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Genetic control of protein and glucose-anabolic-enzyme syntheses by *Saccharomyces cerevisiae* in the fermentation of a Nigerian rice, *Oryza sativa* variety "Igbimo"

Bolatito Esther Boboye*, Helen Adetoun Lawal

Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria.
Email: *boboye_b@yahoo.com

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ABSTRACT

This work aimed at the control of the production of protein and glucose-anabolic-enzyme (GAE) by *Saccharomyces cerevisiae* during the fermentation of a Nigerian rice, *Oryza sativa* variety "Igbimo". The yeast was mutated with ethylmethyl sulphonate. The variants and the parental yeast were separately inoculated into cooked rice and allowed to ferment at 27˚C for 7 days after which protein content and glucose-anabolic-enzyme synthesis were determined using Dinitrosalicylic acid and Biuret reagents techniques. Mutants with varying capacities to form protein and GAE were obtained. Glucose-Anabolic-Enzyme (GAE) activity ranged from 0.25 to 12.06 Units forming five groups (classes 1, 2, 3, 4 and 5) with the average activity of 0.52, 1.52, 2.28, 4.04 and 10.63 Units respectively compared with that (0.38 Unit) of the parent strain. All the mutants synthesized protein although many of them produced it at lower level while others at higher level than the wild-type. The highest (5.92 mg/mL) and lowest (0.10 mg/mL) levels protein producers are mutants 44 and 14 respectively. Mutants No. 4, 7, 22 and 78 formed total protein similar in concentrations (1.46, 1.46, 1.45 and 1.45 mg/mL) to that of the wild yeast (1.46 mg/mL). These three sets of protein concentration have ratios 4.1 (highest), 0.07 (lowest) and 1.0 (normal) to that of the parental yeast.

Keywords: Protein; Glucose-Anabolic-Enzyme; *Saccharomyces cerevisiae*; Rice Fermentation

1. INTRODUCTION

Rice is the second highest grain in the worldwide production after maize [1]. Nigeria ranked highest as both producer and consumer of rice in the sub-Saharan Africa. More than 50% of rice produced in this region are from Nigeria. The crop has been grown for more than 3000 years along the River Niger [2]. About 1.77 Millions Hectares of land is cultivated of rice annually in Nigeria [3]. Rice (*Oryza sativa*) is an indispensable staple food for many parts (one-half) of the world especially in the east, south, southeast Asia, middle east, Latin America and West Indies. It is a good source of carbohydrate with high energy level [1,4]. Rice is however low in protein, sodium, fats and free of cholesterol which makes it useful in treating hypertension [5-7]. It contains higher percentage of lysine than and rate equal in amount of threonine, methionine, cysteine and tryptophan with many carbohydrate rich foods (wheat, maize, millet, sorghum, rye, oats, potato, cassava and yam). It is a good source of vitamins and minerals such as thiamine, niacin, iron, riboflavin, vitamin D and calcium [8]. Rice is important in human diets. It is used to make starch which is employed in the manufacture of baby foods and extruded noddles. It serves as a glucose substitute in oral dehydration solution used for infants suffering from diarrhoea [9]. It is used industrially for the production of many things including snack foods, breakfast cereals, beer, wine, face powder, polish for camera lenses and expensive jewelleries. The hull and straw are used to make construction materials and production of rope, paper and cattle feeds [10].

Fermentation of rice is usually carried out to improve its nutritional quality [11]. Fermenting rice increases food security, preserves it from spoilage, salvage wastes, removes antinutrients, improves nutrition, increase digestibility, increase vitamins and flavour of the rice [12]. Fermentation of rice is most frequently practised in Asian countries [13]. Carbohydrate particularly starch, is an essential part of rice. The carbohydrate is the principal substrate fermented; microbes easily obtain their energy from it to effect good metabolism [14]. In fermentation, the substrate is broken down to give rise to a mixture of end products. Rice is fermented to yield many

products such as milk, mirin, vinegar, wine (sake), Japanese Miso, Angkak (red rice) and Sierra rice (Amarillo) [15-20]. In Nigeria, rice is not fermented for consumption. Remnant cooked rice that naturally ferments is sweet but usually considered spoilt; it is thus thrown away.

