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RESEARCH ARTICLE

DETERMINATION OF THE MICROBIAL PROFILE OF COMPRESSED BAKER'S YEAST AND STUDYING ITS SYMBIOTIC INTERACTIONS

Salah H. Salem[1]*, Mohamed M. Naguib[1], Hussein H. El-Sheikh[2] and Yehia A. Heikal[3]

(1) Food Toxins and Contaminants Dept. National Research Center**,** Cairo- Egypt

(2) Botany and Microbiology Dept. Fac. of Science, Al-Azhar University, Cairo- Egypt

(3) Food Science Dept., Faculty of Agriculture, Ain Shams University, Cairo Egypt

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Corresponding Author

Salah H. Salem

Abstract

Baker's yeast; *Saccharomyces cerevisiae*; is one of the oldest products of industrial fermentation used for bread manufacturing which is a staple food worldwide. Three different brands of compressed baker's yeast were examinedfor their microbial profile. The mean totalyeast viablecounts ranged from 10.26± 0.31 to 10.39± 0.42 log CFU/g with no significant difference between brands. The bacterial counts were determined on nutrient agar, actidione agar and MRS agar media and showed that it ranged from 7.44± 0.58 to 8.24± 0.51 log CFU/g indicating that it's a major constituent of baker's yeast. Randomly, one hundred and forty bacterial strains were isolated from baker's yeast samples and were identified to its corresponding bacterial groups, using biochemical and morphological tests. It showed that it belonged to lactic acid bacteria, Bacilli and Micrococci. The molecular identification of the strains revealed that it is *Bacillus subtilis, B. amyloliquefaciens, B. cereus, Geobacillus stearothermophilus, Lactobacillus plantarum, L. brevis, L. lactis, Micrococcus luteus and Kocuria rosea*. The symbiotic interaction of the 140 isolates with *S. cerevisiae* was tested using disk diffusion method; revealed that six bacterial strains showed antimicrobial activity (inhibition zone ranged from 13.5 to 22.8 mm). The six strains belonged to genera *Bacillus* and *Geobacillus*. Further identification based on sequencing of 16S rDNA gene using universal primers revealed that the strains were identified as *B. subtilis, B. amyloliquefaciens* and *G. stearothermophilus*. The strains sequences were deposited in the GeneBank and have an accession numbers.

INTRODUCTION

Baker's yeast; *Saccharomyces cerevisiae*; is one of the oldest product of industrial fermentation **(Darmola and Zampraka, 2008).** Ancient Egyptians used it for bread makingsince about 6000 years ago **(Yabaya and Jatau, 2009).Beudeker *et al.*, 1990** grouped the baker's yeast formulation into two main types; compressed yeast (about 30% solids) and dried yeast (about 92 % solids). Both are produced in a large fermenters by the fed- batch process on cane or beet molasses supplemented by nitrogen, phosphorous, minerals and vitamins.

The fermenters couldn't be kept completely sterile; therefore they always contain some contaminating microorganisms (**Reed and Nagodawathana 1991).** Bacterial contamination in industrial yeast production is a huge problem. *Lactobacilli* are considered the most frequent contaminant. It thrives under the same growth conditions as

*S. cerevisiae***(Albers *et al.*, 2011).** Contaminating bacteria can provide added value in the form of flavor, taste and so on, but their levels must be maintained within certain limits (**Priest and Campbell, 2003).**
Several studies reported bacterial contaminants of commercial yeast; including predominantly lactic acid bacteria (LAB) **(Reed and Nagodawithana 1991; Lues, 1992)** and occasionally *Enterococcus spp.***(O'Brien*et al.*, 2004)**, *Bacillus* spores **(Bailey and von Holy, 1993; Viljoen and von Holy, 1997).**
Reed and Nagodawithana, 1991 reported that the baker's yeast specification require the absence of *Salmonella* and other pathogens and restrict the number of *coliform* organisms to be less than 1000 CFU/g, and the count of *Escherichia coli* should be less than 100 CFU/g yeast.
Possible sources of contamination include and not limited to raw materials; processing water; chemicals (defoamer); insufficient cleaning of fermentation tanks, storage tanks, dryers, filter cloths and product contact surfaces; airborne contamination or unhygienic handling of yeast products **(Tessendorf, 1991; Lues, 1992).**

This study aimed to determine the microbial profile of Egyptian compressed baker's yeast and studying its interactions.

