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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (1): 115-118, 2006
ISSN 1028-8880

Isolation of a Mutant of *Kluyveromyces marxianus* Resistant to Glucose Repression

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Abstract: In this study, we isolated a mutant of *Kluyveromyces marxianus* resistance to glucose repression. To screen for depression mutants, the strains were treated with UV rays. Fifteen resistant mutant strains were isolated. The mutants were further screened for glucose-repression-resistant mutants in the presence of 2-deoxy-D-glucose, an analog to glucose and lactose as the sole carbon source. In this condition, one glucose-repression-resistant mutant was isolated. The enzyme activity in this mutant strain and the wild type strain was compared using different mediums containing 4% of each lactose and glucose and 2% glucose+2% lactose. The results demonstrated significant decreasing in glucose repression in the mutant strain as compared to the wild type. This mutant was unable to grow anaerobically on glucose in present of antimycin A, the property of *rag1* mutants. This mutant is, therefore, capable of constitutive expression of β-galactosidase, which makes it suitable for industrial purposes.

Key words: β-galactosidase, glucose repression, *Kluyveromyces marxianus, rag1* mutant, glucose-repression-resistant mutant

INTRODUCTION

Saccharomyces cerevisiae and many other yeasts such as *Kluyveromyces* species may thrive on a variety of carbon sources, but glucose is the preferred ones[1-4]. When this sugar is present, the enzymes required for the utilization of alternative carbon sources such as lactose in some *Kluyveromyces* strains are synthesized at low rates or not at all[1,5,6]. This phenomenon is known as glucose repression. Many microorganisms including bacteria, yeasts and fungi produce β-galactosidase with different properties[7,8]. β-galactosidase (Lactase) is produced by several microorganisms including bacteria, yeast and fungi[7].

The lactose uptake and β-galactosidase activity in a number of yeast strains including *Kluyveromyces marxianus* are induced by lactose[9]. However, in other strains such as *Kluyveromyces lactis* (*K. lactis*) the enzyme is produced constitutively without requiring further induction[10,11]. Several strains of *Kluyveromyces marxianus* capable of expressing β-galactosidase activity have been isolated in our laboratory previously[12,13]. Further, analysis of these strains revealed that this activity could be repressed by glucose.

In this study, it was attempted to isolate mutant strains of *Kluyveromyces,* which were resistant to glucose repression. For this purpose, the identified *Kluyveromyces* strains were exposed to ultraviolet radiation as a mutagenic factor, as it has been shown to induce mutations in yeast and bacterial strains effectively[7]. Forty mutants resistance to glucose was isolated of which one was found capable of utilizing lactose as a sole carbon source. The isolation of this mutant, which could be of invaluable industrial application, is discussed.

MATERIALS AND METHODS

Yeast strains: Four strains of *Kluyveromyces marxianus* were isolated from dairy products[13]. These strains were identified using the standard taxonomic key outlined by Kutzman and Fell[14]. This identification were based on different chemical testes including the fermentation of sugars, liquid assimilation of carbon compounds, liquid assimilation of nitrogen compounds, growth at 37°C and 40°C, growth in 50% Glucose and Urase activity.

UV-treatment and isolation of mutants resistant to glucose repression: The strains were cultured into medium containing 20 g L^{-1} of dextrose and peptone and 10 g L^{-1} yeast extract. Ultraviolet radiation has been shown to induce mutations in yeast and bacterial strains[7,14]. For this purpose, the grown yeast strains were spread on Potato Dextrose Agar (PDA) medium and were

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exposed to UV radiation (25 μw cm^{-2}) for different time periods of 0, 5, 10, 15 and 20 min. The plates incubated for 48 h at 25°C. The resistant mutants to UV radiation were isolated. For this screen, the UV mutants isolated were cultured into a medium containing 0.1% 2-deoxy-D-glucose as an analog of glucose that cannot normally be metabolized by these organisms, 10 g L^{-1} lactose as the sole carbon source, 0.4, 0.5, 1 and 5 g L^{-1} NaCl, $MgCl_2$, KH_2PO_4 and $(NH_4)SO_4$, respectively, filter sterilized and were incubated for 12-16 h at 25°C under shaking at 180 rpm. A positive reaction was detected by visual inspection as an increase in the turbidity of the liquid medium. The grown mutants were isolated and transferred to a solid medium containing 0.1% 2-deoxy-D-glucose, 10 g L^{-1} lactose, Nitrogen base medium and 1.5% agar-agar and was incubated at 25°C for 2-3 days. The mutant strain that grown in this medium was again transferred into a liquid medium containing 2-deoxy-D-glucose (see above) and incubated for 12-16 h at 25°C. The mutant strain which was at this condition grown was purified by streaking on Potato Dextrose Agar (PDA) and stored on a PDA slant at 4°C.

