Research Article

Media Optimization for Primary Screening of β-Glucosidase Producing Yeast Strains

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Abstract

Beta glucosidase is the enzyme responsible for enhancing wine aroma or flavor during the process of wine grape fermentation. Many researchers have carried out primary screening of beta glucosidase producing yeast strains by using different media like esculin glycerol agar or media containing arbutin as a substrate. Present study was conducted at ICAR-NRC for Grapes to screen out different yeast strains for beta glucosidase enzyme by using media containing arbutin as a substrate. The satisfactory results were not obtained from medium cited in literature. Two new media were designed to carry out screening of beta glucosidase producing yeast strains. A total of seventeen yeast strains were screened for the purpose. Satisfactory results were observed on newly designed media containing arbutin as a substrate. Two strains namely VSI 1106 and SPR were found positive on both the media namely M2 and M3 and produced brown colour during incubation. However, the brown colour was appeared from the strains very early stage of incubation on M3 in comparison to M2. The results were reconfirmed by using Esculin glycerol agar medium also. Newly optimized media support the growth of yeast and gave better, quick and reliable results as compared to reported medium. Enzyme activities of positive yeast strains were quantified and yeast strain VSI 1106 was found superior over the SPR.

Keywords: Screening, beta glucosidase, arbutin, media optimization, ONPG

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Introduction

Wine quality is comprised by different parameters and influenced by various factors. Among these parameters alcohol, sugar, acidity, pH, colour, phenols, tannins and aroma compounds have own importance. Aroma is an important attribute in wine quality and aroma development in wines occurs as a result of interaction between various components of the grapes themselves and those produced during processing, fermentation and aging [1]. About 800 volatile compounds have been identified responsible for aromas of a particular wine [2]. Flavour compounds in grapes may be present in their free, odour-active form or as non-volatile precursors [3]. Increasing attention has recently been paid to the complex non-volatile glycosides which act as aroma precursors and occur in high concentrations in musts of red wine grapes [4]. The aroma potential is naturally revealed during fruit maturation by endogenous enzymes called β-glucosidases. These endogenous β-glucosidases show low activities and high variability within harvests so cannot liberate the whole aroma potential. The interaction of exogenously supplemented β-glucosidases releases more volatile compounds and give more fruity and floral wines with more intense and complex aroma [5].

Indian wine industry is largely dependent on imported yeast formulations. The ability of these yeast strains in relation to β-glucosidase activity in tropical wine making is unknown. Locally identified non-saccharomyces yeast strains performed well in wine making and are almost at par with commercial cultures [6, 7]. Screening of yeast strains for β- glucosidase activity helps in the identification of suitable strains having ability to produce wines with improved aromas. β-glucosidase activity has been assayed on several culture media and under various growth conditions. The highest activities were obtained in yeast extract peptone medium, however, the activity with a drop of 25% was also detected when grape juice was used as growth medium and anaerobic conditions were employed. β-glucosidase activity has been assayed by using β-glucoside analogues such as p-nitrophenyl-b-D-glucopyranoside (p-NPG) and 4-methylumbelliferyl-b-D-glucopyranoside (4-MUG); however, these substrates are expensive and laborious and they have not shown any direct correlation with glucosidase activity in grape must or wines [8]. Derivatives arbutin or esculin were found most reliable for estimation of β-glucosidase activity when included in solid media and the colonies showing glucosidase activity were easily identified by released brown colour [8-11]. However, there are no reports showing a correlation of this test with natural grape glucoside hydrolysis capacity by

yeasts [8]. The lack of screening methods for glucosidase activity so as to correlate with real wine conditions is one of the constraints in identifying these strains. Considering the importance of yeast strains, screening for β-glucosidase activity, present study was aimed to standardize suitable medium for the screening of yeast strains having β-glucosidase enzyme potential.

Materials and Methods

The present study was carried out at ICAR-National Research Centre for Grapes, Pune (India). Seventeen yeast strains of saccharomyces and non-saccharomyces were evaluated in this study on basic medium (MI) [12]. Two combinations were included as M2 and M3 (**Table 1**). A pH of 5.0 was maintained in case of M1 while it was increased in M2 and M3 to 6.5. Various constituents of media were procured from Hi-media, Sigma Aldrich and Thomas Baker.

