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Effect of Some Composed Media and Mutation on the Growth, Sucrose-degrading-enzyme and Leavening Activities of *Candida versatalis* Strain 'Bol 1'

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Some media were composed namely: cassava-groundnut extract (CGE), maize-groundnut extract (MGE), sweet potato-groundnut extract (SPGE), soybean extract (SBE) and sucrose-locust bean extract (SLBE). Effect of these media on the growth of *Candida versatalis* strain 'Bol 1' was determined. The fungus was mutated chemically with hydroxylamine. The wild-type strain and the mutants were tested for their ability to synthesize sucrose-degrading-enzyme when grown in nutrient broth (NB) and sucrose-minimal-medium (SMM). They were assessed for leavening property in bread making relative to a commercial (instant) baker's yeast. The yeast grew best in MGE with optical density of 1.85 at 670 nm followed by SBE, SLBE, SPGE and CGE in decreasing order of cell density. The rate at which the mutation occurred was 2.2% survived cells. The mutants and wild yeast showed varying levels of sucrose-degrading-enzyme activity. Some mutants synthesized the enzyme in both NB and SMM. The baker's yeast leavened the bread dough best followed by the wild-type strain and mutant numbers 50 and 20.

Key words: Composed media and Mutation effects, *Candida versatalis* strain 'Bol 1'.

Yeasts are single celled organisms that grow by budding or binary fission (Phaff, 1986). Yeasts occur in various habitats more importantly in sugar-rich medium or substrates such as flower parts, pawpaw, palmwine and mapple syrup. Fruit juice is rich in yeast population like *Saccharomyces cerevisiae*, *S. carlbergensis*, *Pichia membranaefaciens*, *P. fermentans*, *Candida krusei*, *C. guiller* and *Torulopsis* (Ayres, 1980). Pawpaw is easily infected with yeasts. Palmwine contains yeasts that belong to the genera *Klockera*, *Pichia* and *Candida* (Obisanya *et al*., 1987; Okafor, 1987; Somiari and Udoh, 1995). Flour dough raising employed in the making of puff-puff, bread and similar products requires the use of yeasts to ferment the sugar contained in the flour. Sucrose is the main sugar used in leavening the flour dough. The sugar is fermented to alcohol and CO_2 (Conn and Stump, 1976) after its breakdown to simple monosaccharides. The alcohol is useful in brewing while the CO_2 raises flour dough. Enzymes involved in the catabolism of sucrose are therefore important for the liberation of abundant amount of CO_2 during fermentaion. This calls for application of genetic and molecular biology tools.

Susceptibility of yeasts to sophisticated genetic, biochemical and molecular manipulations of large scale cultivation and a basic biology that mirrors that of plant and human cells have attracted the attention of food and industrial microbiologists over the years (Sherman, 1998). In order to

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increase the safe application of yeasts for industrial uses, conventional selection, mutation or recombinant DNA techniques should be optimised (Nout, 1994). Genetic analysis and transformation can be performed on a number of taxonomically distinct varieties of yeast with extensive studies limited primarily to the many freely interbreeding species of the budding yeast, *Saccharomyces* and the fusion yeast, *Schizosaccharomyces pombe* (Sherman, 1998). Previously, we found out that *Candida versatalis* strain 'Bol 1' (Oval shape) leavened flour dough than *Candida versatalis* strain 'Bol 2' (elongated) both isolated from a Nigerian pawpaw (Boboye *et al*., 2008 submitted). Here, we present the growth of the yeast *Candida versatalis* strain 'Bol 1' in various composed media, total and sucrose-containing basic media, synthesis and mutational control of the activity of sucrose-degrading-enzyme (SDE) in the yeast.

MATERIALS AND METHODS

Materials

The materials used to compose the growth media were soybean, groundnut, maize, sweet potato, locust bean and cassava. They were purchased from 'Oba' market in Akure, Ondo State, Nigeria. The yeast, *Candida versatalis* strain 'Bol 1' was obtained from the stock cultures of Dr. B. Boboye in the Microbiology Department of the Federal University of Technology, Akure, Nigeria.

