SELECTION OF YEASTS FROM LOCAL DAIRY PRODUCTS FOR THE PRODUCTION OF GLUCOAMYLASE

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Abstract: Yeasts are eukaryotic unicellular chemoorganotrophic organisms. They are common in the environment and can be isolated from various sources like dairy products (curd, lassi and buttermilk), sugar rich materials (skin of fruits), and soil. They are able to produce variety of industrially important enzymes like glucoamylase, invertase, protease, lipase, lactase, zymase, melibiase, endo-tryptase etc. Glucoamylase is an exo-acting enzyme that yields β-D-glucose from the non-reducing chain-ends of amylose, amylopectin and glycogen by hydrolysing α-1, 4 linkages in a consecutive manner. Its main application is on the production of syrups with 96-98 % of glucose. The hydrolysates can either be used as carbon source in fermentations or can be part of the final product, such as in soft drinks, ice-creams, sauces, tinned fruits, breads, etc. About 63 yeasts were isolated from dairy products like curd, lassi and buttermilk obtained from local dairy vendors of various locations of Gujarat, India. These yeasts were grown and maintained on YPD broth and YPD agar medium respectively containing 2% starch. These yeasts were screened for production of enzyme glucoamylase by submerged fermentation in the same nutrient medium. Activity of glucoamylase was measured in units by estimation of product glucose formed by utilization of substrate starch. Glucose was estimated by using DNS method. Yeasts isolated from samples of Bharuch (HYE54) and Dakor (HYE14) were found to be having good potency for production of glucoamylase amongst all the isolates. The amount of glucoamylase produced by HYE54 and HYE14 was 1.23 and 0.93 units respectively..

Key Words: Yeast, Fermentation, Glucoamylase.

Introduction:

Yeasts are eukaryotic unicellular chemoorganotrophic organisms. Some species of yeast may become multicellular through the formation of strings of connected budding cells known as pseudohyphae, or false hyphae, as seen in most molds. [1] They are common in the environment and can be isolated from various sources like dairy products, [2] sugar rich materials like fruit pulp, [3] soil [4] and in some cases in association with insects. [5] Yeasts had found their application in various fields, like in making of alcoholic as well as non-alcoholic beverages, baking, bioremediation, nutritional supplements and probiotics. [6][7][8] Using yeast as part of bioremediation process has also been reported. *Saccharomyce cerevisiae* can be used in order to remove BOD from cheese whey. During removal of BOD, production of single cell protein can also be carried out. [6]

Glucoamylase is a hydrolysing enzyme. It can degrade both amylose and amylopectin by hydrolysing both α-1, 4 and α-1, 6 glycosidic links of starch and produce glucose. [9][10] Glucoamylase [(1, 4)-α-D-glucan glucohydrolase, amyloglucosidase, EC 3.2.1.3 is an exo-acting enzyme. It yields β-D-glucose from the non-reducing chain-ends of amylose, amylopectin and glycogen by hydrolysing α-1, 4 linkages

in a consecutive manner. [11] The α-1, 6 glycosidic bonds are also hydrolysed, but at a much reduced rate. Hence, Glucoamylase can convert starch completely to glucose. Glucoamylase also finds potential applications in a number of industrial processes such as in food, fermentation, textiles and paper industries. [12] Microbial Glucoamylase have successfully replaced the chemical hydrolysis of starch in starch-processing industries. Glucoamylase is used mainly in the production of glucose syrup, high fructose corn syrup and in whole grain and starch hydrolysis for alcohol production. It could be potentially useful in the pharmaceuticals and fine chemicals industries provided that enzymes with suitable properties can be prepared.

Materials & Methods:

Sample collection, Isolation and screening of yeasts:

Since the aim was to get non-pathogenic strain of yeast, various dairy products were chosen for isolation of yeasts. The samples included curd, buttermilk and lassi. More than 30 samples of dairy products were collected from local dairy vendors and some traditional home made curd samples for the purpose of isolation of yeasts from various places of Gujarat, India. Curd, Lassi and Buttermilk were sampled in sterile container and were used for isolation of yeasts.

Isolation of yeasts was carried out by streaking samples on Yeast extract-Peptone-Dextrose agar (YPD) medium. Same medium was also reported to be used by Lamers *et al.* [13] for cultivation of yeasts in recent time. The plates were incubated at 30˚C for 24-48 hours. After incubation the colonies found were studied macroscopically as well as microscopically.