Material and Methods

Samples

Thirty six compressed yeast samples produced in Egypt (yeast cake, containing about 30% solids) had been collected from local markets and named brand (A), (B) and (C). Samples were directly analyzed on the same day of production.

Microbiological media and reagent

Four different microbiological media were used for determination of microbial profile of the collected yeast samples; acidic dextrose agar medium (DA) for yeast, MRS agar for isolation of lactic acid bacteria, nutrient agar (NA) and actidione agar (AC) for isolation of wide range of bacteria **(Atlas, 2006 and ES,191/2000).**
YPD agar media and Tryptic soya broth were used for studying the effect of contaminating bacterial isolates on S. cerevisiae**(Albers *et al.*, 2011 and Atlas, 2006).**
All polymerase chain reaction (PCR) reagents were obtained from Thermo Scientific Inc. including green Taq DNA polymerase, PCR buffer, dNTPs mix, forward and reverse primers

Determination of microbial profile of baker's yeast samples

The total viable cell counts for microbial flora were determined using 0.1% peptone solution serial dilutions and pour plating technique. DA plates were incubated at 30^{o}C for 72hr, while MRS agar, nutrient agar and actidione agar plates were incubated at 35± 2^{o}C for 72hr **(Albers *et al.*, 2011 and Catizeddu *et al.*, 2006).**

Isolation and identification of isolated strains

Random single colonies from different media were isolated and purified by streaking repeatedly on the fresh plates of the corresponding media and incubated at 30^{o}C for 24hr.

Preservation of Isolated cultures

The isolated bacterial strains were cultured on its respective media and incubated for appropriate time and temperature. Disks of media (8mm) were transferred into a sterile 2ml screw top vials containing 1ml sterile 50% glycerol then kept at -20^{o}C until used **(Shepherd *et al.*, 2010).** For long term preservation the isolates were lyophilized.

Morphological examination: the purified isolates were Gram and malachite green stained for examination of cell shape using trinocular microscope (Olympus CX-41).
Catalase test: the catalase test was performed on purified isolates using 3% H_2O_2 solution **(Benson, 2001)**.
Oxidase Production: The production of oxidase was carried out using oxidase testing disks (Himedia, Mumbai-India) according to manufacturer instructions.

Determination of the interactions between bacterial isolates and *S. cerevisiae*

The interaction between *S. cerevisiae* and one hundred and forty isolates were evaluated using a modified disk diffusion method according to **Sihem *et al.*, (2011)** and **Qazi *et al.*, (2009)**. A layer of YPD agar media (15 ml) containing 0.1% v/v of *S. cerevisiae* culture grown for 24hr at 30^{o}C in YPD broth. After solidification, a sterilized filter paper discs (Whatman filter paper no. 1 was used to prepare discs approximately 6 mm in diameter) loaded with bacterial suspension (bacterial isolates grown in Tryptic soya broth for 24hr at 35^{o}C) was applied on the surface of YPD agar plates and incubated at 30^{o}C for 24hr. After incubation, the effect of bacterial suspension on *S. cerevisiae* was observed and expressed as stimulation (+), no effect (±) and inhibition (halo zone diameter in mm)**.**

Molecular Identification of bacterial isolates

The method was based on the procedures of **Sambrook and Russell (2001);** the bacterial paste (0.25g) from overnight culture resuspended in 1 ml TE buffer pH (8.0) and genomic DNA of the purified isolates were extracted**.**Amplification of the 16S rDNA gene from the genome of isolated strains was carried out via the polymerase chain reaction (PCR) using a conserved primers designed to amplify the full length (1500bp) of the 16S rDNA gene according to the *E. coli* genomic DNA sequence. The forward primer was 5`-AGAGTTTGATCMTGGCTCAG-3` and the reverse primer was 5`-TACGGYTACCTTGTTACGACTT-3`. For standard PCR, each mixture (final volume, 50µl) contained two µl of template DNA extract, 1.5 µl from each primer at a concentration of 10 pM, 5 µl dNTPs (at concentration 10 mM), 5 µl of 10X PCR buffer and 1.0 µl dream green Taq polymerase containing 2U. The cyclic reaction, composed 4 min at 94°C for first denaturation step and then 30 cycle of 1 min at 94°C for denaturation, 1 min at 55°C for primer annealing and 2 min at 72°C for extension, followed by an additional 10 min at 72°C for final extension and hold at 4^{o}C. Gel electrophoresis was done for detection of extracted DNA from purified isolates as well as for detection of PCR product. The PCR product was purified and sequenced using dideoxy chain termination method according to **Sanger *et al.*, (1977)** and the isolates were identified to species level by comparing sequences with known sequences in GeneBank using BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical Analysis:

All microbiological counts were converted to the base-10 logarithm of colony forming units per gram (log CFU/g). Data were tested for statistical significance at the 5% level ($p< 0.05$) by analysis of variance (ANOVA) using Origin 6.1softwear.

Results and discussion

Determination of the microbial profile of compressed baker's yeast samples

Total viable yeast counts

Total viable counts of yeast were 10.39± 0.42, 10.26± 0.31 and 10.30± 0.34 log CFU/g for brand A, B and C respectively as seen in table (1) and Fig (1). There is no significant difference between the total yeast counts in different brands. **Rollini *et al.*, 2007** tested four commercial compressed baker's yeast samples and reported that the average total yeast cell counts were 4X 10^{10} cell/ g. **Nasr *et al.*, 2010** studied the microbiological quality of nine brands of active dry yeast (ADY) – indigenous and imported – sold in Egypt and compared them with Egyptian compressed yeast. They found that the means of total viable counts ranged from 8.70± 0.47 log CFU/g to 9.59± 0.27 log CFU/g with significant differences between some brands. The present results confirmed that the viable count in yeast is superior than that of the imported brands and the finding of **Abullhassan and Sayied, 2013** reported that the means of total viable count ranged between 1.1X10^{10}CFU/g (10.04 log CFU/g) and 1.5 X10^{10} CFU/g (10.17 log CFU/g) when three brands of active dry yeast were examined.

Table (1): Means ± Standard Deviations of Total Viable Counts of collected baker's yeast samples on different microbiological media.

Brands	Total count (Log CFU/g) on different media				
	Yeast count	Bacterial count			
	DA	**AC**	**MRS**	**NA**	**Mean**
(A)	10.39 ± 0.42^{c}	7.66 ± 0.70^{ab}	8.04 ± 0.57^{d}	8.24 ± 0.51^{f}	7.98± 0.29^{g}
(B)	10.26 ± 0.31^{c}	8.08 ± 0.40^{a}	7.82 ± 0.52^{d}	8.19 ± 0.33^{f}	8.03± 0.19^{g}
(C)	10.30 ± 0.34^{c}	7.44 ± 0.58^{b}	8.11 ± 0. 35^{d}	8.00 ± 0.29^{f}	7.85± 0.36^{g}

Means in the same column with different letters are significantly different ($P< 0.05$).

Total bacterial counts of compressed baker's yeast:

Table (1) and figure (1) showed the total viable count on actidione agar, MRS agar and nutrient agar. Three microbiological media were used for enumeration and isolation of wide range of bacteria; MRS for enumeration and isolation of lactic acid bacteria (LAB), actidione agar (inhibit yeast growth to allow determination of microbial groups) and nutrient agar for isolation of different bacteria. The means of count on MRS agar were 8.04 ± 0.57, 7.82 ± 0.52 and 8.11 ± 0.35 log CFU/g for brand A, B and C respectively with the highest value for brand C followed by brand A and finally brand B.To the contrary, the counts on nutrient agar were 8.24 ± 0.51, 8.19 ± 0.33 and 8.00 ± 0.29 log CFU/g for brand A, B and C respectively with highest count of brand A, while the brand B have the highest

count on actidione agar with mean 8.08± 0.40 log CFU/g which significantly different with the mean for brand C 7.44 ± 0.58 log CFU/g and have no significant difference with the mean of brand A 7.66 ± 0.70 log CFU/g.

In general; the brand B have the highest bacterial count on the three microbiological media with mean 8.03± 0.19 log CFU/g then brand A 7.98± 0.29 log CFU/g and brand C 7.85± 0.36 log CFU/g with no significant difference between means.

Reale *et al.*, (2013) stated that the commercial yeast samples have a total viable count of higher than 9 log CFU/g and they concluded that the samples have been contaminated with different microorganisms and the extent of the contamination depend on the type of baker's yeast formulation.

