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Isolation and Evaluation of Xylose-Utilizing Yeasts for Ethanol Production

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Abstract

Seventeen xylose-utilizing yeasts were retrieved from different ecological niches and screened for production on xylose-containing medium. Seven of the isolates produced no alcohol at all on xylose. The ten isolates while on xylose produced 1—2%, on dextrose they produced 3—5% (vol/vol) ethanol. Six of the yeast isolates producing 5% (vol/vol) ethanol on dextrose with an ability to ferment xylose as well were screened for ethanol production on a medium containing a total of 10% both of dextrose and xylose (4 : 3 ratio). Isolate 11 produced the maximum of 4.5% (vol/vol) ethanol leaving behind only 0.5% residual sugars. It was further examined for ethanol production on media containing either dextrose or both dextrose and xylose (4 : 3) at 12 and 15% sugar levels. In dextrose production medium, a maximum of 5.5% (vol/vol) ethanol was achieved at an initial sugar concentration of 12% at 72 h. As against this a maximum of 4.7% (vol/vol) ethanol was produced on the medium containing both dextrose and xylose (4 : 3). Morphological and biochemical characteristics of isolate 11 were found to conform well to the genus *Candida*.

Key words : Bioethanol, Xylose, Dextrose, Yeast.

In the twenty-first century, attention is being particularly focused on bioethanol as a source of energy (1). The emission and toxicity of ethanol are lower than those of petroleum and is also known as an "oxygenate" because it contains 37% oxygen by weight, which enhances fuel combustion and therefore contributes to a reduction in exhaust emission (2). In India, there are large number of distilleries which produce alcohol by the conventional batch fermentation process using cane molasses as the substrate and producing only 1.3 billion liters of alcohol per year against an installed capacity of 3.2 billion liters. Besides other raw materials such as molasses and starchy substrates in current use, bioethanol production from lignocellulosic biomass would be the best bet, as it does not compete with food crops and is also less expensive than conventional agricultural feedstocks (3). As the biomass grows it consumes as much carbon dioxide as it forms during the combustion, which makes the net contribution to the green house effect zero. Lignocelluloses consist of three major constituents-cellulose, hemicellulose and lignin in the ratio of 4 : 3 : 3 (4). After the hydrolysis, the most abundant sugars present in the lignocellulosic hydrolysates are glucose and xylose in the ratio 4 : 3. Efficient utilization of not only glucose but also xylose offers an opportunity to reduce the cost of producing fuel ethanol by 25 % (5). The most promising organisms for alcoholic fermentation of lignocellulose sugars are yeasts. The discovery of yeast species capable of converting D-xylose directly to ethanol is a significance advance towards the development of an economic fermentation process (6—10). Till date, no yeasts have been reported to ferment pentoses (or specifically D-xylose) to ethanol effectively, even though many yeasts are capable of both metabolizing pentoses aerobically, and in many cases, producing polyols (e.g., xylitol, and arabitol) as the metabolic by-products. The low ethanol tolerance of xylose-fermenting yeasts is the main limiting factor for industrial ethanol production (11). The present investigation was undertaken to isolate xylose fermenting yeasts and examining their ability to ferment xylose in the presence of glucose.

Methods

Microorganisms, Media and Fermentation

Different yeasts were isolated from various ecosystems viz., soil samples of decomposing wheat

Table 1. Ethanol production by isolated yeasts on XPM.

Isolate	Fermentation time 24 h Ethanol % (vol/vol)	24 h Residual sugars (%)	48 h Ethanol % (vol/vol)	48 h Residual sugars (%)	72 h Ethanol % (vol/vol)	72 h Residual sugars (%)
1	0.7	8.0	1.5	5.0	1.5	4.7
2	0.8	7.6	1.5	5.0	1.5	4.6
3	0.9	8.0	2.0	5.5	2.0	5.0
4	1.0	7.5	2.0	5.0	2.0	4.5
5	1.0	7.8	2.0	5.2	2.0	4.8
6	Nil	7.0	Nil	5.5	Nil	4.5
7	1.0	7.0	1.5	6.0	1.5	5.6
8	Nil	7.7	Nil	6.2	Nil	4.8
9	Nil	7.5	Nil	5.0	Nil	4.5
10	Nil	7.8	Nil	6.2	Nil	5.3
11	1.0	7.9	2.0	5.6	2.0	5.0
12	0.5	8.0	1.0	6.5	1.0	6.0
13	Nil	8.0	Nil	6.0	Nil	5.2
14	Nil	7.4	Nil	6.0	Nil	5.0
15	Nil	7.5	Nil	6.0	Nil	5.5
16	1.0	7.3	2.0	5.8	2.0	4.7
17	1.0	7.2	2.0	5.6	2.0	4.8

straw, sugarcane bagasse, aonla, molasses, fruits (grapes, oranges, blackberries and bananas) using xylose enriched medium. Appropriate dilutions of the samples in water were plated on yeast extract-peptone-xylose (YEPX) medium containing (g/l) xylose, 20 ; peptone, 20 ; yeast extract, 10 and agar 20, pH 5.0 ± 0.2. Morphologically different colonies were picked up and isolated as pure clones by streaking them on

Table 2. Ethanol production by selected ethanologenic yeasts on DPM.

