1983) showed that the fermentation of sucrose becomes faster or slower depending on which of these genes are carried in the yeast.

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