Methods

Test for Growth Effect of Composed Media

Twenty percents (w/v) of sweet potato, groundnut, cassava, locust bean, soybean and maize was prepared individually in distilled water and boiled for 15 min. Equal volume of each extract was mixed to make sweet potato-groundnut (SPGE), cassava-groundnut extract (CGE), sucrose-locust bean extract (SLBE), soybean extract (SBE) and maize-groundnut extract (MGE). Fifteen millilitres of each medium was autoclaved at 121°C for 15 min and inoculated with 24 hours old starter culture of the wild-type yeast. They were incubated at 28°C for 24 hours, after which optical density was read at 670 nm.

Mutation Experiment

Wild-type yeast was grown in acidified nutrient broth (NB) at 28°C for 24 hours. Two millilitres of the grown culture was mutated by the method of Parkinson (1976) as described by Boboye and Alao (2008). An aliquot (0.1 mL) of 0.5M hydroxylamine was mixed with the culture, left at 28°C for 1 h. Cells were spun down at 12,168 x 10^3 g for 10 min (MSE Minor 35 Centrifuge). Cells were washed twice with nutrient broth. They were resuspended in 20 mL nutrient broth, kept at 4°C for 18 h for the mutants to segregate. Aliquot of serially diluted cells suspension was inoculated into nutrient agar by standard pour plate technique. Mutational rate was calculated relative to the colony count of the parent strain. Master plate was made for the mutants and they were used for various tests.

Test for Effect of Mutation on the Growth of Yeasts in Total and Basic Media

Total and basic media used were nutrient broth (NB) and sucrose minimal medium (SMM). Nutrient broth was obtained from Oxoid Company. The SMM contained 10 g peptone and 50 g sucrose in a litre of distilled water. Starter culture of each mutant and parent yeast was prepared in 2 ML acidified nutrient broth (NB). Optical density was read and dilution was made to obtain the same OD reading for all strains. A 0.001 ML was used to inoculate 5 ML NB and SMM. Incubation was done at 28°C for 24 hours after which optical density was read.

Test for Effect of Mutation on the Synthesis of Sucrose-degrading-enzyme (SDE)

Preparation of Enzyme

A 0.1 mL from 24 hours old culture of each mutant and wild-type yeast was inoculated into acidified NB and SMM. They were incubated and spun down at 12,168 x 10^3 g for 15 min (MSE Minor 35 Centrifuge). The supernatant was used as enzyme source.

Quantification of protein content

Protein concentration in the enzyme source for each strain was estimated in milligram by standard Biuret method (Gornall *et al*., 1949) as described by Boboye and Alao (2008).

Assay for Activity of Sucrose-degrading-enzyme

The method described by Boboye and Alao (2008) was employed to make the enzyme mix with little changes followed by slightly modified standard method of dinitrosalicylic acid

(DNSA). Enzyme source (0.5 mL) was added to a test tube containing 0.2 mL sucrose (2%w/v), 0.5 mL potassium phosphate buffer at pH 7.0 and distilled water (0.8 mL). This reaction mix was incubated at 30°C for 1hour and boiled for 3 min to stop the reaction. The DNSA reagent (0.5 mL) was added, boiled for five minutes, cooled after which 4.5 mL distilled water was added. Absorbance was read at 540 nm. Reference was made to a glucose standard curve to obtain the amount of glucose released during enzyme reaction in mg/mL. Activity of sucrose-degrading-enzyme was defined as the amount of glucose (mg/mL) released during enzyme reaction per mg/mL protein.

Microscopic Examination of the Mutants and Wild Yeast

Cells of the wild yeast, two mutants namely: with very low (mutant 20) and highest (mutant 50) enzyme activity were stained with lactophenol-in-cotton blue and examined under a light microscope with × 100 lens.

