Journal of Applied & Environmental Microbiology, 2019, Vol. 7, No. 1, 20-24
Available online at http://pubs.sciepub.com/jaem/7/1/4
Published by Science and Education Publishing
DOI:10.12691/jaem-7-1-4

Bread Fungi: Phytochemical Constituents and Antimicrobial Activity of Isolates

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Received March 17, 2019; Revised April 21, 2019; Accepted May 02, 2019

Abstract Bread is a cereal product which provides essential nutrients to the body. Several fungi have been associated with bread spoilage yet bread fungi may offer some benefits. The studied aimed to assess the phytochemicals present in bread fungi and the antimicrobial effects of the fungal isolates. The fungi were isolated using the pour plate technique with Potato dextrose agar and Tryptone soya agar as the growth medium. The fungi were characterized and identified based on their morphological characteristics. The fungi, *Aspergillus spp*, *Rhizopus spp, Fuasrium spp, Mucor spp, Alternaria spp, Tricothecium spp, Sporendonema spp Trichothecium spp* and *Clasdosporium spp*. occurred most frequently in the bread sample. The fungal isolates contained phytochemicals such as alkaloids, tanins, saponins, phenolics, flavonoids and steroids, which reflects its ability to produce bioactive compounds which could serve both environmental and medicinal purposes. The trends of zone of bacteria inhibition for E,coli was *penicillium* spp< *Aspergillus spp*< *Fuasrium spp*< *Rhizopus spp, for staphyloccus was penicillum spp*< *Fuasrium spp* <*Aspergillus spp etc.*

Keywords: *antimicrobial activity, bread, fungi, phytochemicals*

Cite This Article: Mpamah Ihuoma Chaste, Kanu Chidozie, Kanu Kingsley, Uchendu Udochukwu, and Ochor Nnenne, "Bread Fungi: Phytochemical Constituents and Antimicrobial Activity of Isolates." *Journal of Applied & Environmental Microbiology*, vol. 7, no. 1 (2019): 20-24. doi: 10.12691/jaem-7-1-4.

1. Introduction

Bakery products and cereals are a valuable source of nutrients in our diet, providing us with much of our food calories and protein requirements. A limiting factor to the shelf-life of bread is mould spoilage. It is also important to note that the moulds that grow on food could have phytochemical constituents and a broad zone of inhibition against certain bacterial pathogens.

Phytochemicals are chemicals produced by plants. Although, the term could be generally used to describe chemicals from plants or other organisms that could enhance health status of organisms [1].

Reference [2] reported that various phytochemical studies have shown that fungi isolates contain bioactive constituents such as tannins, flavonoids, alkaloids, and saponins.

Microorganisms grow in different habitats, which gives them the capability to produce unique metabolites. Generally, the production of these metabolites may act as chemical defense as an adaptation of fungi competing for habitat [3]. Reference [4] noted that a lot of work have been done in ascertaining the antimicrobial properties of secondary metabolites derived from various groups of fungi. Antimicrobial activities of an endophytic *Aspergillus* spp. against some clinically significant human pathogens have been noted by various researchers [5], [6,7,8]. Bioactive natural compounds isolated from fungal endophytes have been significant in the search for new drugs and becoming an inspiration for researchers due to their structural diversity and complexity. Studies have been carried out to investigate antimicrobial potential of fungal extracts against different gram positive, gram negative bacteria.

Many secondary metabolites of fungi are of great commercial importance. Fungi naturally produce antibiotics to kill bacteria, limiting their competition in the natural environment. Important antibiotics such as penicillum can be isolated from fungi. The most common secondary metabolites are antibiotics and others include mycotoxins and ergot-alkaloids, the commonly used immune-suppressant cyclosporine and fumigillin as an inhibitor of angiogenesis and a suppresser of tumor growth [9].

These fungal metabolites can be used as the main ingredients for bio-herbicides, bio-insecticides and bio-fungicides [10]. The bioactive metabolites that were present in most fungi includes phenolic compounds, flavonoids. It is necessary to explore, identify, conserve and utilize the vast potential uses of fungal diversities for the benefit of mankind, as well as the environment. Therefore, the main aim of this study was to identify the phytochemicals and antimicrobial activity of fungi present in baked bread samples.

2. Materials and Methods

2.1. Identification of Fungal Isolates on the Bread Samples

For microbial analysis of the bread samples, the procedure described by the International commission for microbiological food safety (1990) modified by [11] was adopted.

Twenty-five grams of each bread sample (the bread samples were baked in the food science laboratory and stored at different temperature for a period of three weeks) were weighed out using a sensitive electronic balance. The bread samples were crushed in a mortar and homogenized thoroughly with 225mls of distilled water to obtain a 10^{-1} dilution factor of the food extract.

