Original Research Article

FAST SCREENING METHOD FOR LYSINE-PRODUCING YEASTS

ABSTRACT

Aim: To screen for active Lysine -producing yeasts

Study Design: Examination of different kinds of fruits

Place and Duration of Study: Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, from January, 2014 and July, 2015

Methodology: Yeast isolates (100) recovered from different fruits were screened for lysine producers on solid agar medium. Halo growth of the lysine auxotroph, *Escherichia coli*, seeded in the agar medium indicate lysine production by the yeast isolate. Lysine accumulation in submerged medium by the isolate was examined.

Results: Five of the yeast isolates observed to be active lysine producers accumulated lysine yields of 0.20µg/ml to 0.90µg/ml in submerged medium. The lysine level accumulated in the broth culture of the yeast was observed to be proportional to the halo growth of the *Escherichia coli* on solid agar medium.

Conclusion: Yeasts are capable of producing lysine and a fast screening method for lysine producers detected.

Key words: Yeasts, Fruits, Lysine production, Halo growth, Screening method

INTRODUCTION

Yeast is a group of fungi in which unicellular form is predominant (1). As a group of microorganisms, yeasts have cosmopolitan distribution (2). They have been isolated from natural substrates like leaves, flowers, sweet fruits, grains, exudates of trees, insects, dung and soil (3).

The useful physiological properties of yeasts have led to their use in the field of biotechnology, fermentation of sugars by yeasts being the oldest and largest of this technology (4). Several investigations have been carried out in different natural and crop growing environment so as to obtain better knowledge of yeast diversity and to define the impact of this on food products (5).

L-Lysine is an essential amino acid mainly used as feed additive in the animal industry for such animals like broilers, poultry and swine (6, 7, 8, 9, 10) and as supplement for humans improving the feed quality by increasing the absorption of other amino acids (11).

Several microbiological organisms including fungi and bacteria are known to produce lysine (13, 14, 15). However, not much is known about yeasts and lysine production.

This study was, therefore, conducted to isolate lysine producing yeast using a fast screening method.

MATERIALS AND METHODS

Isolation of Yeasts

Forty fruit samples (e.g. apple, pineapple, banana, pawpaw, water melon, oranges) were collected randomly from different localities in Awka town, Anambra state. The fruit samples were cut and 10g sliced or mashed and homogenized in a sterile warring blender (Panasonic Mixer Blender) with 100ml of 0.85% sterile physiological saline. The homogenate was then placed in a 250ml Erlenmeyer flask and shaken for 10mins on a rotary shaker (120rpm) to release the yeast cells into the suspension. 1ml of the suspension was diluted tenfold and 0.1ml 10^{-2} spread inoculated on Sabouraud Dextrose Agar (SDA Oxoid) plates. After 24 to 48hr incubation at 27^0C, the isolates were recovered, purified and stored at 4^0C on SDA slants.

Screening of isolates for lysine production on solid agar medium

The isolates were screened for lysine production following a modified method described by Ozulu *et al*. (16). A 100ml minimal agar medium [Glucose, 4.0g; $(NH_4)_2SO_4$, 2.0g; K_2HPO_4, 0.05g, KH_2PO_4, 0.05g; $MgSO_4.7H_20$, 0.1g; Fe $SO_4.7H_20$, 0.001g; $MnSO_4.H_20$, 0.001g; CaCO3, 2.0g; Agar, 15.0g, H_20, 1L, pH adjusted to 6.0 with 6N HCl] in a 250ml Erlenmeyer flask, was sterilized at 115^0C for 15mins, allowed to cool at 40^0C and then aseptically seeded with 2ml of a 24h broth culture of a lysine auxotroph, *Escherichia coli*. The molten agar medium was poured into Petri plates, allowed to solidify and then spread inoculated with the isolates. Uninoculated plates were kept as control. The plates were observed for halo growth of the *E.coli* auxotroph after 96h incubation at 30^0C, which is indicative of lysine

production by the isolates. Further studies were carried out on five of the suspected active lysine producers.

Lysine accumulation by the isolates in submerged medium.

