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Isolation and Morphological Identification of Microbiota of Wheat Bran collected from different locations at Sohag Governorate

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Abstract

The aim of the present research is to study the microbial community associated with 20 wheat bran samples collected during the period between August 2022 to June 2023 in Sohag governorate, Upper Egypt. A total number of 49 species in addition to 3 species-varieties belonging to 15 genera of microbiota, which represented in 38 fungal species and 3 species-varieties appertaining to 9 fungal genera, 10 species of yeast belonging to 5 genera and 1 species of *Streptomyces* appertaining to Actinobacteria were isolated and identified. The most common and prevalent genus was *Aspergillus* (20 spp. and 3 varieties) followed by *Penicillium* (6 spp.), *Talaromyces* (3 spp.) and *Saccharomyces* (3 spp.) with 100%, 100%, 75% and 60% frequencies of occurrence, respectively. Four fungal genera appeared in moderate frequencies of occurrence namely, *Alternaria* (50% of samples & 2 spp.), *Absidia* (40% of samples & 2 spp.), *Acremonium* (40% of samples & 2 spp.) and *Mucor* (30% of samples & 1 spp.) with 2.15%, 1.30%, 0.90% and 1.92% of total count, respectively. The associated microbiota in individual sample ranged between 0.43×10^4 – 5.3×10^4 CFU/g. Moisture content of the of the studied samples ranged between 12.06 ±0.06 - 15.66 ±0.09 with mean 14.19 ±0.18. Chemical analyses of samples revealed the overall mean of crude fat was 5.86 ±0.04, crude protein was 13.95 ±0.11, crude fiber was 12.71 ±0.08, ash was 4.05 ±0.04 and total carbohydrates mean was 49.22 ±0.26. This is the first report of microbes associated with wheat bran samples in Egypt.

Keywords: Wheat bran, Fungi, Yeast, Actinobacteria, Chemical analyses.

INTRODUCTION

Cereals and their products are among the most significant food sources (FAO, 2002). Wheat *(Triticum aestivum)* is a staple and major crop worldwide (Cheng *et al.*, 2021). According to the Food and Agriculture Organization, 794 million tons of wheat are expected to be produced to meet the global demand for this crop (FAO, 2023). A standard wheat grain is mainly composed of the following parts: 80–85% endosperm, 10–14% bran, and 2.5–3.0% germ (Fardet 2010). The outer layers and some associated inner components, known as wheat bran, constitute most of the secondary components of the crop. Its main application is in animal feed, but due to its high dietary fiber content, it has also been used in human cuisine. Thus, wheat bran can be used to create unique commercial products, especially from the aleurone layer, one of the bran layers, which also contains two other components: the outer layer and the middle layer (Chen *et al.*, 2023). Typical wheat bran from wheat flour milling consists of approximately 6–23% of the husk, 6–30% of the outer husk and inner husk, 33–52% of the aleurone layer, and 9–35% of the starchy endosperm (Hemdane *et al.*, 2016). Wheat bran accounts for 15–25% of the weight of wheat grains. Worldwide wheat bran production is estimated at 150 million tons per year (Katileviciute *et al.*, 2019). Chemically, wheat bran consists of dietary fiber (45%) (Cellulose, 6.5–9.9%; hemicellulose, 20.8–33.0%; lignin, 2.2–9.0%), protein (9.6–22.0%), ash (4.0–7.0%), and fat (4.8%–2.9%) as macro-components. Phytic acid (~4.2%), polyphenols (~1.1%), vitamins (~0.04%), minerals (~3.4%), phytosterols (0.16–0.18%), and alkylresorcinols (~0.3%) are among the bioactive compounds (Fardet 2010, Chinma *et al.*, 2015, Ramdan *et al.*, 2016). Wheat bran is high in dietary fiber which ranges from 33.4% to 63.0%; it can be classified as ‘soluble’ and ‘insoluble dietary fiber’ based on their solubility in water. Soluble dietary fiber in wheat bran is <5% of total dietary fiber, and it consists of glucan and xylans (Brouns *et al.*, 2012). Dietary fiber has been defined as remnants of edible plant cell polysaccharides, lignin and other substances which escape hydrolytic enzymatic digestion in the upper gastrointestinal tract. It is the indigestible carbohydrates of plant origin with a heterogeneous chemical structure that is resistant to the effects of digestive enzymes in the human gut (Almeida *et al.*, 2013). Wheat bran is a cheap and abundant source of dietary fiber, which has been linked to improved bowel health and possible prevention of some diseases such as colon cancer. It also contains minerals, vitamins and bioactive compounds such as phenolic acids, arabinoxylans, alkylresorcinols and phytosterols. These compounds have been suggested as an aid in prevention of non-communicable diseases such as cardiovascular disease (Onipe *et al.*, 2015). Dietary fiber increased viscosity in gut and reduced postprandial glycemic response laxative effect, lowered blood cholesterol level and colon cancer prevention (Javed *et al.*, 2012).There are numerous causes of microbial contamination in cereals, but they can all be linked to the conditions under which grains are handled, cultivated, and processed. Cereal grain contamination by microorganisms can occur from a variety of sources, including the air, dust, soil, water, insects, rodents, animals, birds, people, storage and shipping containers. Rainfall, drought, humidity, temperature, sunshine, soil, wind, harvesting equipment, handling, storage conditions are just a few of the environmental factors that affect the microbial contamination of cereals (Bullerman *et al.*, 2008). Nevertheless, molds represent the main source of contamination for cereals and cereal products, both for their visible growth as well as to produce mycotoxins (de Souza 2017). Mold growth can contribute to the spoilage of cereal products, namely bakery and dried products. On the bakery products mold spoilage is evident as white, filamentous, and fuzzy colonies that gradually turn from blue green to black as conidia are produced (Cook and Johnson, 2009). These microorganisms may lead to serious diseases for humans as well as many microorganisms are benefit in food industries in addition to production of different metabolites such as enzymes, antibiotics. etc. Therefore, this research was designed to throw a beam of light on microbiota associated with wheat bran due to its importance as principal source in