Beta-galactosidase activity: The enzyme activity of this mutant was qualitatively prepared with the wild type using X-gal plates versus overly adapted from Duttweiler[15] in different mediums, (4% Glucose, 4% Lactose, 2% Glucose + 2% Lactose). Then the Beta-galactosidase activity of the mutant and wild type was measured quantitatively using the Ortho-nitrophenyl-β-D-galactopyranoside (ONPG) assay as described by Guarente[16]. The assay was carried out in triplicate using 4% of each lactose and glucose and 2% lactose + 2% glucose and the results were averaged.

Anaerobic growth in present of antimycin A: The mutant strain was grown under anaerobic condition at 30°C in a medium containing (per liter): 10 g glucose, 5 g yeast extract, 5 g ammonium sulfate, 2.5 g $MgSO_4.7H_2O$, 2 g KH_2PO_4 and Antimycin A (15 μM; sigma), a respiratory chain inhibitory[17].

RESULTS AND DISCUSSION

Four strains of *Kluyveromyces marxianus* were isolated and identified, which were found to be repressed by glucose (Table 1). Because of the glucose repression, the strain has some limitations in industrial application. To overcome this problem, attempts were put to isolate new mutant resistant to glucose repression. In this study, the grown strains into rich medium were spread on Potato Dextrose Agar (PDA) medium and were exposed to UV radiation for different time periods of 0, 5, 10, 15 and 20 min and incubated in suitable conditions. Fifteen UV resistant mutants were isolated (Table 2). These isolated were considered resistant to glucose repression. For this screen, the UV mutants isolated were cultured into medium containing 2-deoxy-D-glucose and lactose as the sole carbon source. The 2-deoxy-D-glucose is an analog of glucose that can repress the synthesis of β-galactosidase in cell sensitive to glucose repression[4,11]. Under these repression conditions, the sensitive cells are not able to utilize lactose as the carbon source and thus cannot grow. Only the glucose-repression resistant mutants can produce β-galactosidase to digest lactose for growth. Among these, one mutant exhibited a positive reaction. It was transferred to a solid medium containing 2-deoxy-D-glucose, lactose, Nitrogen base medium and

Table 1: Identification of yeasts isolated from dairy producer centers in lab

	Conventional name of yeast			
	M_1	M_2	M_3	M_4
Fermentation				
Glucose	+	+	+	+
Galactose	+	+	+	+
Sucrose	+	+	+	+
Maltose	-	-	-	-
Raffinose	+	+	+	+
Trehalose	-	-	-	-
Assimilation				
Galactose	+	+	+	+
Sucrose	+	+	+	+
Maltose	-	-	-	-
Cellobiose	+	+	-	-
Trehalose	+	-	-	-
Melibiose	-	-	-	-
Raffinose	+	+	+	+
Inuline	+	+	+	+
D-Xylose	+	+	+	+
L-Arabinose	+	+	+	-
D-Ribose	-	-	-	+
L-Rhamnose	-	-	-	-
Glycerol	+	+	+	+
D-Mannitol	-	+	+	-
Citrate	-	-	-	-
Inositol	-	-	-	-
Nitrate	-	-	-	-
l-Lysine	+	+	+	+
Growth in 50% Glucose	ND	ND	ND	ND
Growth in 37	+	+	+	+
Growth in 40	+	+	+	-
Urease activity	-	-	-	-
Yeast species	*K. marxianus*	*K. marxianus*	*K. marxianus*	*K.marxianus*

+: Positive reaction; -: Negative reaction; ND: Not Detection

Table 2: UV treatment of Four strains

	Yeast strains resistant to UV ray after different time radiation (min)				
Strains	5	10	15	20	Growth into 2-DOG + lactose medium
M_1	4	-	-	-	-
M_2	3	1	2	-	-
M_3	5	1	-	-	-
M_4	1	-	2	-	1 sample*