Test for Effect of Mutation on the Leavening Ability of the Yeast and Sensory Characteristics of Breads

Two mutants with very low (mutant 20) and highest (mutant 50) enzyme activity were selected for this experiment. Flour dough was made with flour (25 g), 0.25 g salt, 15 mL warm water, 1.5 g sugar and 10 g fat (Pyler, 1988). Cells of freshly grown wild yeast, mutants 20 and 50 were diluted to the same optical density and added separately to the ingredients, mixed and fermented at 32°C for 2 hours. Baking was done at 200°C for 30 min.

Sensory characteristics (leavening, texture, odour, taste and appearance) of the breads were assessed by trained panellists. The assessement was based on score grades of bad=1, fair=2, satisfactory=3 and good=4.

RESULTS AND DISCUSSION

Effect of Composed Media on the Growth of wild *Candida versatalis* strain 'Bol 1'

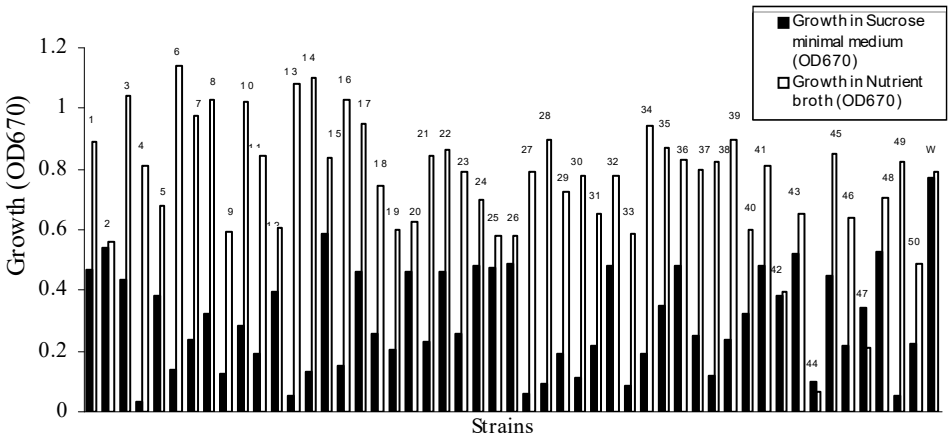
All the media supported the growth of the parent yeast although to varying degrees (Table 1). Optimum growth of the yeast was obtained in the maize-groundnut extract-medium at OD_{670} of 1.8. This signifies that the MGE is the best composed medium that can be used to cultivate this yeast. Similar result was obtained by Xiaola *et al*. (2002) who reported a higher optical density for *Saccharomyces cerevisiae* grown in a corn-steep liquor than in a medium containing peptone and hydrolysate of bean. The MGE medium appeared to contain certain substances that are in the form easily utilizable by the yeast. Maize is a high carbohydrate containing food and groundnut is rich in oil and protein. Maize and groundnut also lack growth inhibitory or toxic substance. Cassava contains cyanide, a toxigenic compound which appeared to have limited the growth of the yeast. Hence, cassava based composed medium supported the least growth of this parent fungus.

Table 1. Effect of composed media on the growth of *Candida versatalis* strain 'Bol 1'

Media	OD_{670}
Sweet potato-groundnut extract (SPGE)	1.2
Sucrose-locust bean extract (SLBE)	1.25
Maize-groundnut extract (MGE)	1.85
Soybean extract (SBE)	1.65
Cassava-groundnut extract (CGE)	0.8

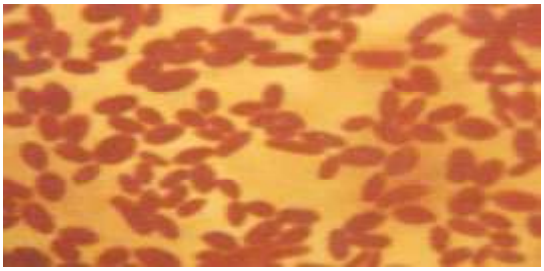
Effect of Mutation on the Growth of *Candida versatalis* strain 'Bol 1'

