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Sustainable Producing of Oxalic Acid from *Aspergillus niger* and *Candida albicans* Isolated from Environmental and Clinical Sources

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ABSTRACT

Oxalic acid is one of the important acids that is used in many fields. It is of medical, industrial and agricultural importance and is used as an acid in foods, building and construction, pharmacy, and others. This acid is produced in multiple ways, and the biogenic method is the best method because it is safe and cheap. Hence, this study came about, where reliance was placed on wheat bran in preparing nutrient medium to produce oxalic acid from *Candida albicans* and *Aspergillus niger* yeast isolated from environmental and pathogenic sources. The study aimed to use wheat bran as an alternative medium for growing fungi and yeasts that produce oxalic acid, and to compare the productivity of this medium with standard media. One hundred fungal isolates were isolated from different environmental and clinical sources, and grown in Sabouraud dextrose agar medium (SDA) and potato dextrose agar to obtain pure isolates of *A. niger* and *C. albicans*. Thirty seven isolates of *C. albicans* and thirty two isolates of *A. niger* were isolated. Thirty one contaminated samples were discarded. The isolates were grown in standard (SDB, PDB) and alternative (wheat bran) media. The amount of acid was estimated by mulching against potassium permanganate. The results showed that wheat bran medium was the most efficient in producing oxalic acid with a rate of 26.2% for *A*. niger and 25.3% for *C. albicans*, compared to standard media. The best temperature for acid production was 32 °C with a production rate of 19.1% for *A. niger* and 22.7% for *C. albicans*. The best pH was 6.5 for *A. niger* with a production rate of 20.2% and 5.5 for *C. albicans* with a production rate of 23.3%. The study conclude from the above that the fungus *A. niger* is the best compared to *C. albicans*, as well as the medium of wheat bran is a promising and effective medium in the production of oxalic acid in an environmentally friendly way.

Keyword: biosynthesis, oxalic acid, wheat bran, *Candida albicans*, *Aspergillus niger.*

INTRODUCTION

Organic acids are chemical compounds that are indispensable in many aspects of life. They are important factors in the field of medicine, where they are important antimicrobial agents, as well as in the field of food preservation, the manufacture of cosmetics, dyes, and building materials, and as a support for plant growth (Gadd, 1999; Hodgkinson, 1977).

This acid is produced in multiple ways, the most important of which is the chemical method that occurs in factories, which requires energy, high heat, raw materials, and a specialized staff for the purpose of producing acid. This method is expensive and dangerous and does not guarantee obtaining a pure product. The other method is the biological method, which includes the use of a living organism that carries out the fermentation process and thus produces acid in a specific medium and special conditions that can be provided for it. It is a cheap and safe method, and we obtain pure acid from it, and its purity can be verified by scanning or by spectrophotometry (Kumar *et al*., 2024; Ahmed and Cruickshank, 1953; Schuler *et al*., 2021).

Aspergillus niger is a filamentous fungus that is found in all environments due to its great ability to adapt and imprint. It does not need special requirements. It grows easily when the minimum nutritional requirements are available. It is characterized by its genetic and phenotypic diversity and can produce a huge number of

reproductive units that work to make it dominant in the environment in which it lives. This fungus is distinguished by its high ability to produce primary and secondary metabolites, including enzymes, organic and amino acids, antibiotics, and others (Al-Mehana *et al*., 2021; Alsudani and Al-Shibli, 2015).

The situation applies to *Candida albicans*, as it is a widespread yeast in the environment on plants, fruits, milk, and cheese. It may also cause various disease conditions such as oral, vaginal, and skin candidiasis. Candida is known for its high fermentative enzymatic capacity, which makes it a target for researchers for the purpose of manufacturing many compounds, including organic acids (Al-Shibly *et al*., 2024; Sudbery, 2011).

Wheat bran is a good medium for the growth of fungi. The wheat grain consists of three main layers, including the bran, the endosperm, and the germ. The bran is the outer solid layer filled with important components. During the milling process, the bran is removed from the grain so that it becomes a by-product of the wheat flour production process. Wheat bran contains many fibers and other nutrients and also contains gluten protein, fruktan sugar, and phytic acid (Al-Shibly *et al*., 2024; Stevenson *et al*., 2012).