manufacture of low-calories bread especially for diabetes humans, athletes and for weight loss in addition to animal feed.

MATERIALS AND METHODS

Wheat bran samples collection:

From August 2022 to June 2023, a total of 20 samples of wheat bran (300 g, each) were gathered from mills and different granaries at Sohag Governorate in Upper Egypt which Bűchler technology (Germany) is used in wheat grains milling and wheat bran extraction as dry grinding stages. Samples were kept in sterile polyethylene bags involved in another sterile polyethylene bags as soon as the wheat was conditioned and milled Then kept at 4-5°C until different mycological surveys and chemical analyses were conducted.

Chemicals:

All the chemicals used in this investigation were purchased from Sigma (Germany) through Al-Gomhoria Company. Deionized water was used.

Methods:

Analytical methods:

According to (AOAC 2012). The overall chemical composition of the collected wheat bran samples was estimated as moisture content, crude fat, crude oil, crude protein, crude fiber and ash content Total carbohydrates estimated according to the following equation:

(%Total carbohydrate) = (100) – (%MC + %ASH + %P + %FAT + %CF)

Microbiota Isolation:

1. Isolation of Fungi:

Dilution-plate method.

Dilution-plate method was used as described by Pitt and Hocking (2009) for microbiota isolation from wheat bran samples. Ten gm of the sample were transferred into sterilized dilution glass contained 90 ml of sterile distilled water, then shaking well for 10 minutes using rotary shaker. Serial dilutions were achieved. Spread-plate technique was used to inoculate 10 plates for each sample with the desired prepared dilution. One ml of supernatant suspension of selected desired prepared dilution was transferred into each plate, spread, pour about 15-20 ml of sterilized used medium. The inoculated plates were rotated clockwise and anticlockwise to spread and thoroughly homogenate the suspension uniformly. Five replicates for each of Czapek‘s-glucose agar and Yeast extract peptone dextrose agar medium for each sample. Then incubated at 28± 2°C for 7 days for fungi and at 35± 2°C for 2 days for yeast and actinobacteria. The developing microorganisms were counted as colony forming units (CFUs), isolated and identified. Pure cultures of identified fungi, yeast and streptomyces were transferred to pure sterilized Czapek's and YPDA agar slants and kept for further studies.

Media used:

Czapek's-glucose agar medium:

This medium was used for isolation, identification and maintenance of isolated fungi from wheat bran samples tested. It was supplemented with rose Bengal and chloramphenicol as bacteriostatic (Smith and Dawson, 1944, Martin, 1950). Its composition (g/l) as employed by Al-Doory, 1980 is as follows; glucose, 10, NaNO3, 3.0, KH2PO4, 1.0, MgSO4.7H2O, 0.5, KCl, 0.5, Agar, 20.0, distilled water, 1000, Chloramphenicol, 0.5, pH, 6.2.

