THE EFFECT OF MEDIA STERILIZATION AND OF VARYING SOURCES AND CONCENTRATIONS OF SUGAR AND NITROGEN ON ALCOHOL PRODUCTION BY *SACCHAROMYCES CEREVISEAE* STRAINS

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ABSTRACT

Several locally isolated and one ATCC yeast strains were tested for their ability to produce alcohol in sterilized and unsterilized media, in various sugar sources, in varying sugar concentrations of molasses and in varying levels of nitrogen supplied by urea. Some strains produced alcohol only in sterilized media while other strains produced as much or sometimes higher alcohol when media was unsterilized. Molasses was the best source of sugar for all strains except MG2065 which produced as much alcohol from brown sugar. All strains except MG2064 produced the highest alcohol concentration at 10% sugar concentration while nitrogen from urea supplied at 10 mg/L was optimal for alcohol production in unsterilized media.

INTRODUCTION

The production of ethanol by *Saccharomyces cereviseae* is commercially undertaken by about 22 distilleries in the Philippines . Previously considered a mere adjunct to an integrated sugar mill, the improvement of alcohol fermentation efficiency has not been given much attention and efficiencies may range from 65-98% in the various alcohol plants.

The present government plan to use alcohol as fuel additive has changed research directions towards improving fermentation efficiency. There is considerable interest in the genetic improvement of yeast for alcohol fermentation. This paper reports results of experiments conducted to determine the reaction of yeast strains to various practices in the fermentation plants. These include the use of varying concentrations of molasses depending upon the season of the year, the use of urea at varying concentrations and the general use of unsterilized wort. Also determined is the reaction of a set of yeast strains to various sugar sources since there is a proposal to use brown sugar for alcohol fermentation.

The production of alcohol from molasses by various strains has been reported (Hipolito *et al*, 1961; Ignacio and Alicbusan, 1967; Flor, 1977; Sabiniano, 1979; Colle *et al*, 1982). There are also several studies on other substrates such as coconut sap. The variation

in sugar concentrations used in fermentation by the industry is most often based on observation and experience gathered through the years. Valera (1955) claimed that it is essential as well as economical to work at 12° Brix. Prescott and Dunn (1949) advised that a sugar concentration of 10-18% usually gives satisfactory results. Hipolito *et al* (1961) found that alcohol-producing yeast was tolerant up to 25% total reducing sugar in molasses medium. Almonte (1977) recommended the use of molasses at 10° Brix and 15% sugar to produce high yield of alcohol. Presently, there is a preference in the industry towards the use of higher sugar concentrations for fermentation to minimize the amount of slops that causes environmental pollution. Shvests and Slyuserendo (1970) studied the effect of sterilization of molasses to minimize decrease in concentration of fermentable matter for yeast. They recommend that molasses must be sterilized at 120°C for ten minutes or at 140°C for 5 minutes. However, the reaction of specific strains to sterilization, varying sugar sources and concentrations and varying N-concentration is not yet reported.

MATERIALS AND METHODS

The MG series of yeast strains were locally isolated from fermenting nipa sap and identified as *Saccharomyces cereviseae* by Ms. Lourdes M. Tapay. The strain ATCC 2345 used as control was obtained from the American Type Culture Collection through Dr. Ernesto J. del Rosario.

All strains were streaked and allowed to grow on MYGP slants. After sufficient growth, each isolate was transferred aseptically to sterile molasses medium containing 5% sugar for inoculum build-up and incubated for 24 hours with shaking. Using ten percent (10%) of the inoculum, the fermentation medium (15% sugar) was incubated for 8 hours on a rotary shaker and then allowed to stand at room temperature for 15 hours. Treatment was varied as follows:

1. Sugar sources – Molasses, brown sugar, white sugar and glucose-sucrose mix were used. Sugar concentration was 15%.

2. Sugar concentration – Molasses was used as source of sugar at 15, 20 and 25% sugar concentration.

3. Nitrogen levels – Nitrogen levels used were 10, 20, 30 and 40 mg/L. The recommended amount of 20 mg/L solution served as control.

Each treatment was replicated three times in 100 mL fermentation media with initial pH of 5.0.

Percent alcohol was determined using an ebulliometer and total sugar using the phenol-sulfuric assay method.

Statistical tests were carried out by CRD two factorial.