The screening step primarily was performed by growing the yeasts on YPD agar plates containing 2% (w/v) soluble starch. The plates were incubated at 30℃ for 48-72 hours. After incubation they were flooded with Lugol's iodine reagent (0.2% iodine - 0.4% KI solution) [14] for its chromogenic reaction with starch producing characteristic blue-purple colour. The plates were observed for the presence of clear zone of starch utilization surrounding the colony.

Screening of glucoamylase producing yeasts:

Since starch is one of the substrates for amylolytic enzymes, it was used as part of screening medium. The yeasts were grown under submerged condition in YPD broth at 30˚C on shaker with 150 rpm overnight. On second day, the grown yeast were centrifuged at 10000 rpm for 10 minutes and the biomass was re-suspended in sterile activation medium containing (g/L) yeast extract 10.0, peptone 20.0 and starch 5.0 and incubated overnight under same conditions to induce production of glucoamylase. After overnight induction, the biomass was again separated aseptically by centrifugation and resuspended in YPS medium containing (g/L) yeast extract 10.0, peptone 20.0 and starch 20.0 till its OD_{600} reaches to 1. This inoculum was used in proportion of 5% to inoculate 100ml production medium (YPS) in 250ml capacity flask. The flasks were incubated on shaker at 30˚C for 48-72 hours. After incubation, sample was aseptically withdrawn from the flasks and centrifuged to remove biomass. The supernatant was used as crude enzyme for performing glucoamylase assay.

Glucoamylase assay:

Glucoamylase assay was performed using 1% soluble starch as substrate prepared in 50mM sodium acetate buffer. Same buffer was used for dilution of crude enzyme wherever required. The amount of glucose released after utilization of substrate starch by glucoamylase was measured using DNS (3, 5-Dinitrosalicylic acid) method. [15] One glucoamylase unit (U) can be defined as the amount of enzyme producing 1 µmole of glucose per minute from soluble starch under the specified conditions.

Result and Discussion:

From different samples of dairy products about 63 yeasts were isolated. The details of yeasts, their sources and sampling places are presented in Table 1. The yeasts produced characteristic colonies

(Figure 1) with medium to large size. The colonies were raised visibly with creamy white appearance. Some of the yeasts appeared little creamy pink in appearance. All of the yeasts were found to be Gram positive since they were observed in violet colour during microscopic study after Gram staining (Figure 2). All the yeasts were able to grow on YPD agar medium supplemented with 2% starch but only those carrying amylolytic activity were able to utilize starch in the medium, which was observed by zone of starch utilization surrounding the colonies after addition of iodine reagent (Figure 3). After observing the results obtained in YPD agar plate with starch, about 12 yeasts were selected for further studies on production of glucoamylase.

On performing enzyme assay, it was found that all the yeasts were able to produce glucoamylase in different amounts. In enzyme assay, the amount of glucose liberated represents the amount of enzyme that acts on non-reducing ends of starch. Maximum amount of glucose was measured in enzyme assay by HYE54. The unit activity of glucoamylase produced by HYE54 was found to be 1.23 units. Yeast HYE14 also displayed remarkable activity as 0.93 units. The remaining yeasts were having very poor amylolytic activity as compared to above two yeasts.