2. Isolation of yeast and actinobacteria: -

The previous dilution technique was used for isolation of different yeast species and actinobacteria.

Yeast extract peptone dextrose agar medium for yeast isolation:

The composition of medium (g/l) as follows; yeast extract, 10; peptone, 10; dextrose, 20, agar 20 and one liter of deionized distilled water as employed by Barnett *et al*., 2007 and Zakpaa *et al*., 2009. Conical flasks containing medium were sterilized at 121^{o}C and 1.5 bar for 20 minutes in autoclave.

Identification of yeast:

1. Germ tube test

germ tube test for *Candida* species was achieved according to Taschdjian *et al.,* 1960.

2. Ascospores formation

The ability of yeast species to form ascospores in yeast extract peptone dextrose agar medium was determined according to Barnett *et al*., 2007.

3. Biochemical tests for isolated yeasts

Fermentation abilities of isolates of yeasts to ferment 2% of different sugar solutions as employed by Kurtzman *et al*., 2011.

Scientific references used in identification of fungi and yeasts and Streptomyces:

The morphological characteristics based on macro- and microscopic characteristics of hyphae and spores were used for identification of fungi, yeast and Streptomyces to species level. The following scientific references were consulted for identification:- (Samson *et al*., 2004), (Domsch *et al*., 2007), for fungi in general, (Ellis 1971, 1976), for Dematiaceous Hyphomycetes, (Moubasher 1993, David 1989), for fungi in general, (Pitt 1979, 1985), for *Penicillium* species, (Raper and Fennell 1965), for the genus *Aspergillus*, and (Barnett *et al*., 2007, Kurtzman *et al*., 2011) for yeast species, and (Berge's manual 1984,1994,2005) for Streptomyces species.

Statistical analysis

Data were analyzed statistically by general linear model using SPSS software (IBM SPSS Statistics, version 22). When the difference was statistically significant ($p < 0.05$), Duncan test 1955 was used to determine the differences among the mean.

RESULT AND DISCUSSION

I-Chemical Analyses:

I.1. Moisture content determination:

The moisture content values fluctuated between 12.06 ±0.06 - 15.66 ±0.09 with mean 14.19 ±0.18. The great majority of moisture content values of samples (75%) were less than 15%, while 25% were more than 15% and ranged between 15.62 ±0.09 - 15.66 ±0.09. The lowest value recorded in sample number 2, while the richest one recorded in sample number 15 as shown in Table 1. The previous results were in full harmony with that recorded by Magan and Lacey 1988 and Tančinová and Labuda 2009.

I.2. Gross chemical analyses of wheat bran: -

Data represented in Table 1 of chemical analyses revealed that crude fat values ranged between 5.27 ±0.08 in sample No.20 to 6.16 ±0.04 in sample No.12 with mean value was 5.86 ±0.04. The crude protein ranged between 12.58 ±0.07 in sample No.17 to 15.13 ±0.09 in sample No.10 with mean value was 13.95 ±0.11. While crude fiber ranged from 12.13 ±0.15 in sample No.5 to 13.52 ±0.37 in sample No.16 with mean value was 12.71 ±0.08, whereas ash value ranged between 3.57 ±0.09 in sample No.13 to 4.33 ±0.03 in sample No.6 with mean value was 4.05 ±0.04. Total carbohydrates of wheat bran samples tested ranged from 46.60 ±0.37 in sample No.6 to 51.37 ±0.27 in sample No.19 with mean value was 49.24 ±0.26. The obtained results of chemical analysis of wheat bran agreed with that obtained by (Tančinová and Labuda, 2009) in Ivanka pri Nitre mill (Slovakia). (Sorour *et al.,* 2022) in Assiut city, Upper Egypt.

Table (1): Gross chemical composition for some types of wheat bran (%wet weight basis).