* This sample was resistant to glucose repression

Fig. 1: 1) Mutant strain in 2-DOG + lactose medium, 2) Mutant strain in lactose medium, 3) Wild type in 2-DOG + lactose medium

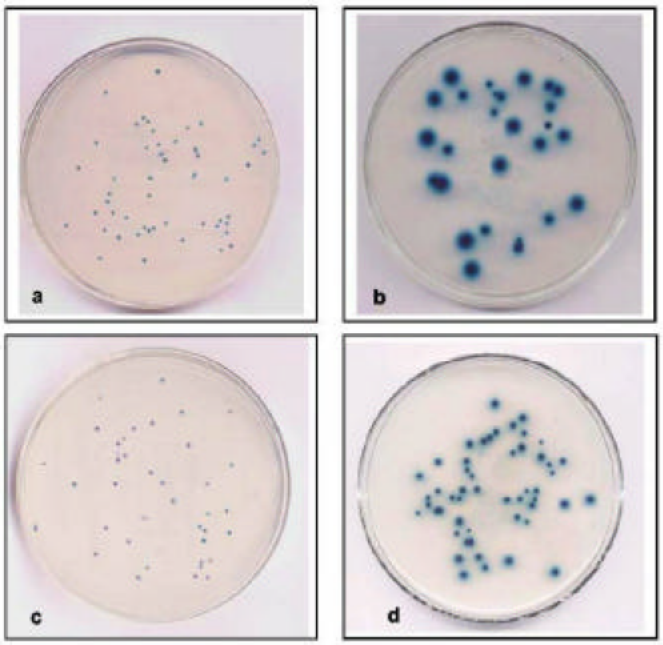

Fig. 2: Qualitatively preparation of beta-galactosidase activity of the mutant with wild strain using X-gal plates versus overly adapted in different medium:
a and b: wild type and mutant strain, respectively in 2% glucose + 2% lactose medium,
c and d: wild type and mutant strain, respectively in 4% glucose medium

agar-agar (Fig. 1). One colony of the mutant on the above medium was again transferred into liquid medium containing 2-deoxy-D-glucose and then grown mutant strain was purified on PDA. Qualitatively preparation of beta-galactosidase activity of this mutant with wild strain using X-gal plates versus overly adapted in 4% Glucose, 4% Lactose, 2% Glucose + 2% Lactose mediums was performed. This preparation indicated significant differences between the mutant and wild type in 4% Glucose and 2% Glucose + 2% Lactose mediums (Fig. 2). The β-galactosidase activity of the mutant and wild type using the Ortho-nitrophenyl-β-D-galactopyranoside (ONPG) assay in present 4% lactose, 4% glucose and 2% lactose + 2% glucose, was quantitatively measured (Table 3). In the 4% lactose the enzyme activity of mutant and wild type were 5844 and 5298 U mL^{-1}, respectively. The difference of beta-galactosidase activity between the mutant strain and wild type in the medium with 4% glucose as the only carbon source and in the 2% glucose + 2% lactose medium was considerable (Table 3). In the 4% glucose medium the beta-galactosidase was very low in wild type, 150 U mL^{-1}, whereas in the mutant was

Table 3: Enzyme activity of wild type and mutant strain in different medium

	β-galactosidase activity (U mL^{-1})	
Medium	Wild type	Mutant
4% lactose	5298	5844
4% glucose	150	2611
2% lactose+2% glucose	959	5305

2611 U mL^{-1}. When a complex of 2% glucose and 2% lactose was used together, the enzyme activity of the mutant reached 5305 U mL^{-1} whereas in the wild type was only 959 U mL^{-1}. All these results demonstrated that the repression effects of glucose on the mutant strain decreased significantly as compared to that in the wild type strain.

In fermentative condition in present of antimycin A, the mutant strain was unable to grow on glucose[18]. This is the characteristic property of *rag1* mutant in *Kluyveromyces*[9,19]. *Rag1* encodes an inducible low-affinity hexose transporter[2,19] and several of the *rag* genes seem to be involved in the regulation of this gene[19]. *rag1* is necessary for fermentative growth on high concentration of glucose, so the *rag1* mutant unable to grow anaerobically on high concentration of glucose in present of a respiratory chain inhibitor such as antimycin A[19,20]. *rag1* is induced by several hexoses but induction is rather slow[2].