Table 1: Details of Yeast isolates

Sr	Name	Collected from	Location	Village/city	District	Gram reaction
1	HYE01	Curd	Khadiya	Ahmedabad	Ahmedabad	Positive
2	HYE02	Curd	Khadiya	Ahmedabad	Ahmedabad	Positive
3	HYE03	Curd	Amod	Amod	Bharuch	Positive
4	HYE04	Curd	Amod	Amod	Bharuch	Positive
5	HYE05	Curd	Boriyavi	Boriyavi	Anand	Positive
6	HYE06	Curd	Boriyavi	Boriyavi	Anand	Positive
7	HYE07	Curd	CHikhodara	Chikhodara	Vadodara	Positive
8	HYE08	Curd	CHikhodara	Chikhodara	Vadodara	Positive
9	HYE09	Curd	Dabhoi	Dabhoi	Vadodara	Positive
10	HYE10	Curd	Dabhoi	Dabhoi	Vadodara	Positive
11	HYE11	Curd	Dabhoi	Dabhoi	Vadodara	Positive
12	HYE12	Curd	Dandiabazar	Vadodara	Vadodara	Positive
13	HYE13	Curd	Dandiabazar	Vadodara	Vadodara	Positive
14	HYE14	Curd	Dakor	Dakor	Kheda	Positive
15	HYE15	Curd	Dakor	Dakor	Kheda	Positive
16	HYE16	Curd	Dakor	Dakor	Kheda	Positive
17	HYE17	Curd	Dakor	Dakor	Kheda	Positive
18	HYE18	Curd	Deesa	Deesa	Banaskantha	Positive
19	HYE19	Curd	Deesa	Deesa	Banaskantha	Positive
20	HYE20	Curd	Anand	Anand	Anand	Positive
21	HYE21	Curd	Anand	Anand	Anand	Positive
22	HYE22	Curd	Gotri	Vadodara	Vadodara	Positive
23	HYE23	Curd	Khambholaj	Khambholaj	Anand	Positive
24	HYE24	Lassi	Madhvi Foods	Mehsana	Mehsana	Positive
25	HYE25	Lassi	Madhvi Foods	Mehsana	Mehsana	Positive
26	HYE26	Lassi	Madhvi Foods	Mehsana	Mehsana	Positive
27	HYE27	Curd	Anand	Anand	Anand	Positive
28	HYE28	Curd	Navli	Anand	Anand	Positive
29	HYE29	Curd	Jhagadiya	Bharuch	Bharuch	Positive
30	HYE30	Curd	Jhagadiya	Bharuch	Bharuch	Positive
31	HYE31	Curd	Jamnagar	Jamnagar	Jamnagar	Positive

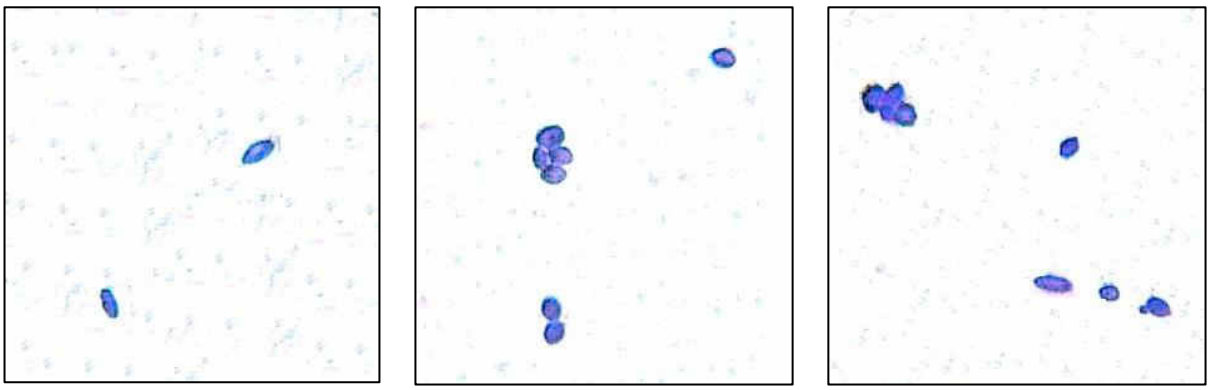
32	HYE32	Curd	Jamnagar	Jamnagar	Jamnagar	Positive
33	HYE33	Curd	Jamnagar	Jamnagar	Jamnagar	Positive
34	HYE34	Curd	Kamla	Kamla	Kheda	Positive
35	HYE35	Lassi	Madhvi Foods	Mehsana	Mehsana	Positive
36	HYE36	Curd	Mehsana	Mehsana	Mehsana	Positive
37	HYE37	Curd	Mehsana	Mehsana	Mehsana	Positive
38	HYE38	Curd	Mehsana	Mehsana	Mehsana	Positive
39	HYE39	Curd	Nandesari	Nandesari	Vadodara	Positive
40	HYE40	Curd	Nandesari	Nandesari	Vadodara	Positive
41	HYE41	Curd	Nadiad	Nadiad	Kheda	Positive
42	HYE42	Curd	Anand	Anand	Anand	Positive
43	HYE43	Curd	Navabazar	Vadodara	Vadodara	Positive
44	HYE44	Curd	Navabazar	Vadodara	Vadodara	Positive
45	HYE45	Curd	Vadodara	Vadodara	Vadodara	Positive
46	HYE46	Curd	Vadodara	Vadodara	Vadodara	Positive
47	HYE47	Curd	Vadodara	Vadodara	Vadodara	Positive
48	HYE48	Curd	Vadodara	Vadodara	Vadodara	Positive
49	HYE49	Curd	Rarod	Rarod	Vadodara	Positive
50	HYE50	Curd	Rarod	Rarod	Vadodara	Positive
51	HYE51	Curd	Anand	Anand	Anand	Positive
52	HYE52	Curd	Motakarala	Motakarala	Vadodara	Positive
53	HYE53	Curd	Bharuch	Bharuch	Bharuch	Positive
54	HYE54	Curd	Bharuch	Bharuch	Bharuch	Positive
55	HYE55	Curd	Thasra	Thasra	Kheda	Positive
56	HYE56	Curd	Thasra	Thasra	Kheda	Positive
57	HYE57	Curd	Thasra	Thasra	Kheda	Positive
58	HYE58	Buttermilk	Una	Una	Gir somnath	Positive
59	HYE59	Curd	Unava	Unava	Mehsana	Positive
60	HYE60	Curd	Unava	Unava	Mehsana	Positive
61	HYE61	Curd	Visnagar	Visnagar	Mehsana	Positive
62	HYE62	Curd	Visnagar	Visnagar	Mehsana	Positive
63	HYE63	Curd	Visnagar	Visnagar	Mehsana	Positive