NO	Sample Source	%Moisture	%Crude Fat	%Crude Protein	%Crude Fiber	%Ash	%Total Carb.
1	**WBRFLM**	14.94^{c}±0.07	5.80^{bcd}±0.09	12.65^{a}±0.12	12.16^{a}±0.18	3.60^{a}±0.06	50.84^{d}±0.05
2	**WBRCLM**	12.06^{a}±0.06	5.94^{cd}±0.08	13.99^{bc}±0.01	13.04^{ab}±0.34	4.10^{b}±0.06	50.87^{d}±0.32
3	**WBRFSM**	12.10^{a}±0.09	5.99^{cd}±0.11	13.95^{bc}±0.06	12.66^{ab}±0.04	4.07^{b}±0.09	51.24^{d}±0.09
4	**WBRCSM**	13.93^{b}±0.36	6.12^{d}±0.5	14.14^{bc}±0.14	13.34^{b}±0.52	4.17^{b}±0.03	48.31^{c}±0.94
5	**WBRFLM**	14.83^{c}±0.09	5.75^{bcd}±0.08	12.71^{a}±0.08	12.13^{a}±0.15	3.70^{a}±0.12	50.89^{d}±0.12
6	**WBRCLM**	15.65^{d}±0.09	5.88^{cd}±0.19	15.08^{d}±0.08	12.45^{ab}±0.32	4.33^{b}±0.03	46.60^{a}±0.37
7	**WBRFSM**	12.13^{a}±0.10	5.97^{cd}±0.12	14.00^{bc}±0.11	12.69^{ab}±0.02	4.13^{b}±0.12	51.08^{d}±0.26
8	**WBRCSM**	13.92^{b}±0.35	6.12^{d}±0.08	14.17^{bc}±0.13	13.39^{b}±0.57	4.27^{b}±0.07	48.12^{abc}±0.95
9	**WBRFLM**	14.92^{c}±0.08	5.65^{abc}±0.23	12.66^{a}±0.17	12.20^{a}±0.16	3.63^{a}±0.07	50.95^{d}±0.13
10	**WBRCG**	15.63^{d}±0.12	5.78^{bcd}±0.26	15.13^{d}±0.09	12.56^{ab}±0.33	4.20^{b}±0.15	46.71^{ab}±0.23
11	**WBRFG**	12.09^{a}±0.08	6.04^{cd}±0.11	13.92^{bc}±0.07	12.69^{ab}±0.02	4.10^{b}±0.10	51.17^{d}±0.13
12	**WBRCG**	13.86^{b}±0.41	6.16^{d}±0.04	14.15^{bc}±0.08	13.38^{b}±0.51	4.27^{b}±0.12	48.19^{bc}±0.87
13	**WBRFG**	14.94^{c}±0.09	5.40^{ab}±0.15	12.62^{a}±0.10	12.21^{a}±0.17	3.57^{a}±0.09	51.27^{d}±0.22
14	**WBRCG**	15.62^{d}±0.09	5.84^{bcd}±0.14	15.02^{d}±0.05	12.62^{ab}±0.44	4.17^{b}±0.07	46.73^{ab}±0.45
15	**WBRFG**	15.66^{d}±0.09	5.90^{cd}±0.18	15.05^{d}±0.10	12.49^{ab}±0.32	4.27^{b}±0.09	46.63^{a}±0.33
16	**WBRCG**	13.90^{b}±0.33	6.12^{d}±0.03	14.23^{c}±0.12	13.52^{b}±0.37	4.23^{b}±0.09	48.00^{abc}±0.74
17	**WBRFG**	14.93^{d}±0.08	5.81^{bcd}±0.04	12.58^{a}±0.07	12.19^{a}±0.16	3.70^{a}±0.06	50.79^{d}±0.11
18	**WBRCG**	15.64^{d}±0.05	5.87^{cd}±0.13	14.89^{d}±0.17	12.57^{ab}±0.25	4.20^{b}±0.10	46.82^{abc}±0.38
19	**WBRFG**	12.14^{a}±0.11	5.86^{cd}±0.18	13.88^{b}±0.07	12.66^{ab}±0.04	4.10^{b}±0.06	51.37^{d}±0.27
20	**WBRCG**	14.98^{c}±0.07	5.27^{a}±0.08	14.24^{c}±0.13	13.43^{b}±0.44	4.25^{b}±0.10	47.83^{abc}±0.59
Overall mean		**14.19±0.18**	**5.86±0.04**	**13.95±0.11**	**12.72±0.08**	**4.05±0.04**	**49.22±0.26**
P-value		**<0.001**	**0.003**	**<0.001**	**0.019**	**<0.001**	**<0.001**

Abbreviations:
WBRFSM wheat bran raw fine from short milling stages.
WBRCSM wheat bran raw coarse from short milling stages.
WBRFLM wheat bran raw fine from long milling stages.
WBRCLM wheat bran raw coarse from long milling stages.
WBRCG wheat bran raw coarse from granaries WBRFG wheat bran raw fine from granaries.