One millilitre of the sample extract was inoculated at $10^{-}1$ dilution into eight Petri dishes in triplicates. Fifteen millilitres of sterile tryptone soya agar (TSA) were poured inside each of the plate and swirled carefully to mix very well and incubated at 37°C for 24hours. The same procedure was repeated using potatoe dextrose agar (PDA) maintaining the same pour-plate technique of inoculation. These second set plate were to indicate the growth of fungi and to enumerate the Total Fungal Count of the samples in colony forming unit. The plates were incubated at room temperature for 48hours and 72 hours respectively. At the end of the incubation period, the PDA plates were observed for growth of both bacteria and fungi. The microbial colonies were counted in a colony counter machine and the results presented as colony forming units (cfu) per gram of bread sample. The identification method by (24) was adopted in identifying the fungi species.

2.2. Phytochemical Screening Analysis

An analysis of phytochemicals from the solvent free extract of mycelium was carried out using various qualitative tests for alkaloids (Mayor's test), flavonoids (Alkaline reagent test), phenols (Ferric chloride test) and saponins (Froth test) using the method as described by [12].

2.3. Determination of Antibacterial Activity of the Fungal Isolates

Selected fungi isolated from the bread samples (*Aspergillus* spp, *Penicillium* spp, *Alternaria* spp, *Rhizopus* spp, *Fusarium* spp *and Mucor* spp*)* were tested for antibacterial activity. Potato dextrose broth (High media) was dissolved in distilled water. This was distributed in 100ml conical flasks and were sterilized in an autoclave at 121°C for 15min. On cooling to 45°C the medium was poured into sterilized petri dishes. The antifungal activity of fungal extract was evaluated by Agar well diffusion method [13]. Inocula were spread over the surface of agar plates with sterile glass spreader. Five wells were made at equal distance using sterile cork borer. To test the antibacterial activity of extracts were made in concentrations of 100mg/ml. of extract was poured into each well and then plates were incubated for a period of 24h at 37°C in an incubator. After incubation, the diameter (mm) of the clear inhibitory zone formed around the well was measured. All determinations were conducted in duplicate.

3. Results and Discussion

Table 1 shows strict morphological and cultural characterization. The growing organisms identified were single cell fungi and many multi-cellular fungi. A total of fourteen fungi species were observed and isolated during the periods of storage from only those samples stored at room temperature. It was observed that the toxigenic ones were, *Aspergillus* spp, *Fusarium* spp, *Rhizopus* spp, *Clasdosporium* spp, *Alternaria* spp, *Penicillum* spp *Tricothecium* spp, *Sporendonema* spp especially *Sporedonema sebi, Starchybotrys* spp also showed toxgenic properties and *Syncephalastrum* spp.

Table 1. Identified fungi and their morphological characteristics

fungus spp	presence of budding	presence of spores	presence of sporomgia	presence of poromgiophore	presence of conidia	presence of conidiosphore	colour of mycellia	appearance of mycellia	presence of septa	presence of stolons	presence of rhizoids	presence of fragments	presence of pigments	presence of athrosphores	presence of ascospores	presence of chlomydospores	presence of vescicles	presence of phialid
Sacchoromyces	+	+	-	-	-	-	Pale white	fu	-	-	-	-	-	-	+	-	-	-
Pichia	+	-	-	-	-	-	Pale white	fu	-	-	-	-	-	-	+	-	-	-
Rizopus	-	+	+	+	-	-	Black	Web like	-	+	+	-	+	-	-	-	-	-
Monilla	-	-	-	-	+	+	White	Cotton wool	+	-	-	-	-	+	-	-	-	-
Fusarium	-	+	+	+	-	-	Salmon pink	Net work	+	-	+	+	+	-	-	+	-	-
Cladosporium	-	-	-	-	+	-	Green	Net work	+	-	-	-	+	-	-	+	-	-
Alternaria	-	-	-	-	+	+	yellow brown	web	+	-	-	+	+	-	-	-	-	-
Mucor	-	+	+	+	-	-	Blue yellow	Cotton wool	-	-	-	-	+	-	-	-	-	-
Trichothecium	-	-	-	-	+	+	brown white	Net work	+	-	-	-	+	-	+	-	-	-
Sporendonema	-	+	-	-	+	+	brown	web	+	-	-	-	+	+	-	-	-	-
Botrytis	-	-	-	-	+	+	grey	cluster	+	-	-	+	+	+	-	-	-	-
Stachybotrys	-	-	-	-	+	+	black	cluster	+	-	-	+	+	-	-	-	-	-
Aspergillus	-	-	-	-	+	+	green	web	+	-	-	-	+	-	-	-	+	+
Penicillum	-	-	-	-	+	+	green	Brush like	-	-	-	-	+	+	+	-	-	+

Fu- forming units , += present, -= absent.