Isolate	Fermentation time 24 h Ethanol % (vol/vol)	24 h Residual sugars (%)	48 h Ethanol % (vol/vol)	48 h Residual sugars (%)	72 h Ethanol % (vol/vol)	72 h Residual sugars (%)
1	2.1	4.5	3.5	2.5	5.0	Nil
2	2.2	4.0	3.4	3.0	4.1	0.8
3	2.4	3.8	3.6	2.4	5.0	Nil
4	2.1	4.5	3.5	2.5	5.0	Nil
5	2.2	4.5	3.4	2.5	5.0	Nil
7	2.1	5.0	3.3	3.0	3.9	1.2
11	2.3	4.5	3.5	2.8	5.0	Nil
12	1.5	6.0	2.5	5.0	3.0	1.5
16	2.1	4.5	3.3	2.5	4.0	0.8
17	2.1	4.0	3.3	2.0	5.0	Nil

Table 3. Ethanol production by selected ethanologenic yeasts on DXPM containing dextrose and xylose in ratio 4 : 3.

Isolate	Fermentation time 24 h Ethanol (%) (vol/vol)	24 h Residual sugars (%)	48 h Ethanol % (vol/vol)	48 h Residual sugars (%)	72 h Ethanol % (vol/vol)	72 h Residual sugars (%)
1	1.8	5.5	3.0	4.0	3.7	1.4
3	2.2	4.0	3.0	3.0	3.5	1.8
4	2.0	4.5	3.0	3.5	3.5	2.0
5	2.1	5.8	3.2	3.5	3.7	1.2
11	2.0	5.0	3.2	3.0	4.5	0.5
17	2.0	3.5	3.5	2.0	3.8	1.2

the plates containing the same medium. The stock cultures of the isolated yeasts were maintained on YEPX slants by regular transfers and stored at 4C in a refrigerator.

Inoculum for fermentation was prepared using medium containing (g/liter) xylose, 10 ; peptone, 5 ; yeast extract, 2 ; and malt extract, 2, pH 5.0± 0.2. Fermentation media were similar to inoculum media except that the xylose was raised to 10—12% or re-

Table 4. Batch fermentation of ethanol production by isolate 11 at various concentrations of Dextrose / Xylose and Dextrose (3 : 4 ratio) in production medium.

Total sugar conc.	Fermentation time 24 h Ethanol (%) (vol/vol)	24 h Residual sugars (%)	48 h Ethanol (%) (vol/vol)	48 h Residual sugars (%)	72 h Ethanol % (vol/vol)	72 h Residual sugars (%)
12% (Dextrose)	2.6	6.0	4.5	3.5	5.5	Nil
12% (Xylose+ Dextrose)	2.2	6.0	3.8	3.2	4.7	1.5
15% (Dextrose)	2.8	7.5	4.7	4.5	5.5	2.0
15% (Xylose+ Dextrose)	2.8	8.0	3.9	5.0	4.7	2.5

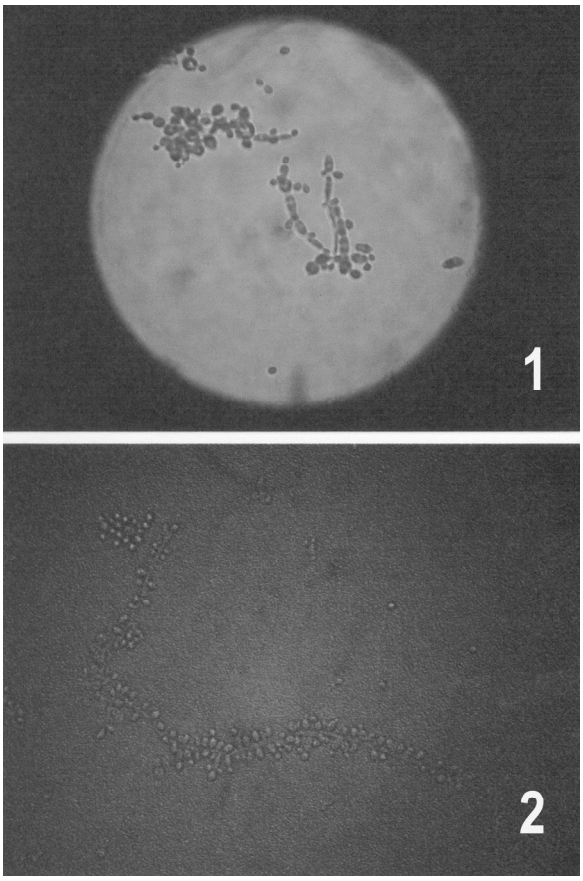

Figure 1. Vegetative cells of isolate 11 (100 X) in YEPX. **Figure 2.** Sporulating cells of isolate 11 (40 X).

placed by dextrose.Alternatively, it contained both the sugars in a ratio of 3 : 4.