Agricultural waste plays a major role in environmental pollution because of the increase in agricultural production, which has led to an increase in the amount of waste. Plant waste is one of the most naturally occurring and renewable organic materials. It can be treated with materials to become ready for use in other fields, such as fish feeding (Algburi, and AL-Amari, 2023; Mohsin *et al*., 2024). as it is still present in large quantities in some areas (Wanzenböck *et al*., 2017). The agricultural waste using as eco-friendly solution for removing Cu^{2+} ions from industrial effluents, by production of activated carbon (Ghibate *et al*., 2024).

Wheat bran, a cheap lignocellulosic biomass, is the primary by-product of wheat flour production, constituting approximately almost 16% of the weight of wheat grains. This makes wheat bran an ideal, abundant, and low-cost raw material for producing functional food ingredients and feed additives (Stevenson *et al*., 2012).

Wheat bran consists of approximately 13–18% protein, 12% water, 56% carbohydrates and 3.5% fate, dietary fiber about 55% arabinoxylan, lignin (3–5%), remaining fiber composed of cellulose (9–12%), fructan (3–4%), mixed-linked β-glucan (2.2–2.6%). Notably, around 95% of dietary fiber is insoluble (Onipe *et al.*, 2015).

Wheat bran possesses several essential nutrients. For every 100 grams, it contains approximately 333 calories, 3.33 grams of fat, 13.33 grams of calcium, 133 milligrams of protein, 66.67 grams of carbohydrates, 40 grams of dietary fiber, 9.6 milligrams of iron, 0.567 milligrams of riboflavin, and 13.33 milligrams of niacin (Chen *et al*., 2023).

Aim of study

The goal of the current study is to find a medium that is cheap and highly efficient in producing oxalic acid in a biological way compared to common mediums that are expensive and difficult to prepare. This medium is wheat bran, which is a by-product of the flour-making process.

MATERIALS AND METHODS

Isolation, ptrification and identification of Isolated Fungi

One hundred samples were collected from various sources (environmental samples including soil, water, air, fruits, vegetables, bread molds, and milk, as well as clinical samples from different fungal infections of the skin, eyes, middle ear, head, legs, nails, hair, mouth, vaginal infections in pregnant women, nose, respiratory tract, and saliva). These samples were cultured on SDA medium, and growth was observed on Petri dishes during the isolation and diagnosis period to obtain fungal isolates represented by the two species (*A. niger* and *C. albicans*). Identification was based on morphological features such as shape and color, as well as microscopic features such as the shape, size, and color of spores and other structures using classification keys provided in sources that addressed the classification and study of fungi in the current study (Watanabe, 2002; Frey *et al*., 1979).

After obtaining them and diagnosing them visually, the isolates were cultured in plates to obtain pure isolates. (Yakop *et al*., 2019; Gautam and Avasthi, 2019). The isolates were seduced to select the isolates most capable of production, and only 30 isolates were settled, the highest in acid enforcement, including 15 isolates of *Aspergillus niger* and 15 isolates of *Candida albicans*.