The mutational rate of the hydroxylamine was 2.2% survived cells. This shows that the chemical has caused some changes in the genome of the yeast. The mutation affected the growth of the microbe as shown in fig. 1. The wild-type and the mutants except mutant 44 grew in both nutrient broth (NB) and sucrose minimal medium (SMM). They grew optimally in NB than SMM except for mutants number 42, 44 and 47 that showed higher absorbance in SMM than in NB and no considerable difference in the optical density values of mutant No. 42 when grown in NB and SMM. About 66% of the mutants grew better than the parental strain in NB. None of the mutants grew below optical density of 0.1 in NB in contrast to mutants 4, 13, 27, 28, 33 and 49 grown in SMM. Nutrient broth is a total medium containing all ingredients necessary for the growth of microorganisms in general. The growths of the parental strain of the yeast in both media were relatively similar.

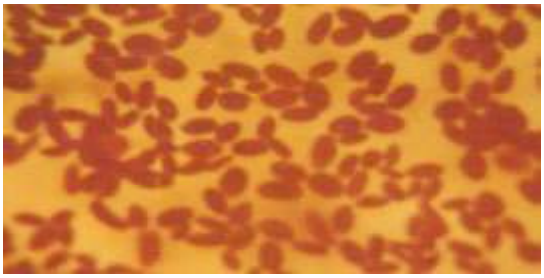


1 to 50 are mutants number 1 to 50. W is the wild-type strain.

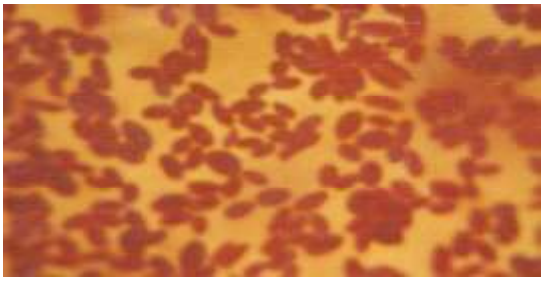
Fig. 1. Growth of mutants and wild-type of *Candida versatalis* strain 'Bol 1' in sucrose minimal medium and nutrient broth.



Mutant 20



Mutant 50



Wild-type

Fig. 2. Microscopic appearance of mutants and wild-type of *Candida versatalis* strain 'Bol 1'.

Microscopic Forms of the Mutants and Wild Yeast.

The microscopic morphology of the yeast and two mutants (20 and 50) are shown in fig. 2. The mutants appeared thinner and bigger than the wild-type strain respectively. The former mutant was a little thinner than the latter. This shows that the mutation had changed the shape of the yeast.

Effect of Mutation on Synthesis of Sucrose-Degrading-Enzyme (SDE)

The yeast and its mutants were able to degrade sucrose at varying levels when grown in NB and SMM (Fig. 3); an indication that the enzyme (SDE) was synthesized extracelllarly. Many of the mutants exhibited higher activity of the enzyme when cultivated in SMM than in NB despite better growth shown in NB, except for mutants 41, 45, 46 and 47. Nutrient broth being a total medium contained some undefined components which could cause hinderance in expression of the enzyme. It also means that enzyme activity is specific for sucrose.

Effect of Mutation on Dough Leavening Ability of the Yeast and Sensory Characteristics of Breads made with the Yeasts

There is no considerable difference between the wild-type strain and commercial yeast in all the parameters tested (texture, aroma, odour, taste and appearance) but in dough raising (Fig. 4). Commrecial baker's yeast showed highest leavening (dough raising) activity followed by the