In a previous work, Nigerian indigenous rice variety "Igbimo" was fermented in order to employ nutrient enrichment of rice during fermentation. Chemical analysis of the fermented rice showed that there was improvement in the nutrient contents of the rice [21]. Boboye [22] showed that the chemical features of condiments composed using naturally fermented Nigerian rice ("Igbimo") and locust beans, at certain mix ratios, were similar to that of a commercial condiment (Maggi-star).

Starter cultures are commonly used in the fermentation of rice to prevent contamination with pathogenic and toxigenic microbes and competition with unwanted microbes [23,24]. This increases rate of fermentation thus reducing fermentation time and minimizes dry matter losses. Boboye and Alabi [25, unpublished] showed that individual microorganisms isolated during the natural fermentation of the rice carried out specific functions in the synthesis and catabolism of nutrients. The use of starter culture should be optimised to achieve desired result. This will increase the level of safety consumers require in the manufactured product [26]. This is done by conventional selection, mutation and recombinant DNA Technology. Selection involves screening for natural strains that possesses the desired trait/s while recombinant DNA Technology is a modern technique of developing desired strains involving recombination processes (conjugation, transformation and transduction). Mutation is a heritable change in the nucleotide sequence of DNA. It occurs spontaneously which is a rare event (usually 1 in 10^9 cells) or induced (using physical, biological and chemical mutagens) [27]; causing various changes in living systems including modification of bases for mispairing to take place [28,29], tautomerism by base analog subsitution [27,29], intercalation [30], deamination [31] and alkylation [32]. Mutants generated will possess phenotype different or similar to the wild-type strain such as change in specific enzymatic pathway [33] or requirement for nutrient supplements [27], change in antibiotic production/reaction of strain to drug or chemicals, pathogenicity/virulence, temperature sensitivity, morphological/cultural appearance [27,34,35].

Sweet taste formed during natural fermentation of Nigerian rice ("Igbimo") is related to the amount of simple sugars released from polysaccharides in the fermented rice. In order to study the genetical basis of the process involved in the production of the simple sugar (glucose) released from polysaccharide (starch), we mutagenized *Saccharomyces cerevisiae* which considerably digested carbohydrate better than most of the microbes isolated [25, unpublished]. Effect of the mutation on protein biosynthesized by the fungus was studied. Activity of glucose-anabolic-enzyme (GAE) in each mutant was investigated.

2. MATERIALS AND METHODS

2.1. Mutation Experiment

Saccharomyces cerevisiae was grown in 5 mL potato dextrose broth at 27˚C for 18 hours with agitation at 80 rpm. Using ethylmethyl sulphonate (EMS), the culture was mutated at 28˚C according to the method of Parkinson [36] modified as described by Boboye and Alao [37]. Mutational rate was estimated. Mutants were screened for the synthesis of protein and glucose-anabolic-enzyme (GAE) during the fermentation of rice.

2.2. Fermentation of Rice with Mutants and Wild-Type Yeast

Each mutant was inoculated into 5 mL of potato dextrose broth and incubated at 27˚C for 24 hours after which cells were spun down at 12,168 × 10^3 g for 15 min (MSE Minor 35 Centrifuge). Cells were washed and resuspended in sterile distilled water. Equal cell number of each mutant (0.867 at 670 nm) was used to inoculate 4 g of cooled rice previously sterilized for 15 min at 121˚C. The wild-type strain was used as a control at the same optical density. Fermentation was carried out for 7 days at 27˚C after which protein content and glucose-anabolic-enzyme (GAE) were determined.