RESULTS AND DISCUSSION

1. *Effect of media sterilization*

Sterilization of media caused a very dramatic increase in alcohol production by the majority of yeast strains tested (Table 1). MG2S13, MG2S15, MG2S16 and MG2S19 showed marked reduction in alcohol production in unsterilized molasses medium compared with sterilized molasses medium. This probably indicates that these strains are more sensitive to the presence of contaminants in molasses medium or it could also be due to the inability of the strains to utilize sucrose. Sterilization inverts the sucrose into glucose and fructose specially at low pH. Hence, either Strains MG2S14, MG2S18, MG2S20, MG2S21 and MG2S35 are more resistant to contamination or they possess more active invertases since their alcohol production in sterilized as well as unsterilized media were comparable. Strains MG2S14 produced higher alcohol yield in unsterilized medium implying that it is most resistant to contamination or it possesses the most active invertase system.

2. *Effect of sugar source and sterilization on alcohol production*

The alcohol production by five yeast strains, MG 2063, MG 2064, MG 2065, MG 2A33 and ATCC 2345 is presented in Table 2. Statistical analysis of the data indicated significant differences among the five strains and therefore their performance is very much affected by the sugar source and sterilization. All strains produced alcohol best in molasses medium whether sterilized or not. The highest alcohol concentration was produced by MG 2064. MG 2065 produced the same amounts of alcohol in sterile and unsterile molasses medium. But the other three strains produced slightly higher amounts of alcohol in sterilized than in unsterilized molasses medium. Brown sugar was the next best source of sugar to molasses. The higher amounts of alcohol produced in molasses compared with the other sugar sources indicates the positive effect on alcohol fermentation of organic substances such as vitamins, amino acids and other sugars and mineral salts found in molasses. Such substances could also be found in brown sugar but in lesser amounts which explains why brown sugar was the second best source. MG 2065 produced as much alcohol in unsterilized brown sugar medium as in sterile and unsterile molasses medium indicating its utilization of additional heat-sensitive substances in the brown sugar medium. Alcohol production in unsterilized white sugar and unsterilized glucose-sucrose mix was comparable in all strains. This is expected since these fermentation media have not been supplemented with mineral salts necessary even for normal growth, the only supplement being urea as N-source. The least amount of alcohol was produced by all strains in sterilized white sugar.

The production of slightly more alcohol in sterilized molasses medium compared with unsterilized molasses medium could be due to the presence of more contaminants in unsterilized medium. The possible effect of sucrose inversion to glucose and fructose could not explain this difference because in white sugar and glucose-sucrose mix media, the reverse was observed. More alcohol was produced in these unsterilized medium, probably because in these media, alcohol production was affected by loss of trace organic nutrients due to heating during sterilization.

3. *Effect of varying sugar concentrations and sterilization on alcohol production*

As expected, the production of alcohol increased with increasing sugar concentrations between 15 and 25% (Table 3) but only in sterilized medium. In unsterilized medium, the tendency for all strains except Strain MG 2064 was a decrease in alcohol production when the sugar concentration was increased from 15 to 25%. This could be due to the higher population of contaminants since in media with higher sugar concentration, more molasses was added. Strain MG 2064 was probably quite resistant to contamination; its alcohol production in sterile as well as unsterilized media was comparable in media with 15% and 20% sugar. However, at 25% sugar concentration, MG 2064 produced lower alcohol in unsterilized medium than in sterilized medium since there was probably a higher initial contaminant population.

4. *Effect of varying levels of nitrogen on alcohol production*

The yeast strains' reaction to increasing levels of urea from 10 mg-40 mg/L was a decrease in alcohol production (Table 4). All strains produced their highest alcohol yields at 10 mg/L in unsterilized medium but Strain ATCC 2345 produced comparable levels in unsterilized medium supplemented with 20 mg urea and Strain MG 2064 in sterilized medium with 20 mg urea. In this set of experiments, the difference in alcohol produced due to the use of sterile and unsterile media was more apparent with a general trend for all strains to produce higher alcohol amounts in unsterilized media. This is especially true when the concentration of urea used was 10 mg/L. These results show that urea at 10 mg/L in unsterilized wort was most favorable for alcohol fermentation by all five strains tested.

SUMMARY AND CONCLUSIONS

1. The ability to produce high amounts of alcohol in sterile or unsterile molasses medium is strain-specific.

2. Molasses was the best source of sugar for all strains tested, except Strain MG 2065 which produced comparable alcohol amounts in both sterilized and unsterilized molasses and unsterilized brown sugar.

3. The optimum sugar concentration for alcohol production using unsterilized wort in all strain was 15%, except Strain MG 2064 which produced higher alcohol at 20% sugar.

4. Urea of 10 mg/L was found to be optimal for alcohol production in all strains.

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Table 1. Alcohol production by some yeast strains in sterlized and unsterilized molasses media containing 15% sugar and 20 mg urea/L at pH 5*.