Table 2. Qualitative phytochemical screening of selected fungal isolates

Fungi Isolates	Phytochemicals					
	Alkaloid	Saponin	Tanins	Flavonoids	Steroids	Phenolics
Aspergillus spp	+	+	+	-	-	+
Fusarium spp	+	-	-	-	+	+
Penicillium spp	+	-	+	+	+	+
Rhizopus spp	-	-	+	-	+	+
Mucor spp	-	-	+	-	-	+
Alternaria spp	+	+	-	-	-	+
Yeast	-	-	-	-	-	-

KEYS
+ = Detected
- = Not Detected.

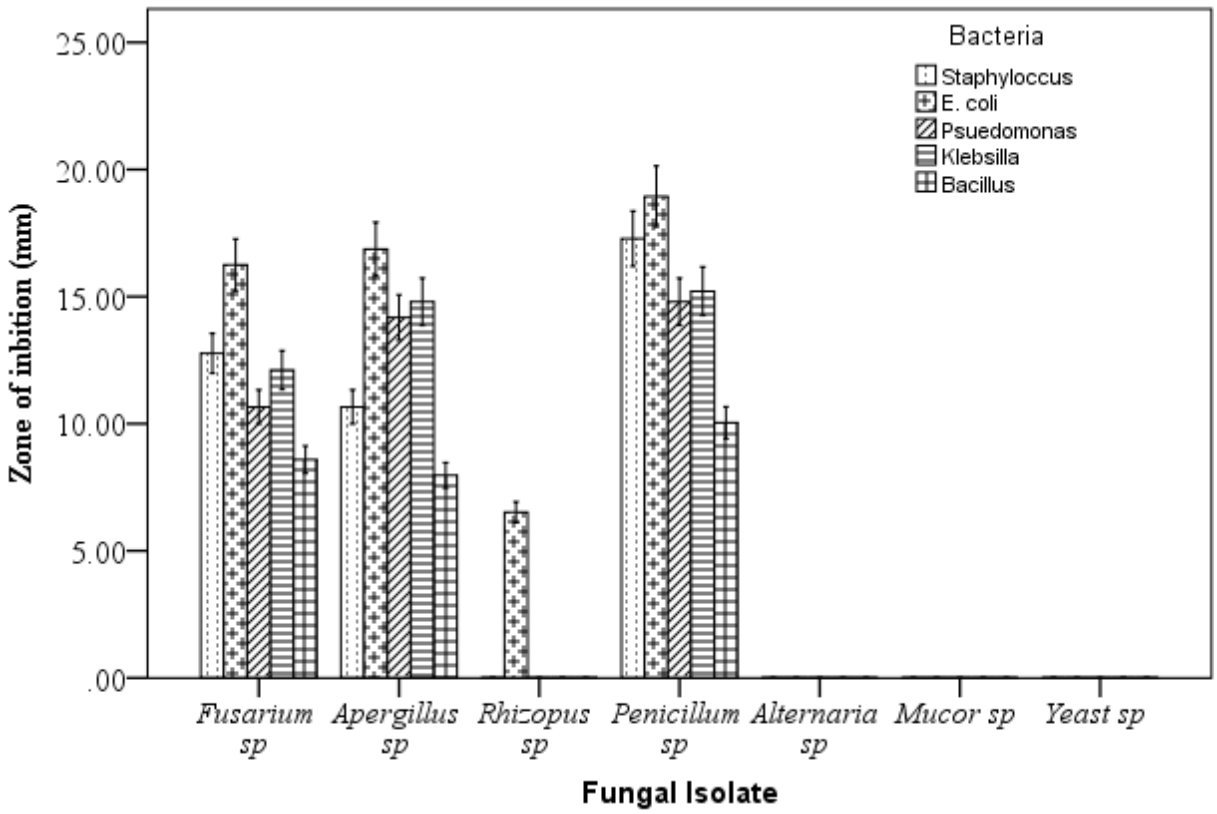

Figure 1. Antimicrobial activity of extracts of fungal isolate from chemically preserved bread

Table 2 shows the phytochemicals present in the fungi isolated in the bread samples. Alkaloids were detected in *Aspergillus, Penicillium, Fusarium and Alternaria* spp. Phenolic were present in all the isolates except yeast. Flavonoids were only present in *Penicillium*.