Microorganisms of wheat bran:

Microorganisms play a significant role in deterioration and spoilage of commodities depending on moisture content, storage conditions and organic matter content. Food safety is a global public health threat with frequent incidents of foodborne diseases (Pitt and Hocking, 1997; Hanson *et al.*, 2012, Callejón *et al*., 2015). This study was designed to determine the microbiological status and the distribution of microorganisms in wheat bran collected from different mills and granaries in Sohag governorate, Upper Egypt. The data obtained cleared that forty-nine species and 3 species-varieties belonging to 15 genera were isolated and identified from 20 wheat bran samples. Thirty-eight species and 3 species-varieties appertaining to 9 genera of fungi, while from yeast; ten species belonging to 5 genera, whilst from actinobacteria, only one species (*Streptomyces coelicolor*) was isolated and identified as shown in Table 2. Fungi represent the main source of contamination and spoilage of cereals and cereal products as reported by de Souza, 2017. The total count of microorganisms in samples fluctuated between $0.43 \times 10^4 – 5.3 \times 10^4$, and the great majority of total count of microorganisms in samples was less than 1×10^4 CFU/sample. The obtained data were in full agreement with that reported by Berghofer *et al*., 2003 and Tančinová and Labuda 2009. Our results demonstrated that only in a single case (sample No. 15) microbiota count exceeded this value that 5.3×10^4 CFU/sample and contained the highest level of moisture content among samples tested (15.66^{d}±0.09). The moisture

content of most samples was below than 17%, which limit and inhibit fungal growth of wheat bran samples analyzed in this study which in full agreement with that recorded by (Magan and Lacey 1988) and (Tančinová and Labuda 2009). *Aspergillus, Penicillium, Saccharomyces* and *Talaromyces* were quite the most common genera contributed the broadest spectra of microbial species. *Aspergillus* was represented by 20 species in addition to three species varieties which belong to seven groups of the 18 as described by (Raper and Fennell 1965), (Pitt 1979, 1985) as listed in Table 2. *Aspergillus* was the most prevalent genus regarding the number of cases of isolation. It appeared in total samples (100%) tested comprising (53.5% &196914 CFU) of the gross total count of micoorganisms isolated. *Aspergillus niger* (23156 CFU, 8 samples out of 20 tested accounting 6.24% of total count with moderate occurrence), *A. flavus* (14450 CFU, 8 samples accounting 3.89% with moderate), *A. amstelodami* (13560 CFU, 6 samples, 3.65% of total count with moderate), *A. carbonarius* (9110 CFU, 6 samples, 2.45% of total count with moderate) and *A. awamori* (5334 CFU, 6 samples, 1.44% of total count with moderate) were the most pre dominant species occurring in moderate frequences. While the other 12 species and 3 species-varieties were isolated in low occurrence (3-5 cases out of 20 tested), whereas A. chevalieri was the only fungal species isolated in rare frequency accounting, 11520 CFU and 3.10% of total count. *Penicillium* was the second most dominant genus among isolated microbial genera in this study recorded in all samples (100%), accounting 57855 CFU & 15.58% of total count. It was represented by 6 species, of which *P. palitans and P. viridicatum* appeared in moderate occurrence (8 & 6 cases out of 20 tested), accounting 23992 CFU & 6.46% of the samples and 7569 CFU & 2.03% of the total count, respectively. Whilst *P. cyclopium, P. cyaneo-flavum, P. digitatum and P. nigricans* were isolated in low occurrence (4 cases for each) accounting 7622 CFU & 2.05%, 6666 CFU & 1.80%, 6652 CFU & 1.79% and 5354 CFU & 1.43% of total count. Respectively. The obtained data are in full harmony with that recorded by Tančinová and Labuda 2009, who reported that the most prevalent genera isolated from wheat bran samples collected from Ivanka pri Nitre mill (Slovakia) were *Penicillium (*20 spp.) followed by *Aspergillus* (10 spp.) and *Cladosporium* (3 spp.) with 100%, 89% and 72% frequencies of occurrence, respectively. As well as Saleemi *et al.*, 2017. determined mycological contamination of 67 samples of wheat and 17 samples of wheat bran collected from Faisalabad district of Pakistan. *Aspergillus* was the most common (44.77%) genus followed by *Penicillium, Fusarium* and *Alternaria. Penicillium verucosum* (30.64%) was the most frequently isolated species followed by *A. niger, A. flavus, A. parasiticus, P. chrysogenum, A. ochraceous, A. carbonarius* and *A. fumigatus*. Among *Aspergilli*, *A. niger* representing (46.67%) were most frequently isolated species. *Talaromyces* occupied the third order among microbial species collected in this investigation. It was represented by 3 species; *T. funiculoses*, *T. luteus*, isolated in low occurrence (4 cases for each) while, *T. verruculosus* appeared in rare occurrence (2 cases out of 20 tested) comprising (12442 & 3.35%, 8666 CFU & 2.33% and 2222 CFU & 0.60% of microorganisms total count), respectively. *Saccharomyces* ranked the fourth place among isolated microorganisms representing 31228 CFU, 8.41% of the samples appearing in 12 cases as high frequency of occurrence. It represented by 3 species: *S. cerevisiae* (18128 CFU,4.88% of the samples & 8 cases in moderate occurrence. While *S. baynus* and *S. uvarum* appeared in 2 cases, rare occurrence for each accounting 8434 CFU & 2.27% and 4666 CFU & 1.26% of the samples, respectively. Six microbial genera isolated from wheat bran samples in moderate frequencies of occurrence (6-10 cases out of 20 tested) in this study. These include four fungal genera, *Absidia* (*A. croymbifera* and *A. repens*), *Acremonium* (*A. rutilum* and *A. strictum*), *Alternaria* (*A. alternata* and *A. tenuissima*) and *Mucor* which represented by *M. circinelloides.* From yeast, *Rhodotorula* (15328 CFU, 4.13% of the samples & 8 cases) was represented by 2 species *R. glutinis* (13330 CFU, 3.59% & 6 cases) and *R. mucilaginosa* (1998 CFU, 0.54% of samples and 2 cases) and *Trichosporon* appeared in 8 cases in moderate occurrence (13998 CFU & 3.77%) was