O'Brien *et al.*, (2004) studied the presence of *Enterococcus, Coliform* and *E. coli* in a commercial yeast manufacturing process. They found that the mean ± standard deviations of *Enterococcus, Coliform* and *E. coli* in compressed yeast samples (30% solids) were 3.80± 0.44, 2.89± 0.29 and 1.10± 0.40 log CFU/g, respectively. They used a preliminary incubation technique at 30^{o}C for 24hr to boosting bacterial count. The means ± SD of *Enterococcus, Coliform* and *E. coli* counts after preliminary incubation were 7.35 ±0.25, 2.14± 0.42 and 3.87± 0.23, respectively which indicates that the preliminary incubation technique was demonstrated to be suitable for the detection of low numbers of bacterial contamination in commercial yeast. In a previous study **(Salem, 2010)** it was reported that the means± SD of contaminating bacterial counts in different yeast samples was 7.10±0.22 CFU/g, 7.14±0.25 CFU/g and 7.00±0.23 CFU/g for liquid yeast suspension, compressed yeast and active dry yeast respectively with no significant difference between bacterial counts of yeast cream and yeast cake samples but there was a significant difference between them and bacterial count of active dry yeast samples.

Reed and Nagodawathana (1991) stated that the overwhelming proportion of contamination in compressed yeast and active dry yeast is lactic acid- producing organisms often called diplos (diplococci) in the industry. In the past; these organisms thought to be useful as contributors to the flavor of bread. This belief is certainly not true for the very short dough fermentations at very high levels of yeast inocula typical of modern methods of backing. Despite massive amounts of time and effort spent on these matters, bacterial contamination is still a serious problem and a threat also to the successful development of commercial bio- based fuel production **(Albers *et al.*, 2011).** The total bacterial counts of yeast was reported by **Carlin (1958)** as 2-3 X 10^{9} CFU/g of yeast, but these values was much higher than could be ascertained in continuous sampling of compressed yeast and active dry yeast samples while the actual counts are likely to vary between 10^{4}- 10^{8} per gram of yeast **(Reed and Nagodawathana1991)**. These organisms always or almost always belong to heterofermentative lactic acid producing organisms of the genus *Leuconostoc* or to homofermentative bacteria of the genus *Lactobacillus*. Some *coliform* organisms, occasionally a few *E. coli*, can be found. Active dry yeast made by drying compressed yeast contains the same kind of microorganisms, but the total count generally decreased during drying and upon subsequent storage of the dried yeast. Specifications for rope spores require an upper limit of 200 per gram of active dry yeast **(Reed and Nagodawathana1991)**.

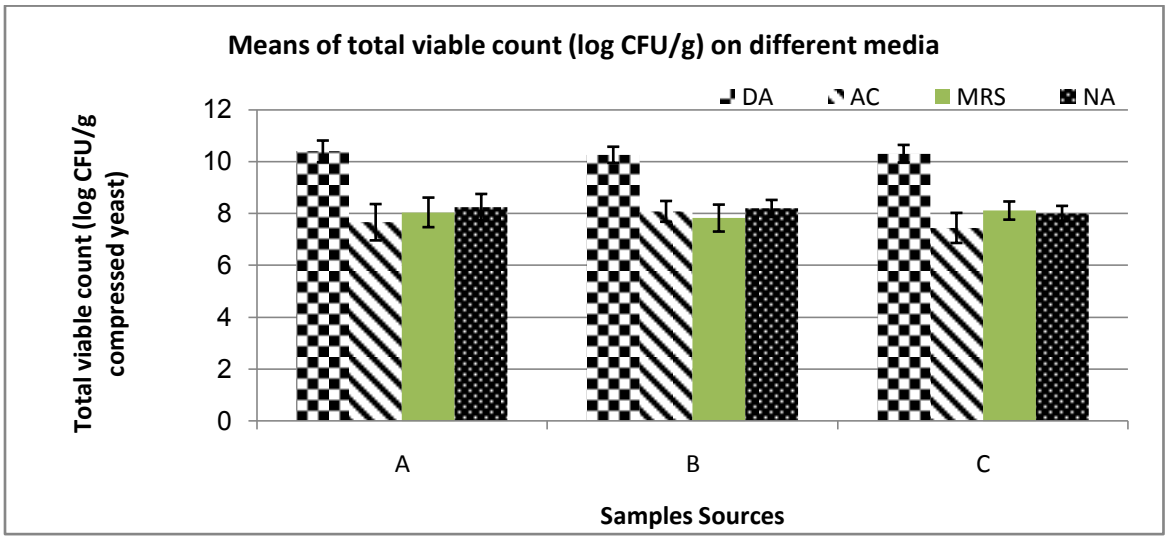

Fig (1): Means of total viable count of three brands of baker's yeast samples collected from local market on four different microbiological media (bars indicating standard deviations)