Figure 1: Macroscopic characterization of yeasts on YPD agar.

HYE18

HYE63

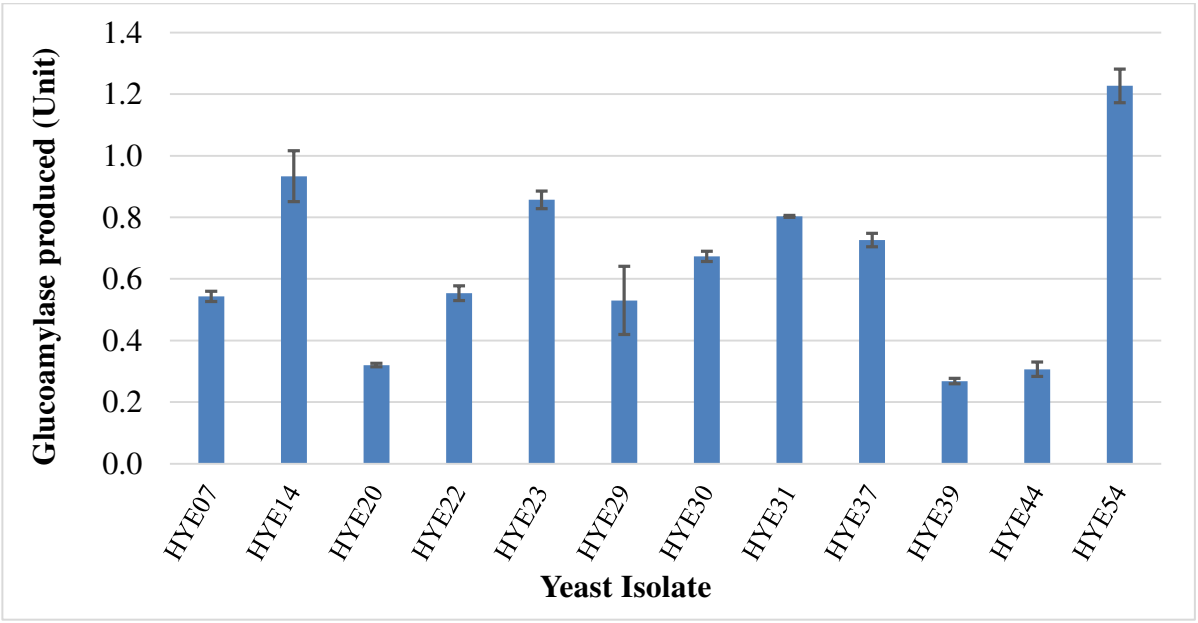
Figure 2: Microscopic characterization of yeasts after Gram staining.



HYE17 **HYE58** **HYE63**

Figure 3: Utilization of starch by yeast HYE54 in YPD medium containing starch.

Figure 4: Production of Glucoamylase from yeast isolates.



Conclusion:

After isolating and screening of 63 different yeast isolates from about 30 different samples of dairy products, it was found that about 12 yeasts were able to produce glucoamylase. So, about only 19% yeasts from the isolated yeasts were able to hydrolyse starch while others were not amylolytic. Amongst all of these isolates HYE54 and HYE14 were found to be good producers for glucoamylase that

produced 1.23 and 0.93 units of glucoamylase respectively. These yeasts can be further studied for production of glucoamylase and the production of glucoamylase can be enhanced by optimizing different parameters like pH and temperature required for production as well as utilizing different substrates too.

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