represented by *T. asteroids* (8666 CFU, 2.33% and 4 cases) and *T. asahii* (5332 CFU, 1.44% and 4 cases) as shown in Table 1. The other five genera *Cladosporium* (*C. cladosporioides*), *Rhizopus* (*R. stolonifer*) from fungi, from yeast *Candida* (*C. glabrata & C. railenensis*) and from actinobacteria (*Streptomyces coelicolor*) were isolated and identified in low frequencies of occurrence while *Pichia* (*P. kudriavzevii*) only was isolated in rare frequency of occurrence (2 cases out of 20 tested). These results were in full harmony with that obtained by Berghofer *et al*., 2003 who investigated wheat flour and bran and reported that the most dominant fungi isolated were *Aspergillus*, *Penicillium*, *Cladosporium* and *Eurotium* spp. As well as, the genera of *Saccharomyces*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Candida*, and *Zygosaccharomyces* are involved in spoilage in cereal and cereal products in rare cases (Cook and Johnson, 2009). This is the first report on microbiota associated with wheat bran in Egypt.

Table (2): Average total counts, number of cases of isolation and occurrence remarks of fungi, yeast and actinobacteria genera and species isolated on Czapek's- glucose agar medium at 28±2°C for eight days.

Genera & Species	Average total count. Colonies/g	% Total count	Number of cases of isolation	OR
Gross total count.	**371178**	**100**	**20**	**H**
Fungi				
Absidia	4862	1.31	8	M
A. croymbifera	3110	0.83	4	L
A. repens	1752	0.47	4	L
Acremonium	3334	0.90	8	M
A. rutilum	2000	0.54	4	L
A. strictum	1334	0.36	4	L
Alternaria	7998	2.15	10	M
A. alternata	7332	1.97	6	L
A. tenuissima	666	0.18	4	L
Aspergillus	196914	53.05	20	H
A. amstelodami	13560	3.65	6	M
A. awamori	5334	1.44	6	M
A. candidus	6000	1.62	4	L
A. carbonarius	9110	2.45	6	M
A. chevalieri	11520	3.10	2	R
A. chevalieri var *intermedius*	6000	1.62	4	L
A. ficuum	11332	3.05	4	L
A. flavus	14450	3.89	8	M
A. flavus var *columnaris*	15110	4.17	4	L
A. flavo-furcatis	12886	3.47	4	L
A. foetidus	7338	1.98	4	L
A. foetidus var. *acidus*	2000	0.54	4	L
A. fumigatus	3328	0.90	4	L
A. japonicus	7106	1.91	4	L
A. niger	23156	6.24	8	M
A. parasiticus	2888	0.78	4	L
A. phoenicis	8664	2.33	4	L
A. pulverulentus	4444	1.20	4	L
A. repens	6666	1.80	4	L
A. ruber	7332	1.98	4	L