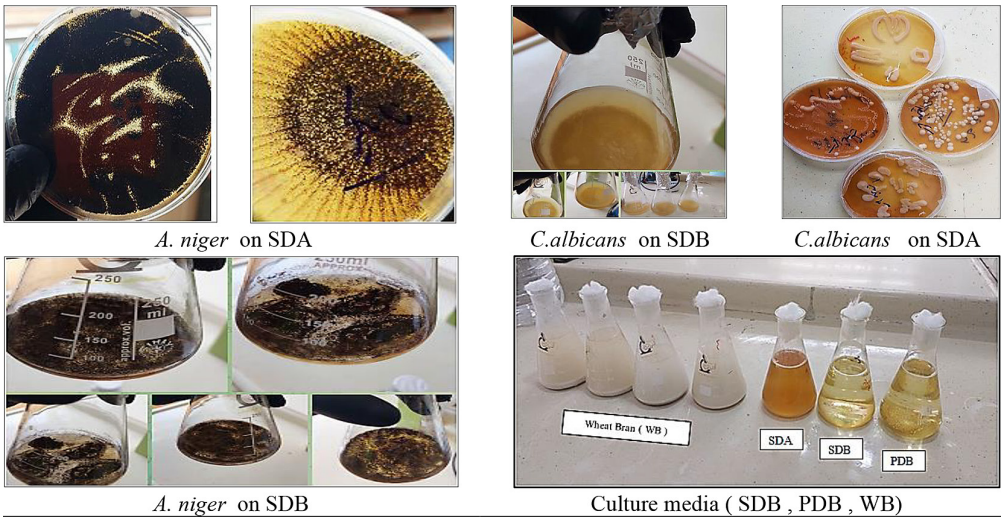

Figure 1. Growth of fungal colonies on media

Preparation of culture media

Standard culture media were prepared as shown in Figure 1 according to the instructions of the manufacturers of each medium, considering the conditions and influencing factors such as temperature, acidity, and sterilization during preparation. The standard media used in the study are Sabouraud dextrose agar (SDA), potato dextrose agar (PDA), sabouraud dextrose broth (SDB) and potato dextrose broth (PDB) (Brown, 1923; Crowther *et al.*, 2018).

Wheat bran culture media

The wheat bran (WB) liquid medium was prepared as for the alternative growing medium to produce oxalic acid from fungi according to the supervisor's instructions, Table 1 appear the percent of content wheat bran culture media (Al-Shibly *et al*., 2024).

Table 1. Components for preparing wheat bran medium

No.	Substance	Weight, volume
1.	Wheat bran	100 grams
2.	Distilled water	1 liter
3.	Ammonium sulfate	5 grams
4.	Magnesium sulfate	5 grams
5.	Sodium chloride	1 gram
6.	Iron sulfate	1 gram
7.	Manganese sulfate	1 gram

Steps for preparation and use (Irfan et al., 2012)

1. Washing wheat bran – thoroughly wash WB with distilled water to remove any impurities.
2. Preparation of metal solution – in a separate container, dissolve ammonium sulfate, magnesium sulfate, sodium chloride, iron sulfate, manganese sulfate, and zinc sulfate in distilled water. Ensure that all salts are completely dissolved before proceeding.
3. Preparation of liquid wheat bran medium – in a large container, mix the washed WB with the prepared metal solution. Ensure thorough mixing to obtain a homogeneous mixture.
4. pH adjustment – measure the pH of the wheat bran medium using a pH meter. The pH of the medium should be around 5.5.
5. Sterilization – sterilize the liquid wheat bran medium by placing it in a tightly sealed container and autoclaving it at 121 °C for 15 minutes.
6. Cooling – after sterilization, allow the liquid WB medium to cool to room temperature.
7. Storage – the liquid medium WB can be stored in the refrigerator for up to two weeks, then it should be thoroughly shaken before use.

Estimation of the oxalic acid production

The amount of oxalic acid was estimated after the growth of fungi on the liquid media mentioned in the previous paragraph, and then the cultures were nominated by the filter papers to obtain a clean and pure filtrate, then the titration was done against potassium permanganate until the color changed to pink and the color stabilized,

Figure 2. Filtration, purification and calibration

then the acid percentage was calculated according to the equation below.

The percentage of oxalic acid was determined according to the method described by Al-Rikabi and AL-Shibli (2022), via titration until a pink or pinkish color appeared, as illustrated in Figure 2. The percentage of the acid was calculated because every 1 ml of potassium permanganate (0.02 N) is equivalent to 1.2653 mg of oxalic acid, according to the Equation 1:

$$Percentage\ (OA) = Volume\ KMnO_4 \times 1.2653 \quad (1)$$

RESULTS AND DISCUSSION

Isolation and identification

Thirty pure fungal isolates were obtained for the fungi *C. albicans* (15 isolates) and *A. niger* (15 isolates), other isolates were excluded because of contamination or weak production of oxalic acid. The isolates were screened for oxalic acid production to determine and select the most efficient fungal isolate in acid production. Additionally, the influencing factors in production, such as temperature and pH value, were determined. These isolates were activated on Petri dishes containing SDA medium. The percent's of fungal isolates show in Figure 3.