REFERENCES

1. Carlson, M., 1999. Glucose repression in yeast. Curr. Opin. Microbiol., 2: 202-207.
2. Chen, X.J., M. Wesolowski and H. Fukuhara, 1992. Glucose transport in the yeast *Kluyveromyces lactis*. II. Transcriptional regulation of the glucose transporter gene *rag1*. Mol. General Gene., 33: 97-105.
3. Gustafsson, L., 2004. Switching the mode of metabolism in the yeast *Saccharomyces cerevisiae*. EMBO Reports, 5: 532-537.
4. Wei, W., S. Wang, X. Zhu and W. Wan, 1999. Isolation of a mutant of *kluyveromyces* sp. Y-85 resistant to catabolite repression. J. Biosci. Bioeng., 87: 816-818.
5. Carlson, M., 1998. Regulation of glucose utilization in yeast. Curr. Opin. Gene. Develop., 8: 560-564.
6. Fredlund, E., L.M. Blank, J. Schnurer, U. Sauer and V. Passoth, 2004. Oxygen and glucose-dependent regulation of central carbon metabolism in pichia anomala. Applied Environ. Microbiol., 70: 5905-5911.
7. Klaus, W. (Ed.)., 1996. Nonconventional Yeasts in Biotechnology. A Handbook. Springer-Verlag Berlin Heidelberg New York, pp: 139-201.
8. Uhlig, H., 1998. Industrial Enzymes and Their Applications. Translated and updated by Elfriede M. Linsmaier-Bednar. John Wiley and Sons, Inc.
9. Schaffrath, R. and K.D. Breunig, 2000. Genetics and molecular physiology of the yeast *Kluyveromyces lactis*. Fungal Gene. Biol., 30: 173-190.
10. Breunig, K.D., M. Bolotin-Fukuhara, M.M. Bianchi, D. Bourgarel, C. Falcone, I. Ferrero, L. Frontali, P. Goffrini, J.J. Krijger, C. Mazzoni, C. Milkowski, H.Y. Steensma, M. Wesolowski-Louvel and A.M. Zeeman, 2000. Regulation of primary carbon metabolism in *Kluyveromyces lactis*. Enz. Microb. Technol., 26: 771-780.
11. Gancedo, J.M., 1998. Yeast carbon catabolite repression. Microbiol. Mol. Biol. Rev., 62: 334-361.
12. Moeini, H., I. Nahvi and M. Tavassoli, Improvement of SCP production and BOD removal of whey with mixed yeast culture. Elec. J. Biotechnol., 7: 249-255.
13. Nahvi, I. and H. Moeini, 2004. Isolation and identification of yeast strains with high beta-galactosidase activity from dairy products. Biotechnology, 3: 35-40.
14. Kurtzman, C.P. and J.W. Fell (Ed.), 1998. The Yeasts: A Taxonomic Study. Elservier: Amsterdam.
15. Duttweiler, H.M., 1996. A highly sensitive and non-lethal beta-galactosidase plate assay for yeast. Trends Gene., 12: 340-341.
16. Guarente, L., 1983. Yeast Promoters and *Lacz* Fusions Designed to Study Expression of Cloned Genes in Yeast. In: Methods in Enzymology (Wu, R., L. Grossman and K. Moldave Eds.). Acad. Press, New York, 101: 181-191.
17. Slater, E.C., 1967. Application of inhibitors and uncouples for a study of oxidation phosphorylation. Method Enzymol., 10: 48-57.
18. Goffrini, P., A.A. Algeri, G. Donnini, M. Wesolowski-louvel and I. Ferrero, 1989. *rag1* and *rag2*: Nuclear genes involved in the dependence/independence on mitochondrial respiratory function for growth on sugars. Yeast, 5: 99-106.
19. Wesolowski-Louvel, M., P. Goffrini, I. Ferrero and H. Fukuhara, 1992. Glucose transport in the yeast *Kluyveromyces lactis*. I. Proterties of an inducible low-affinity glucose transporter gene. Mol. Gen. Gene., 33: 89-96.
20. Boumans, H., L.A. Grivell and J.A. Berden, 1998. The respiratory chain in yeast behaves as a single functional unit. J. Biol. Chem., 273: 4872-4877.