Follow Table (2)

Genera & Species	Average total count. Colonies/g	% Total count	Number of cases of isolation	OR
A. terreus	5332	1.44	4	L
A. tubingensis	7788	2.10	4	L
A. versicolor	5570	1.50	4	L
Cladosporium cladosporioides	3332	0.90	4	L
Mucor circinelloides	7144	1.92	6	M
Rhizopus stolonifer	3885	1.05	4	L
Penicillium	57855	15.58	20	H
P. cyclopium	7622	2.05	4	L
P. cyaneo-flavum	6666	1.80	4	L
T. digitatus	6652	1.79	4	L
P. nigricans	5354	1.43	4	L
P. palitans	23992	6.46	8	M
P. viridicatum	7569	2.03	6	M
Talaromyces	23330	6.28	15	H
T. funiculosus	12442	3.35	4	L
T. luteus	8666	2.33	4	L
T. verruculosus	2222	0.60	2	R
Yeast				
Candida	2198	0.60	4	L
C. glabrata	1332	0.36	2	R
C. railenensis	866	0.23	2	R
Pichia kudriavzevii	1332	0.36	2	R
Rhodotorula	15328	4.13	8	M
R. glutinis	13330	3.59	6	M
R. mucilaginosa	1998	0.54	2	R
Saccharomyces	31228	8.41	12	H
S. cerevisiae	18128	4.88	8	M
S. baymus	8434	2.27	2	R
S. uvarum	4666	1.26	2	R
Trichosporon	13998	1.91	8	M
T. asahii	5332	1.44	4	L
T. asteroides	8666	2.33	4	L
Actinobacteria	5330	1.43	4	L
Streptomyces coelicolor	5330	1.43	4	L

OR: Occurrence Remarks: -

H = High occurrence; more than 10 cases out of 20 tested.

M = Moderate; between 6-10 cases. **L** = Low occurrence less than 3-5 cases.

R = Rare occurrence less than 3 cases**.**

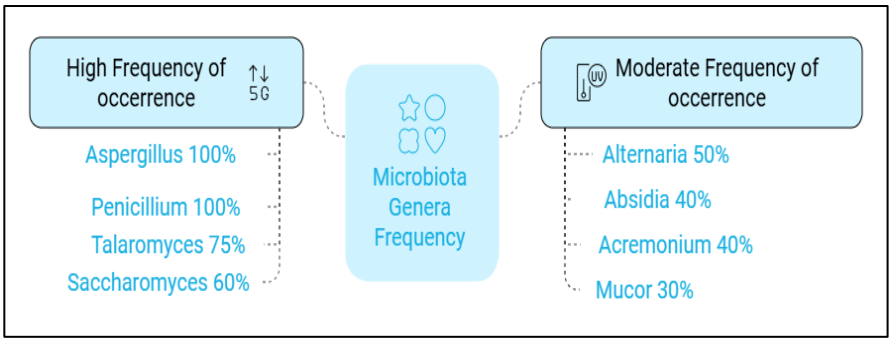

Figure 1. Occurrence frequency of microbiota genera recovered from wheat bran samples collected from different mills and granaries in Sohag governorate, Upper Egypt.

CONCLUSION

This is the first report of the microbiota associated with wheat bran samples in Egypt. Wheat bran is used in bakery process especially low-calories bread. This study threw a beam of light that wheat bran samples tested had narrow spectrum of associated fungi, yeast and actinobacteria. The samples tested had low moisture content less than 17% which very safe to limit and inhibit fungal growth, so, it is very well for using it in food industries such as bakery process, as well as the isolated microorganisms will be used in future studies for extract many enzymes used in improve the quality of bread.

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