Seed inoculum: the medium for seed culture [peptone, 10.0g; yeast extract, 10.0g; NaCl, 5.0g, H_20 1L; pH adjusted to 6.0 with 6N HCL] was sterilized at 121^0C for 15min. Two loopful of the isolate was inoculated into a 100ml Erlenmeyer flask containing 20ml of the seed medium. The flask was incubated for 18h on a rotary shaker (120rpm) at 30^0C.

Shake Flask Fermentation

Lysine production by the isolates in submerged medium was investigated following the method described by Ekwealor and Obeta (15). Fermentation medium [Glucose, 20.0g; $(NH_4)_2SO_4$, 10.0g; K_2HPO_4, 0.02g; KH_2PO_4, 1.0g; $MgSO_4.7H_20$, 0.4g; $FeSO_4.7H_20$, 0.002g, $MnSO_4.7H_20$, 0.4g; $FeSO_4.7H_20$, 0.002g, $MnSO_4.4H_20$,, 0.002g; $CaCO_3$, 20.0g; Agar, 15.0g; water, 1L; pH adjusted to 6.0 with 6N HCl] was sterilized at 115^0C for 10min. A 2ml volume (10%V/V) of the seed culture was inoculated into 100ml Erlenmeyer flask containing 20ml of the fermentation medium. Duplicate flasks were prepared and uninoculated flasks served as control. After 72h incubation on a rotary shaker (140rpm) at 30^0C, lysine accumulation in the broth culture was determined.

Estimation of lysine in the broth culture

Lysine produced in the broth culture of the isolate was determined by acidic ninhydrin method of Chinard (17). A 5ml volume of the culture broth was centrifuged at 5,000Xg for 20min. To 1ml of the supernatant in a test tube was added 1ml of glacial acetic acid and 1ml of reagent solution (an acid mixture of 0.4ml of 6M orthophosphoric acid, 0.6ml of glacial acetic acid and 25mg of ninhydrin per ml of the acid mixture). The blank test tube contained 1ml of glacial acetic acid, 1ml of the reagent solution without ninhydrin and 1ml of the supernatant. Both tubes, the reacting mixture and the blank were capped, mixed properly and

heated to a temperature of 100^0C in a water bath for 1h. The tubes were cooled rapidly under tap water and the content of each tube brought to a final volume of 5ml with 2ml glacial acetic acid. The value of the reacting mixture was obtained from a spectrophotometer (VWR DS2 – 500 - 2) at 515nm. The lysine present in the supernatant was extrapolated from a standard lysine curve.

Results and Discussion

A total of 100 yeast isolates recovered from different kinds of fruits were screened for lysine production on solid agar medium seeded with lysine auxotroph, Escherichia coli. Halo growth of the *E.coli* auxotroph on the surface of the agar medium spread inoculated with the yeast isolate is an indication that the isolate produces lysine. This observation is similar to that reported by Ozulu *et al.*(16), in their search for methionine-producing bacteria. They noted that only bacterial isolates that released methionine into the agar medium stimulated halo growth of the *E.coli* auxotroph, seeded on the agar.

Five of the yeast isolates observed to be active lysine producers were studied for lysine production in submerged medium. Table 1 shows the lysine yields of the yeast isolates and their halo growths on solid agar medium.

Table 1. Lysine yields of the yeast isolates in submerged culture.

Isolate No	Halo growth of *E.coli*	Lysine (mg/ml)
MS1	+ + +	0.953
MS2	+ + +	0.842
WM	+ +	0.440
PN	+ +	0.516
MS3	+	0.200

It is important to observe (Table 1) that the lysine accumulated in the broth cultures of the yeasts were proportional to the halo growths of the *E.coli* on solid medium. The more the halo growth of the *E.coli* the

more likely the production of a high lysine yield by the yeast isolate. This observation is supported by the work of Ekwealor and Obeta (18), who noted a high lysine yield in submerged medium with increased halo growth of the bacterial isolate on the solid agar medium.

CONCLUSION

The experimental work shows that yeasts are a group of organisms capable of producing lysine, and a fast screening method for the isolation of lysine producers detected.

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