Table 1 Media composition for screening of yeast strains

Components	Media		
	M1	M2	M3
Peptone	0.0 g	2.0 g	2.0 g
Yeast extract	0.0 g	1.0 g	1.0 g
Dextrose	0.0 g	2.0 g	2.0 g
Arbutin	0.5 g	0.5 g	0.5 g
Agar agar	2.0 g	2.0 g	2.0 g
YNB	0.67 g	0.0 g	0.0 g
Ferric Ammonium citrate	0.0 ml	2 ml	0.0 ml
$FeCl_3$	0.0 g	0.0 g	0.03 g

Radial streaking of yeast cultures were made on defined media *viz*., M1, M2 and M3. The prepared plates were inoculated by 0.1 mL test yeast and placed at average room temperature of 26 to 28 °C for incubation. The observations on release of colour from colonies were noted at the interval of 24 h up to 10 days. The colonies which produced brown coloration around were treated as glucosidase producing colony of a particular strain. These strains were again placed on esculine glycerol agar having pH of 6.5. The ability of modified media to response against material having β-glucosidases enzyme was again confirmed by inoculation of aqueous extract of almond seeds (known source of enzyme) soaked for overnight and soil usually having all microbes with dilution of 10^{-6}.

The yeast strains positive to *β*-glucosidases activities were quantified by permeabilized cell assay [13]. ONPG (O-Nitrophenyl-β-D-galactopyranoside) reaction was stopped by 0.5 ml of Na_2CO_3 stock solution when the samples in tubes had developed a pale yellow color. Activity of enzyme (x) was calculated and presented as µmol ONPG min^{-1}

$$x = \frac{\text{OD420}}{\text{OD600 of assay culture} \times \text{volume of assay} \times \text{time}}$$

Results and Discussion

The behavior of yeast strains on different media for a given incubation duration was noted and observations are presented in **Tables 2-4** for media M1, M2 and M3, respectively. No response was noted by any studied yeast strain on M1 as showed in Table 2. Relatively very few colonies were observed on the medium and not a single organism clearly showed browning around the colony. These results may be due to insufficient nutrients availability in the medium. The results obtained from M2 and M3 showed growth in colonies which may be due to availability of sufficient nutrients and clearly observed by comparing the plates of both types of media (Fig. 1). Growth of yeast streaked on medium M2 was found to be very good which can be easily observed on plate and browning was observed around the colony of yeast having ability to produce glucosidase enzyme. Same response was noted for crude enzyme from the almond when inoculated on M2 and M3. Out of seventeen cultured yeast strains only two strains namely VSI 1106 and SPR showed browning around own colonies on 8^{th} and 3^{rd} day, respectively when incubated on M2 medium (Table 3). The incubation on M3 medium gave results very early and both the previous strains produced brown colour on 3^{rd} day after inoculation (Table 4).

The results of this study are showed in **Table 5**. Medium M3 is found superior for screening of yeast strains. M3 having ferric chloride (0.03 g) instead of 2 ml of ferric ammonium citrate in M2. Strauss et al. [14] also suggested that

the most reliable of these β-glucoside analogues were the arbutine or esculine derivatives. When these compounds were included in solid media, colonies showing glucosidase activity can be easily identified by their brown colour. If glucosidase activity is produced by the tested strain, the natural β-glucoside esculin is split into esculetin (6,7-dihydroxycoumarin) and glucose, and then the free esculetin reacts with the ferric ions present in the medium resulting in a brown precipitate. Those cultures showing positive results with both modified media viz.; M2 and M3 containing arbutin as substrate also showed positive results on esculine glycerol agar (Second media suggested in literature for quick screening of glucosidase producers) also. The medium composition affects the activity of β-glucosidase production from *Aspergil lusniger* [15].