2.3. Quantification of Protein

Protein concentration in the rice sample fermented with each mutant and wild-type was estimated in milligram by standard Biuret method [38] according to Boboye and Alao [37].

2.4. Assay for Glucose-Anabolic-Enzyme (GAE) Activity

Synthesis of Glucose-Anabolic-Enzyme (GAE) was determined by DNSA reagent and Biuret procedures [37]. One unit of GAE activity was defined as amount of glucose released from rice starch when 1mg/mL protein was made.

3. RESULTS AND DISCUSSION

The mutation was carried out at 65% rate. Different types of mutant were obtained, all of which had ability to produce protein. The mutants expressed protein as did the unmutated yeast, although many variants produced protein at lower (56%) and higher (40%) levels than latter strain (**Figure 1**). The highest (5.92 mg/mL) and

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lowest (0.10 mg/mL) levels protein producers are mutants 44 and 14 respectively. Only four mutants (Mutants No. 4, 7, 22 and 78) formed total protein similar in concentration (1.46, 1.46, 1.45 and 1.45 mg/mL) to that of the wild yeast (1.46 mg/mL). These three sets of protein concentration have ratios 4.1 (highest), 0.07 (lowest) and 1.0 (normal) to that of the parental yeast.

The mutants formed Glucose-Anabolic-Enzyme (GAE) at activity ranging from 0.25 to 12.06 Units (**Table 1**). Some mutated cells produced GAE at lower levels and others at higher levels than the wild-type yeast. Five groups of the variants were obtained with induction ratios of 1.37, 4.00, 6.00, 10.63 and 27.97 when the average GAE activity (0.52, 1.52, 2.28, 4.04 and 10.63 Units) of the corresponding classes 1, 2, 3, 4 and 5 was compared with that (0.38 Unit) of the parent strain. About 20% of the mutants (class 1) were able to synthesize GAE at the same level with the parental strain, these are Normal-Poor-Level (NPL) GAE synthesizing mutants. None among these variants formed 0 Unit of GAE but seven members of the group produced GAE less than that of the mother yeast. Generally mutants in class 1 are poor GAE producers when compared with strains in classes 2 to 5. The moderate level producers of GAE had activity ranging from 2.0 to 2.99 Units. Forty eight percents mutants (Class 2) have GAE activity at low level (1.0 - 1.99 Units), although higher than that of the wild-type (**Table 1**). Good (3.0 - 5.99 Units) (Class 4) and super-producers (6.0 and above 6.0 Units) (Class 5) of this GAE were very few constituting only 10% of the total mutants. Average level synthesizing GAE variants constituted the moderate class 3.

The yeast used in this work is naturally poor in GAE synthesis since its activity is similar to the Normal-Poor-Level GAE synthesising mutants as determined under the growth conditions used in this experiment. The