Identification of Bacterial flora in baker's yeast samples

One hundred and forty bacterial strains were isolated from appropriate plates of different media. All isolates were subjected to Gram and malachite green stain, catalase and oxidase tests to differentiate them into different bacterial groups. Isolates were examined under microscope to determine the cell shape. Table (2) showed that all bacterial isolates were Gram positive; thirty eight (27.1%) of them were cocci while the other 102 (72.9%) isolate were rod shape. The using of different media enabled better understanding of the microbial profile of baker's yeast. The spore forming rods constitute 84.2% from the counts recorded on Actidione agar. Meanwhile a Gram positive rod represents 81.4% of the counts on MRS agar medium. The highest occurrence of cocci was recorded on nutrient agar medium being 40.7%. The aforementioned results revealed that the presence of bacteria in baker's yeast is almost the sum of counts recorded on different media; thus its symbiotic interaction is worthy to be considered.
From different isolated groups; twenty four random strains were further identified using PCR for 16S rDNA to confirm the morphological and biochemical characteristics. The results of PCR identification demonstrate the species level of isolated genera as *L. plantarum, L. brevis, L. lactis, B. cereus, B. subtilis, B. amyloliquifaeciens, G. stearothermophilus, M. luteus and K. rosea*; (Table3).
The present results are in harmony with previous findings. **Nasr *et al.*, 2010** studied the microbiological quality of nine brands of active dry yeast sold in Egypt and detected *B. cereus* in eight brands with mean count of 1.53± 2.03 log CFU/g. They also detected *Staphylococcus aureus* in eight brands with means ranged from 3.86±0.79 and 0.72±1.54 log CFU/g. **Bailey and Von Holy (1993)** reported that the high *Bacillus* spore contamination of brown bread was originated from yeast.

Table (2): Biochemical and morphological characterization of bacterial isolates associated with baker's yeast samples.

Media for Isolation	No. of isolates	Morphological shape	Percent (%)	Gram stain	Catalase test	Oxidase test	Spore forming	Genus
Nutrient agar	59	Rods (35)	59.3%	+	+	+	+	*Bacilli*
		Cocci (24)	40.7%	+	+	+	-	*Micrococci*
Actidione agar	38	Rods (32)	84.2%	+	+	+	+	*Bacilli*
		Cocci (6)	15.8%	+	+	+	-	*Micrococci*
MRS agar	43	Rods (35)	81.4%	+	-	-	-	LAB
		Cocci (8)	18.6%	+	-	-	-	LAB
Total isolates	140	Rod (102)	72.9%					
		Cocci (38)	27.1%					

Among the thirty four colonies randomly selected by **Barrette *et al.*, (1999)** from higher dilution of high density yeast suspension, and they 41% of the isolated bacteria were found to be aerobic sporulating rods identified as *Bacillus* sp., while the other 59% of the isolates were cocci, catalase and oxidase negative which identified as*Leuconostoc, Aerococcus* and *Lactococcus*.**Vilijoen and lues (1993)** conducteda survey about the microbial populations associated with post-fermented dough and compressed baker's yeast. They found that ten different bacterial genera comprising *Lactobacillus, Leuconostoc, Micrococcus, Pediococcus, Lactococcus, Bacillus, Pseudomonas, Acinetobacter* and *Alcaligenes* were isolated from the different stages. **Ismail (2006)** observed that the main bacterial group in baker's yeast was lactic acid bacteria. On the other hand, total gram negative bacteria were recorded as 5% of total bacterial count. Finally there are no *coliform* bacteria, *Salmonella sp.* and *Listeria sp.* were detected in any tested yeast strains which revealed the good sanitation of fermentation process which is in consistent with that the result obtained by **Abullhassan and Sayied 2013**.
Barrette *et al.*, (1999) suggest that *Bacillus sp.* would constitute a significant fraction of fresh baker's yeast contaminants on contrary with **Vilijoen and lues (1993)**as they reported that *Lactobacilli* were the main contaminating bacteria of compressed baker's yeast.