Table 2 Reaction of yeast strains on M1 medium after inoculation

S No.	Culture code	Days after inoculation on M1 medium incubated at 28 ^{0} C									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
1	VSI 1005	-	-	-	-	-	-	-	-	-	-
2	VSI 1101	-	-	-	-	-	-	-	-	-	-
3	VSI 1104	-	-	-	-	-	-	-	-	-	-
4	VSI 1019	-	-	-	-	-	-	-	-	-	-
5	VSI 1106	-	-	-	-	-	-	-	-	-	-
6	VSI 1039	-	-	-	-	-	-	-	-	-	-
7	VSI 1050	-	-	-	-	-	-	-	-	-	-
8	VSI 1038	-	-	-	-	-	-	-	-	-	-
9	VSI 1037	-	-	-	-	-	-	-	-	-	-
10	VSI 1051	-	-	-	-	-	-	-	-	-	-
11	RS 2	-	-	-	-	-	-	-	-	-	-
12	RS3	-	-	-	-	-	-	-	-	-	-
13	SB	-	-	-	-	-	-	-	-	-	-
14	PC	-	-	-	-	-	-	-	-	-	-
15	CS	-	-	-	-	-	-	-	-	-	-
16	SPR	-	-	-	-	-	-	-	-	-	-
17	SPW	-	-	-	-	-	-	-	-	-	-

Table showing results of glucosidase activity of yeast cultures on medium cited in literature M1 ('-' sign indicates no browning around colony while '+' sign indicates browning around colony)

Table 3 Reaction of yeast strains on M2 medium after inoculation

S No.	Culture code	Days after inoculation on M2 medium incubated at 28 ^{0} C									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
1	VSI 1005	-	-	-	-	-	-	-	-	-	-
2	VSI 1101	-	-	-	-	-	-	-	-	-	-
3	VSI 1104	-	-	-	-	-	-	-	-	-	-
4	VSI 1019	-	-	-	-	-	-	-	-	-	-
5	VSI 1106	-	-	-	-	-	-	-	+	+	+
6	VSI 1039	-	-	-	-	-	-	-	-	-	-
7	VSI 1050	-	-	-	-	-	-	-	-	-	-
8	VSI 1038	-	-	-	-	-	-	-	-	-	-
9	VSI 1037	-	-	-	-	-	-	-	-	-	-
10	VSI 1051	-	-	-	-	-	-	-	-	-	-
11	RS 2	-	-	-	-	-	-	-	-	-	-
12	RS3	-	-	-	-	-	-	-	-	-	-
13	SB	-	-	-	-	-	-	-	-	-	-
14	PC	-	-	-	-	-	-	-	-	-	-
15	CS	-	-	-	-	-	-	-	-	-	-
16	SPR	-	-	+	+	+	+	+	+	+	+
17	SPW	-	-	-	-	-	-	-	-	-	-

Table showing results of glucosidase activity of yeast cultures on newly designed medium M2 ('-' sign indicates no browning around colony while '+' sign indicates browning around colony)

Table 4 Reaction of yeast strains on M3 medium after inoculation

Sr No.	Culture code	Days after inoculation on M3 medium incubated at 28 ^{0} C									
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day	10th day
1	VSI 1005	-	-	-	-	-	-	-	-	-	-
2	VSI 1101	-	-	-	-	-	-	-	-	-	-
3	VSI 1104	-	-	-	-	-	-	-	-	-	-
4	VSI 1019	-	-	-	-	-	-	-	-	-	-
5	VSI 1106	-	-	+	+	+	+	+	+	+	+
6	VSI 1039	-	-	-	-	-	-	-	-	-	-
7	VSI 1050	-	-	-	-	-	-	-	-	-	-
8	VSI 1038	-	-	-	-	-	-	-	-	-	-
9	VSI 1037	-	-	-	-	-	-	-	-	-	-
10	VSI 1051	-	-	-	-	-	-	-	-	-	-
11	RS 2	-	-	-	-	-	-	-	-	-	-
12	RS3	-	-	-	-	-	-	-	-	-	-
13	SB	-	-	-	-	-	-	-	-	-	-
14	PC	-	-	-	-	-	-	-	-	-	-
15	CS	-	-	-	-	-	-	-	-	-	-
16	SPR	-	-	+	+	+	+	+	+	+	+
17	SPW	-	-	-	-	-	-	-	-	-	-

Table showing results of glucosidase activity of yeast cultures on newly designed medium M3 ('-' sign indicates no browning around colony while '+' sign indicates browning around colony)