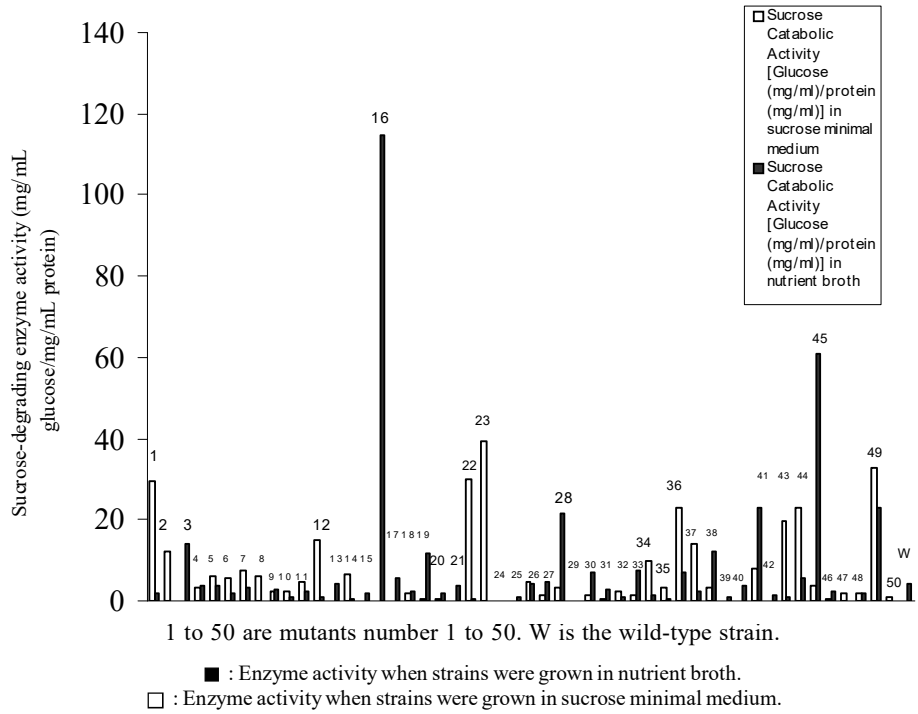
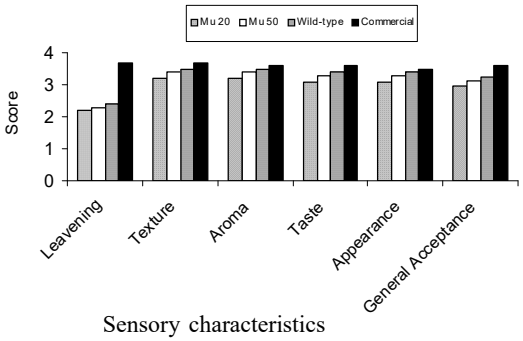


Fig. 3. Sucrose-degrading-enzyme activity in mutants and wild-type of *Candida versatalis* strain 'Bol 1'.

parent yeast, mutant 50 and 20. The wilt-type and the mutants used scored below satisfactory grade in leavening. In all the sensory properties, commercial yeast was rated the best. In general, breads made with all the yeasts and mutant 50 were satisfactory (Fig. 4). Mutant 20 produced bread with total average rating of 2.96.



Mu 20: Mutant 20 and Mu 50: Mutant 50 are mutants with very low and highest sucrose-degrading enzyme activity respectively.

Fig. 4. Organoleptic properties of baked fermented doughs made with representative mutants and wild-type of *Candida versatalis* strain 'Bol 1'.

The insignificant difference observed between the wild-type and mutants in leavening is an indication that the hydroxylamine mutation did not alter the gene encoding breakdown of glucose to CO_2 appreciably although SDE was synthesized highly by the mutant 50. It also means that gene coding for SDE could not ferment glucose and fructose to release CO_2 gas. Another gene/s are therefore involved in the production of the gas. These genes appeared not to have been changed to produce more CO_2 than the wild-type. Hydroxylamine is a deaminating agent. It deaminates DNA cytosine with subsequent replication resulting in the transition of G:C to A:T base pairs (Madigan *et al*., 2001).

CONCLUSION

Maize-groundnut extract medium supported the optimum growth of the yeast. The medium could be used in the large scale cultivation of the yeast. Sucrose degradation was highly favoured by hydroxylamine. This will make a lot of glucose available for fermentaion to release CO_2

gas required for leavening. However, effective dough raising needs good expression of appropriate gene in the yeast. This mutation appeared to have adversely affected the gene encoding CO_2 gas liberation. There is need to carry out gene manipulation in the mutant 50 which had the highest SDE activity.

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