Yeast	AMOUNT OF ALCOHOL PRODUCED AFTER 24 HOURS INCUBATION (% v/v)	
	Sterilized	Unsterilized
MG-2S-13	2.29	0.03
-14	4.25	7.62
-15	7.48	1.39
-16	4.13	0.00
-18	2.33	1.60
-19	6.33	0.00
-20	3.09	2.60
-21	1.75	2.44
MG-2S-35	8.88	7.72

*Each value represents the average of three replicates.

Table 2. Alcohol production (% v/v) * by five yeast strains in sterilized and unsterilized wort with initial pH 5 and supplemented with various sugar sources equalized at 15% total sugars and 20 mg urea/L after 24 h fermentation.

Strain	Sugar source **							
	Molasses		White sugar		Brown sugar		Glucose-sucrose mix	
	SM	UM	SM	UM	SM	UM	SM	UM
MG-20-63	6.17^{abcde}	5.17^{bcdef}	0.22^{k}	3.55^{fghij}	4.48^{defgh}	4.59^{cdefgh}	2.49^{ij}	3.15^{ghij}
MG-20-64	7.43^{a}	7.05^{a}	0.55^{k}	3.58^{fghij}	3.89^{fghij}	4.59^{cdefgh}	3.67^{fghi}	3.50^{fghij}
MG-20-65	5.88^{abcde}	5.96^{abcde}	0.08^{k}	3.58^{fghij}	3.85^{fghij}	6.40^{abc}	2.44^{ij}	3.63^{fghij}
MG-2A-33	6.53^{ab}	4.75^{bcdefg}	0.38^{k}	4.26^{efghi}	4.59^{cdefgh}	$4.98^{bcdefgh}$	2.21^{ij}	3.82^{fghij}
ATCC 2345	6.24^{abcd}	5.05^{bcdefg}	0.38^{k}	3.74^{fghi}	3.41^{fghij}	$4.89^{bcdefgh}$	3.01^{ghij}	3.74^{fghij}

*Each value is the average of 3 replicates

**Means followed by the same letter are not significantly different from each other at 5% using DMRT

SM – Sterilized media

UM – Unsterilized media

Table 3. Alcohol production (% v/v)* of five yeast strains using different sugar concentrations supplemented with 20 mg/L urea at initial pH 5.0 in sterilized and unsterilized molasses media at 24 h fermentation.

Isolate	Sugar concentration (% Total sugar**)					
	15		20		25	
	SM	UM	SM	UM	SM	UM
MG-2O-63	6.48^{fe}	6.69^{de}	6.79^{de}	5.78^{ghi}	8.44^{b}	4.26*
MG-2O-64	6.69^{de}	6.37^{efg}	7.56^{c}	7.29^{cd}	9.65^{a}	7.24^{cd}
MG-2O-69	6.61^{de}	6.84^{de}	7.24^{cd}	5.26^{hijk}	8.83^{b}	4.32^{e}
MG-2A-33	6.65^{de}	6.32^{efg}	6.69^{de}	5.92^{fgh}	9.48^{a}	5.65^{ghi}
ATCC 2345	4.88^{kl}	6.52^{fe}	5.88^{fgh}	6.33^{efg}	6.60^{de}	5.26^{hijk}

*Each value is the average of 3 replicates

**Means followed by the same letters are not significantly diferent from each other at 5% using DMRT

SM – Sterilized media

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Table 4. Alcohol production (% v/v) * by yeast strains in sterilized and unsterilized molasses media supplemented with urea after 24 h fermentation.

Isolate	Nitrogen concentration (mg urea/L)**							
	10		20		30		40	
	SM	UM	SM	UM	SM	UM	SM	UM
MG-2O-63	3.50^{jklm}	6.16^{abc}	4.21^{ghijk}	5.73	3.23^{jklmno}	4.44^{fghij}	2.97^{lmnop}	3.93^{ijklm}
MG-2O-64	4.88^{efghi}	7.14^{a}	6.06^{abcd}	5.40^{cdefg}	2.60^{nop}	4.95^{defghi}	0.06^{q}	2.68^{nop}
MG-2O-69	4.79^{efghi}	6.15^{abc}	4.25^{ghijk}	4.82^{efghi}	3.16^{klmno}	2.88^{lmnop}	3.05^{klmno}	2.11^{op}
MG-2A-33	2.69^{nop}	6.80^{ab}	4.15^{hijkl}	5.30^{cdefgh}	3.34^{jklmno}	4.42^{fghij}	3.32^{jklmno}	3.12^{klmno}
ATCC 2345	2.55^{nop}	6.84^{ab}	3.74^{ijklmn}	6.10^{abcd}	2.15^{op}	5.60^{cdef}	1.81^{p}	4.08^{ijklm}

*Each value is the average of 3 replicates

**Means followed by the same letters are not significantly different from each other at 5% using DMRT

SM – Sterilized media

UM – Unsterilized media