3.1. Fungi Isolated from Bread Samples

The present study was performed to give information on the effects of different preservatives on mould development with the aim of increasing the shelf-life of bread produced with various preservatives and to determine the presence of pathogenic microorganisms in the bread samples. In this investigation some fungi and yeast species were isolated as shown in Table 1 among the twelve moulds identified *Aspergillus which* is a common mould found on bread [14] was most commonly encountered. In the conidial stage *Aspergillus* produces green and in the ascosporic stage yellow, reddish yellow pigment. Like *Penicillium*, it appears in bread sample that is kept moderately moist [15], a favourable temperature for the growth of this fungi is 22 to 30°C. Some species of *Aspergillus* also produce mycotoxins, such as aflatoxin from *A. flavus*, both a toxin and a carcinogen. Our results are in agreement with the above studies and are supported by many researches. The findings; [14] are consistent with our results that revealed some fungi as they isolated from thier bread samples such fungi as *Mucor* sp, *Aspergillus* sp, and *Rhizopus* sp which we also observed in our samples. In Iran, the fungi contaminating wheat samples were mainly *Aspergillus* spp, *Fusarium* spp and *Penicillium* spp [16]. A study in Algerian study in 2008 also revealed the high level of contamination of wheat samples by species of *Fusarium*, *Penicillium*, *Aspergillus* [17]. The abundance of *Aspergillus* species in cereals and their products indicates inadequate preservation methods of the cereals.

According to Ref [18], the main yeasts which may be present in the seeds of cereals and its by-products are the species *Candida*, *Pichia*, *Sporobolomyces*, *Rhodotorula*

and *Trichosporon.* In this study, *Pichia* and *Saccromyces* were identified in the bread samples. According to a study by ref [19] the fungi identified in the bread samples used for their experiment include *Mucor* sp, *Fusarium* sp, *Aspergillus* and *Rhizopus spp* which were also identified in our study. Bread moulds are common because of the ingredients present in bread. Bread is an excellent source of nutrition for many moulds to grow and thrive. It also contains low moisture content which is why mold can grow well instead of bacteria or yeast that requires higher moisture levels to survive.

Yeasts such as *Saccromyces* sp and *Pichia* sp were also identified in the bread samples. According to [20], problems due to spoilage yeasts in bread usually due to post-baking contaminations. The most frequent and troublesome yeast is *Pichia butonnii,* which is known as chalk mould, this yeast can multiply rapidly on bread, with visible growth apparently before mold occurs. Yeast spoilage in my work was characterized by white patches on the bread samples.

3.2. Phytochemical Constituents of Isolated Fungi

Based on my results on Table 2. Some fungal isolates were selected and further attempt was made to determine the phytochemicals such as alkaloids, flavonoids, phenols and saponins that were present in the fungal extract of some selected fungi isolates. The preliminary analysis of the extracts in Table 2 showed that *Aspergillus* sp contained alkaloids, saponin, tannins, phenolics except steroids, *Penicillum* sp contained all the phytochemicals tested for saponin. The phytochemicals were not tested for in the yeast species. This fungal extracts are used to inhibit pathogens due to the presence of ample concentration of phytochemical. That may be the reason responsible for its medicinal properties. The considered phytochemicals were not isolated in the yeast species. It should be noted that steroid compounds are of great importance and interest in drug interaction due to the relationship of such compounds with sex hormones of man and other organisms [21].

3.3. Antimicrobial Activity of Selected Fungal 1solates

Figure 1 shows the antimicrobial activity of the different bread fungal isolates against some known bacterial pathogens. The result shows highest inhibition against *Staphylococcus aureus* by *Penicillium* extract with inhibition zone diameter of 16.7mm while the least was 10.3mm by *Aspergillus* extract.

Ref [22] noted that *Aspergillus* genus is the main contributor of antimicrobial compounds of fungal origin.

There were variations in the potential of the fungal extracts against the test bacterial pathogens. Comparatively, *Penicillium* extracts had more inhibition activity against the tested bacteria as none was resistant to its extracts. Also, the Gram positive bacteria (*E. coli* and *Pseudomonas)* were more sensitive to the fungal extract "drugs" than the Gram positive ones (*Staphloccus* and *Bacillus*). Furthermore, the extract of three of the seven fungal isolates showed antimicrobial activity against all the bacterial above as *Rhizopus* extract was weakly active (6.3) against E. coli only. A previous study by [23] showed antibacterial activity of *Aspergillus terreus* against *S.aureus, Enterococcus faecalis, B.Subtilis, Pseudomonas aeruginosa and E.coli.*

4. Conclusion

From the findings above it is apt to conclude that fungi isolated from bread samples contain phytochemicals, which may be responsible for the antimicrobial properties of the fungal isolates. We therefore recommend that further study be carried out to determine the antimicrobial activity of these isolates against bacteria pathogens.

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