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Fast Screening Method for Detecting Lysine-producing Yeasts

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Authors' contributions

This work was carried out in collaboration between all authors. Author LOCM designed the study, managed the experimental process and wrote the protocol. Author IAE supervised the research and wrote the first draft of the manuscript. Author TCA assisted with analysis of the study and the spectroscopy analysis. Author C. C. Ekwealor managed the literature searches. Author C. Chibor-Ekweanya assisted with yeast sources. All authors read and approved the final manuscript.

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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

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of 0.20 µg/ml to 0.90 µg/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 capable of producing lysine were isolated from fruits and a fast screening method for their detection on solid agar medium have been identified.

Keywords: Yeasts; fruits; lysine production; halo growth; screening method.

1. 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 [6-10] 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 [12-15]. 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.

2. MATERIALS AND METHODS

2.1 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 10 g sliced or mashed and homogenized in sterile water using a warring blender (Panasonic Mixer Blender) with 100 ml of 0.85% sterile physiological saline. The homogenate was then placed in a 250 ml Erlenmeyer flask and the flask shaken for 10min on a rotary shaker (120 rpm) to release the yeast cells into the suspension. The suspension was submitted to rest and 1 ml of the non-sedimented portion diluted tenfold. 0.1 ml of 10^{-2} dilution was spread inoculated on Sabouraud Dextrose Agar (SDA Oxoid) plate containing 0.05 mg/ml chloramphenicol, to prevent bacterial growth. After 24 to 48 hr incubation at 27℃, the isolates were recovered, purified and stored at 4℃ on SDA slants.

2.2 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 minimal agar medium [Glucose, 4.0 g; $(NH_4)_2SO_4$, 2.0 g; K_2HPO_4, 0.05 g, KH_2PO_4, 0.05 g; $MgSO_4.7H_20$, 0.1 g; Fe $SO_4.7H_20$, 0.001 g; $MnSO_4.H_20$, 0.001 g; CaCO3, 2.0 g; Agar, 15.0 g, H_20, 1L, pH adjusted to 6.0 with 6N HCl] in a 250 ml Erlenmeyer flask, was sterilized at 115℃ for 15mins, allowed to cool to 40℃ and then aseptically seeded with 2 ml (ca. 5.8 x 10^8 cells/ml) of a 24 h 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 96 h incubation at 30℃, which is indicative of lysine production by the isolates. Further studies were carried out on the suspected active lysine producers.

2.3 Lysine Accumulation by the Isolates in Submerged Medium

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

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

2.5 Assay 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 5 ml volume of the culture broth was centrifuged at 5,000Xg for 20 min. To 1 ml of the supernatant in a test tube was added 1 ml of glacial acetic acid and 1 ml of reagent solution (an acid mixture of 0.4 ml of 6M orthophosphoric acid, 0.6 ml of glacial acetic acid and 25 mg of ninhydrin per ml of the acid mixture). The blank test tube contained 1ml of glacial acetic acid, 1 ml of the reagent solution without ninhydrin and 1 ml of the supernatant. Both tubes, the reacting mixture and the blank were capped, mixed properly and heated to a temperature of 100℃ in a water bath for 1 h. The tubes were cooled rapidly under tap water and the content of each tube brought to a final volume of 5 ml with 2 ml glacial acetic acid. The value of the reacting mixture was obtained from a spectrophotometer (VWR DS2 – 500 - 2) at 515 nm. The lysine present in the supernatant was extrapolated from a standard lysine curve.

3. 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	Yeast source	Halo growth of *E. coli*	Lysine (mg/ml)
MS1	Orange	+ + +	0.953
MS2	Grapes	+ + +	0.842
WM	Water melon	+ +	0.440
PN	Pineapple	+ +	0.516
MS3	Grapes	+	0.200

It is important to observe (Table 1) that the lysine accumulated in the broth cultures of the yeasts was 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.

4. CONCLUSION

The experimental work shows that yeasts capable of producing lysine can be isolated from fruits, and a fast screening method for their detection on solid agar medium identified. Lysine produced in the broth culture of the yeast was observed to be proportional to the halo growth of the *E. coli* auxotroph on solid medium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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