The symbiotic Interaction between bacteria and *S. cerevisiae*in Baker's yeast

A total of one hundred and forty bacterial isolates were tested for their symbiotic interaction with *S. cerevisiae* using the disk diffusion method. Only six isolates showed inhibitory effect against *S. cerevisiae* (Fig. 2) and the remaining strains didn't reveal any stimulation effect. Table (4) showed the inhibition zone diameter in mm developed on *S. cerevisiae* inoculated plates. It ranged from 13.5±0.65mm to 22.8±2.3 mm. These results agree with that achieved by **Sihem *et al.*, (2011)** as they examined the antifungal activity of two *Bacillus* isolates B27 and B29 against *S. cerevisiae* which showed inhibitory effect with inhibition zones of 22.5±0.25 and 24±0.40 respectively. Also **Qazi *et al.*, (2009)** studied the antifungal effect of bacteria from soil samples on *S. cerevisiae*. He reported that *B. cereus* exhibited the activity of *S. cerevisiae*. The six isolated *B. cereus* strain showed inhibition zones ranging from 11.5mm to 16.25mm. Also two *Bacillus* species and *B. pumilis* have been isolated from soil samples by **Kumar *et al.*,(2009)** and showed inhibitory effect against *S. cerevisiae*.

Oliva-Neto and Yakoya (2001) stated that the microbial contaminant may cause many problems due to the competition between bacteria and yeast for the same substrate and **Paramithiotis *et al.*, (2006)** assessed the interaction between *S. cerevisiae* and lactic acid bacteria in wheat sourdoughs and concluded that the presence of lactic acid bacteria had no effect on *S. cerevisiae* final cell yield but affected negatively the maximum growth rate.

Hsieh *et al.*, (2008) reported that *B. subtilis* strains often produce a variety of antimicrobial cyclic lipopeptides, including iturins, fengycins, and surfactins. Iturin A, one member of the iturin group, shows a strong antibiotic activity with a broad antifungal spectrum. They found 12 *Bacillus* isolates out of these *Bacillus* strains inhibit *S. cerevisiae* which found to be *B. subtilis, B. amyloliquefaciens* and *B. circulans* when identified by PCR.

Table (3): Molecular identification of bacteria isolated from baker's yeast using BLAST search

No.	Isolates Code	Identification	Homology % with GeneBank	Media for isolation
1	NS-00	*B. subtilis*	98	NA
2	NS-21	*B. subtilis*	99	NA
3	NS-57	*B. amyloliquefaciens*	99	NA
4	NS-63	*B. amyloliquefaciens*	99	NA
5	NS-135	*B. subtilis*	99	NA
6	NS-138	*G. stearothermophilus*	98	NA
7	NS-36	*B. cereus*	98	AC
8	NS-27	*B. cereus*	98	NA
9	NS-28	*B. subtilis*	99	NA
10	NS-75	*B. subtilis*	99	NA
11	NS-80	*B. cereus*	99	NA
12	NS-82	*B. amyloliquefaciens*	99	NA
13	NS-93	*B. subtilis*	99	NA
14	NS-96	*B. subtilis*	99	NA
15	NS-65	*Lactobacillus plantarum*	98	MRS
16	NS-66	*Lactobacillus brevis*	83	MRS
17	NS-67	*Lactobacillus plantarum*	98	MRS
18	NS-90	*Lactobacillus plantarum*	98	MRS
19	NS-91	*Lactobacillus brevis*	86	MRS
20	NS-70	*Lactococcus lactis*	97	MRS
21	NS-92	*Lactococcus lactis*	98	MRS
22	NS-139	*Lactococcus lactis*	96	MRS
23	NS-01	*Micrococcus luteus*	98	NA
24	NS-08	*Kocuria rosea (Micrococcus* group*)*	95	NA