A loopful of 24 h old yeast isolate was transferred to 50 ml of the sterilized inoculum medium and incubated at 30 + 2^{0}C on a rotary shaker (100 rpm). After 18 h, 10 ml of the inoculum was transferred to 90 ml production medium. The inoculated production medium was incubated under stationary conditions at 30 ± 2 C.

While ethanol in the fermented broth was estimated according to the spectrophotometric method of Caputi et al. (12), the residual sugars as total reducing sugars were estimated by dinitrosalicyclic acid (DNS) method as described by Miller (13).

Results and Discussion

Isolation of Xylose-Utilizing Yeasts

Seventeen yeast isolates were retrieved from different ecological niches. While grapes were found ecologically the most suitable source of xylose-utilizing yeasts offering five isolates, the molasses was close second to follow, producing three isolates. Blackberry, banana and sugarcane bagasse containing soil sample produced two each. Orange, aonla and decomposing wheat straw containing soil sample produced one each. Interestingly, grapes which contain glucose and fructose but no xylose at all produced larger number of xylose- utilizing yeasts than sugarcane bagasse and wheat straw where xylose represent 60 and 58% respectively, of the total sug-

ars. It sounds yet more interesting that no isolate was retrieved from corn cob (65% xylose), another rich source of xylose.

Screening of Yeast Isolates for Ethanol Production on Xylose-Containing Production Medium (XPM)

All the isolated xylose-utilizing yeasts were screened for ethanol production over a period of 72 on XPM containing xylose @ 10%. While each of the isolates 3, 4, 5, 11,16 and 17 produced 2% (vol/vol) ethanol, isolates 1, 2 and 7 produced 1.5% and isolate 12 produced only 1%. The remaining seven, although could not even produce a trace of ethanol, yet continued to utilize the carbon source xylose, as was evident from the residual values of sugars in the fermenting media (Table 1). May be xylose being utilized in these yeasts is being diverted to xylitol (14).

Although we did not quantify the amount of xylitol produced, we presume that xylitol production by these ethanologenic isolates may be responsible for low ethanol yields. Maximum ethanol concentration was achieved at 48 h as has been observed by Du Preeze et al. (15, 16). There have been instances where maximum ethanol concentration was achieved at 72 h or later (17, 18).

Performance of Selected Ethanologenic Yeast Isolates for Ethanol Production on Dextrose-Containing Production Medium (DPM)

All the ethanologenic yeast isolates (1, 2, 3, 4, 5, 7, 11, 12, 16 and 17) which produced ethanol on xylose were screened for ethanol production on DPM. It was observed that isolates 1, 3, 4, 5, 11 and 17 produced 5% (vol/vol) ethanol whereas 12, 7, 16 and 2 produced between 3—4.1% (vol/vol) ethanol at the end of 72 h (Table 2).

The reason behind the better ethanol production by the same strains on glucose rather than xylose may be because glucose produces three folds increase in the specific activity of the plasma membrane ATPase in comparison to the activity measured with xylose as the carbon source which constitutes a key mechanism to counterbalance deleterious effects of ethanol and ultimately increases the ethanol tolerance of the strain (19, 20).

Ethanologenic Performance of Selected Yeasts on Dextrose and Xylose-containing Medium (DXPM)

The six ethanologenic yeast isolates (1, 3, 4, 5, 11 and 17), each producing 5% (vol/vol) ethanol on dextrose, were screened for ethanol production on DXPM over a period of 72 h.

It was observed that isolate 11 produced the maximum ethanol (4.5%) from mixed carbon source followed by others which produced ethanol between 3.5%—3.8% (Table 3).

In this case glucose will be the preferred substrate and it is consumed before initiation of xylose fermentation. This may be due to the repression of xylose uptake by glucose (21, 22).

Optimization of Isolate 11 for Ethanol Production using Various Concentrations of DPM / DXPM

The isolate 11 was examined for ethanol production on media containing either dextrose or both dextrose and xylose at 12 and 15% sugar level. In the latter, the two sugars were always in a ratio of 4 parts dextrose and 3 parts xylose.

In the fermentation medium containing dextrose, a maximum of 5.5% (vol/vol) ethanol was achieved at an initial sugar concentration of 12% at 72 h (23). Similar results were obtained at an initial sugar concentration of 15%.

Similarly, on the medium containing both dextrose and xylose, a maximum of 4.7% (vol/vol) ethanol was obtained at an initial sugar concentration of 12% at 72 h, which did not increase further as the initial sugar concentration was increased to 15% (Table 4).

Conclusion

It is concluded that among the seventeen xylose-utilizing isolates, isolate 11 gave better results for the conversion of sugars consisting of mixtures of dextrose and xylose (4 : 3 ratio) or xylose or dextrose alone, to ethanol. Isolate 11 has been identified as *candida* spp. (Figs 1 and Fig. 2).

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