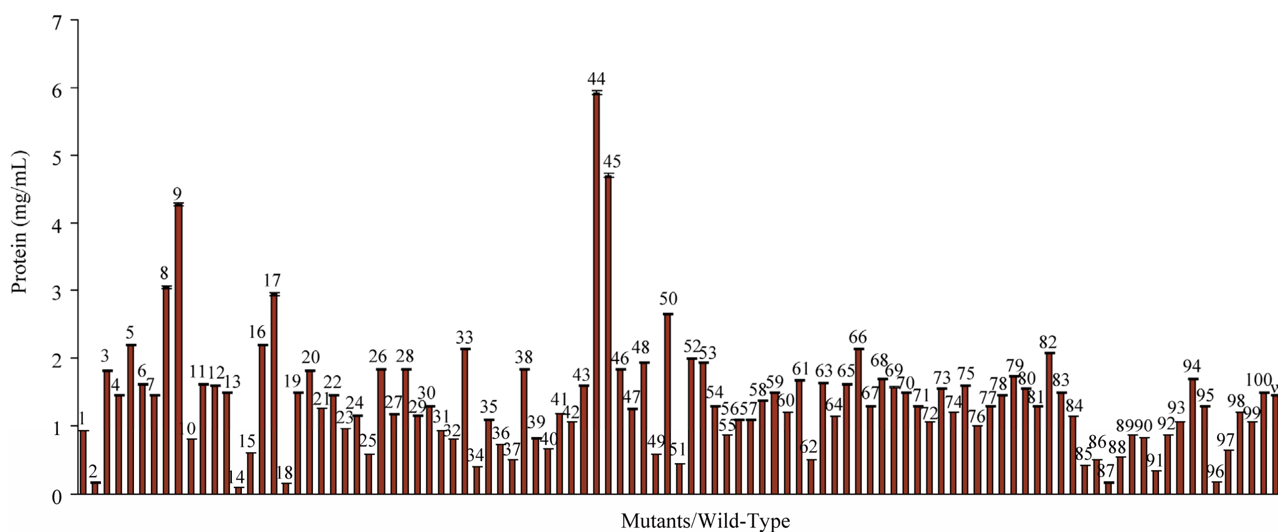

Figure 1. Concentration of protein synthesized by mutants and wild-type *Saccharomyces cerevisiae* during the fermentation of a Nigerian rice, *Oryza sativa* var. "Igbimo". 1 to 100 are mutants 1 to 100 and w is the wild-type strain.

Table 1. Effect of mutation on Glucose-Anabolic-Enzyme activity synthesized by *Saccharomyces cerevisiae* during the fermentation of a Nigerian rice, *Oryza sativa* var. "Igbimo".

Class	Glucose-Anabolic-Enzyme Activity			Mutants
	(GAE Units)	Induction Ratio	Level	
1	0 - 0.99	1.37	Normal-Poor-Level (NPL)	3, 7, 9, 13, 16, 17, 29, 36, 37, 39, 44, 45, 46, 50, 52, 84, 85, 91, 92, 95.
2	1.0 - 1.99	4.00	Low Level (LL)	1, 4, 5, 8, 10, 11, 12, 18, 19, 20, 21, 22, 23, 24, 26, 27, 28, 30, 31, 33, 35, 38, 40, 42, 48, 53, 58, 59, 60, 61, 63, 66, 68, 69, 70, 73, 75, 77, 79, 80, 82, 86, 88, 89, 93, 94, 98, 100.
3	2.0 - 2.99	6.00	Moderate Level (ML)	6, 15, 41, 43, 47, 49, 55, 56, 57, 64, 65, 67, 71, 72, 76, 78, 81, 83, 87, 90, 97, 99.
4	3.0 - 5.99	10.63	Good Level (ML)	25, 32, 34, 51, 54, 62, 74, 96.
5	6.0 - above 6.99	27.97	Super Level (SL)	2, 14.

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variations observed in the production of Glucose-Anabolic-Enzyme and protein mean that the mutagen (ethylmethyl sulphonate, EMS) had hit the genome at various loci; thus there was a great change in the genes coding for the traits examined in this work. The EMS hit the genome to various extents, hence, the differences in the level of expression of the GAE and formation of the protein encoding gene/s in each mutant. The changes appeared to have endowed some mutants with ability to synthesize protein and GAE better than the wild-type yeast. The mutants in class 1 appeared relatively unaffected by the EMS while those in group 2 were slightly modified in contrast to those mutants in classes 3, 4 and 5 that showed good and very high levels of GAE production. These last sets of variants and those able to produce protein at high levels have their genes encoding the traits induced. Genetic induction can be caused by various genetic manipulations including mutation [27]. Ethylmethane sulphonate (EMS), used for the mutation in this experiment is a mono-functional akylating agent that acts by adding methyl group to guanine causing faulty pairing with thymine in nucleotides, resulting in the transition of G-C to A-T [27,34,39]. This mutagen controlled the synthesis of protein and glucose-anabolic-enzyme by producing the mutants with varying capabilities thus regulating the genes encoding these traits.

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