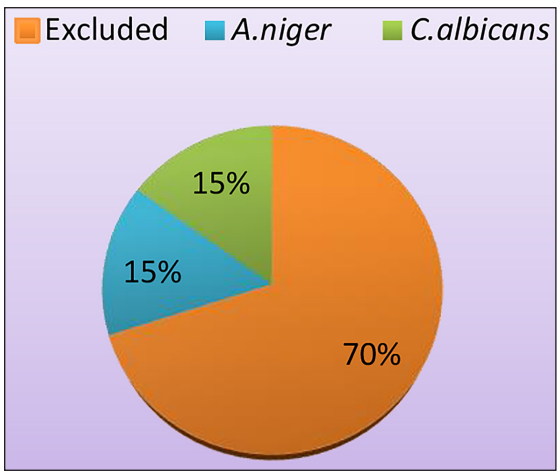

Figure 3. Percentage of Fungal isolates

The two fungi used in the study of fungi widespread has isolated from the soil and milk, fruits and vegetables and from the air, water and foods, as well as isolated from various pathological conditions of the skin, mouth, vagina, hair, urine and others and these fungi have the ability to adapt and resist difficult conditions due to the ability to change in the external form and in the genetic structure according to the surrounding conditions and have the ability also to produce enzymes, toxins, antibiotics and other metabolites that make them more compatible and more able to growth and reproduction (Pauwels *et al*, 2023; Denning, 2024).

Oxalic acid production

Through current study, noted a difference in the ability of the studied fungi to produce oxalic acid, and the best fungi produced are *Aspergillus niger*, especially on the medium, wheat bran 26.2%, followed by the Sabouraud dextrose agar by 21.5%, and the medium potato dextrose broth came in the last order 3.7%. As for as *Candida albicans* were 25.3, 20.6 and 8.8% respectively. Figure 4 show that percentage.

These results are consistent with what the sources said that wheat bran is a very rich food containing sugars, proteins, salts and elements supporting growth and production, as it surpassed many common food media in the production of organic acids, on the other hand, the two fungi used in the study are able to ferment the nutrient medium by the large enzymatic arsenal, which gives different metabolic products, including organic acids (Al-Mehana *et al*., 2021).

This aligns with what was mentioned by (Al-Shibly *et al*., 2024) when using this medium as an

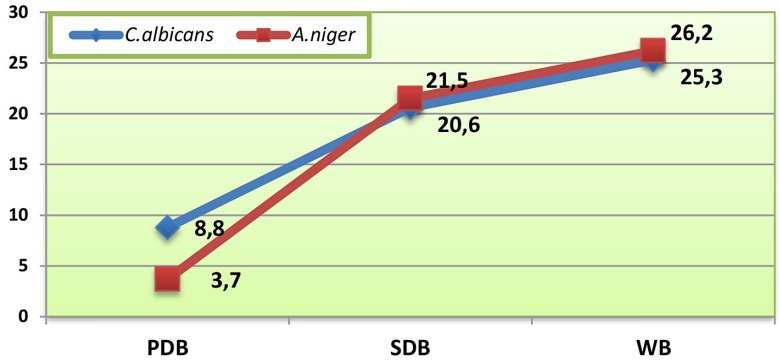

Figure 4. Acid production estimation of oxalic acid percentage in study sample

alternative to standard media for cultivating fungal isolates producing oxalic acid.

The genus *Aspergillus* is known for its prolific production of oxalic acid, attributed to its rapid growth and adaptability to diverse and harsh environments. This genus thrives without complex nutritional requirements, owing to its metabolic system's capacity to generate a wide array of enzymes, particularly hydrolytic ones. *Aspergillus* produces numerous spores that disperse through air currents, germinating upon contact with moisture to form fungal colonies that produce various metabolites, including oxalic acid. The variability in oxalic acid production among species is genetically determined, with higher gene expression correlating with increased production (Huang *et al*., 2023). As for the results regarding white yeasts, they were similar to black molds in terms of efficiency in acid production and ranked similarly for the media, with the alternative medium WB being the best in fungal growth and increasing their productivity for oxalic acid.

CONCLUSIONS

The current study showed the great ability of both *Aspergillus niger* and *Candida albicans* to produce oxalic acid, but the fungus *Aspergillus niger* was the most capable of production, knowing that the isolates of the same fungi also vary among themselves in the ability to produce due to the differences of the scene, environmental and genetic. Using this medium on a commercial level is recommended.

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