Table 5 Comparison of media in terms of response and vivid results

S. No	Name/Code of culture	Media		
		M1	M2	M3
1	VSI 1005	-	-	-
2	VSI 1101	-	-	-
3	VSI 1104	-	-	-
4	VSI 1019	-	-	-
5	VSI 1106	-	Positive response on 8th days	Positive response on 3rd day
6	VSI 1039	-	-	-
7	VSI 1050	-	-	-
8	VSI 1038	-	-	-
9	VSI 1037	-	-	-
10	VSI 1051	-	-	-
11	RS 2	-	-	-
12	RS3	-	-	-
13	SB	-	-	-
14	PC	-	-	-
15	CS	-	-	-
16	SPR	-	Positive response on 3rd day	Positive response on 3rd day
17	SPW	-	-	-

"-" No positive response after 10 days

Hernández et al. [16] also noted effect of medium composition and recorded highest activity of β-glucosidase enzyme when cultured on Yeast Extract Peptone contained medium. M2 and M3 also contained yeast and peptone. New designed media are superior as compared to already cited medium in literature. It supports the growth of yeast and gave better, quick and reliable results as compared to previous medium. Comparative evaluation of both media have clearly carried out the differentiation and proved better quality of medium for primary screening using arbutin as substrate. As medium has made significant improvement in primary screening of β-glucosidase producing yeasts strains, so it will be helpful in identification of more strains having β-glucosidase activities and their further use in producing wines with improved aromas. Each yeast strain has own ability to produce β-glucosidase. As per recorded data, VSI 1106 recorded more activity in comparison to other yeast strain which was found positive in present results

(**Table 6**). Variations in β-glucosidase activities related to different yeast strains have been recorded by different workers [10, 12] also. Further studies may be carried out on fermentation of wine grapes and identification of aroma compounds whose presence is affected by strains having ability to produce β-glucosidase.

Table 6 Activity of β-galactosidase (µmol ONPG/min) in positive strains

Strain	Activity
SPR	0.044
VSI 1106	0.050

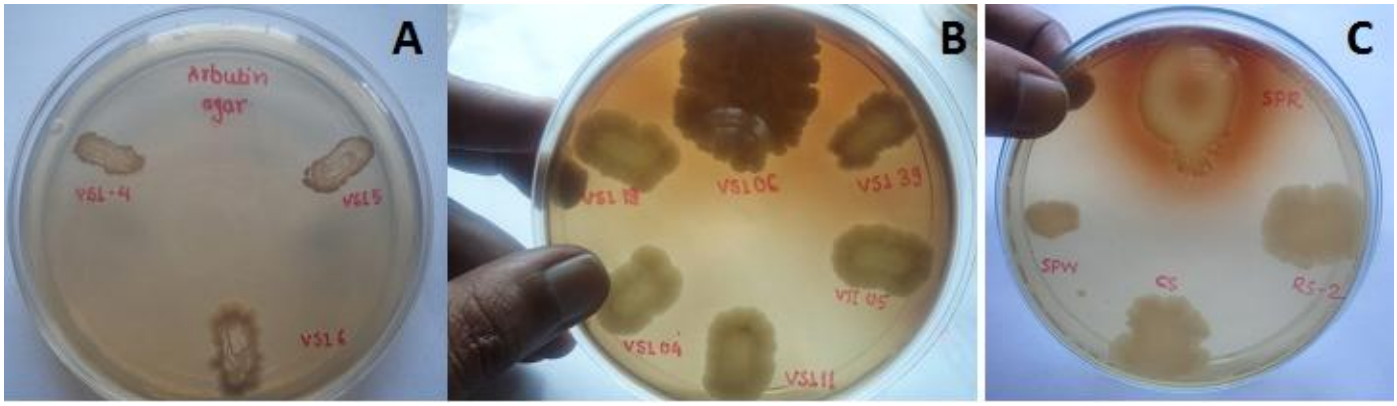

Figure 1 Petriplates a (M1) b and c (M3) showing the results for screenings of glucosidase producing yeast strains

Conclusion

On the basis of results obtained from present study it may be concluded that M3 medium which contains $FeCl_3$ and maintained pH 6.5 was found better than M2 medium. Early results were obtained in M3 medium and this medium can be adopted for screening of yeast strains for β-glucosidase activity. The strains namely VSI 1106 and SPR, showed positive results for recorded activity of β-glucosidase during quantification by following permeabilized cell assay.

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