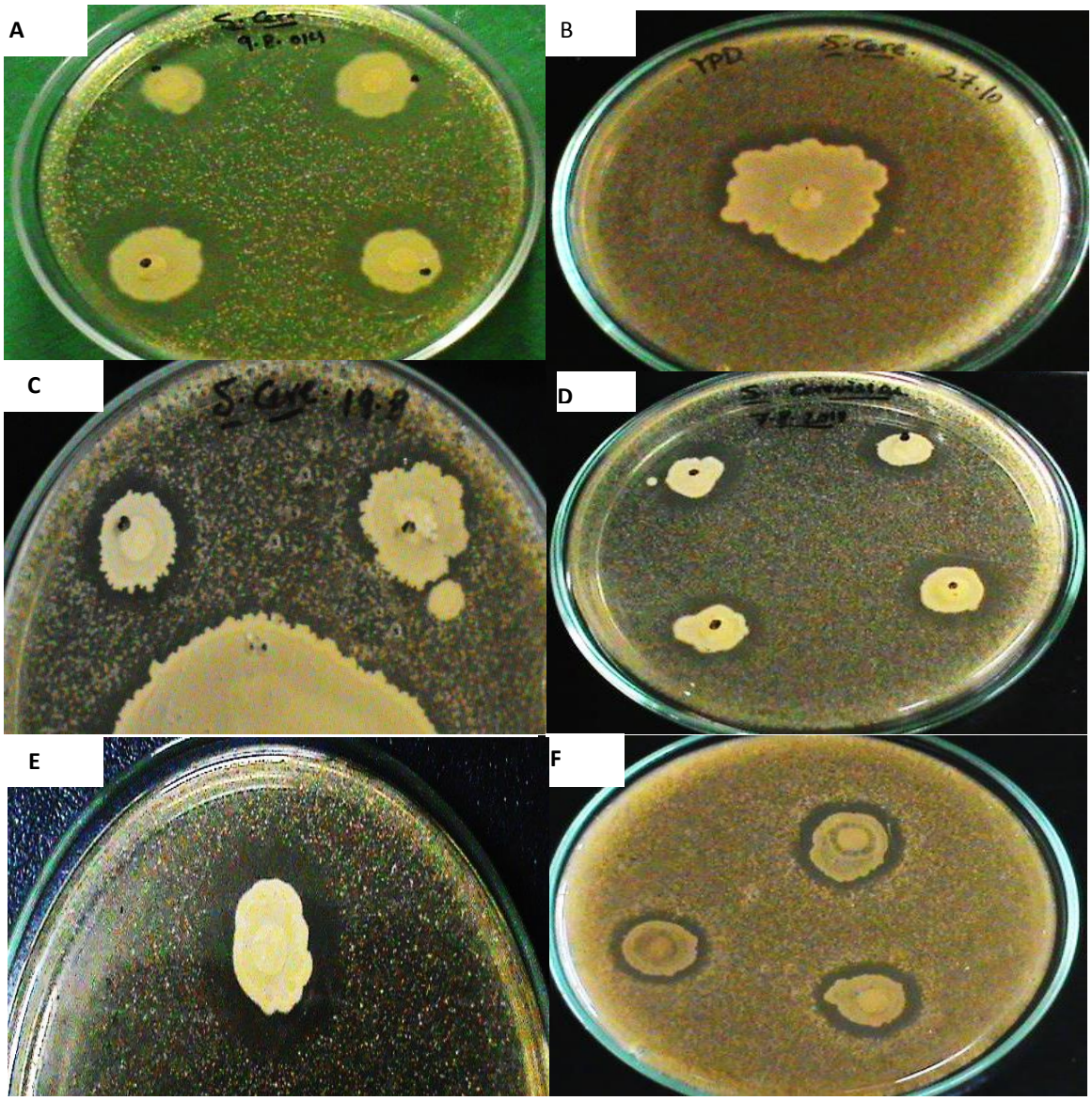

Fig (2): Effect of bacterial isolates against *S. cerevisiae:*
- A, D and F: *B. subtilis*;- B:*G. stearothermophilus* - C and E: *B. amyloliquefaciens*

Identification of inhibitory Bacilli species isolated from Baker's yeast

Figure (3) shows agarose gel (1%) electrophoresis of purified PCR products for 16S rRNA gene of purified DNA isolate. The amplified products were sequenced and the sequencing data obtained were analyzed using nucleotides BLAST search computer based program where this sequence was compared with that of any other rRNA genes that have been sequenced so far. The resulting data revealed that the isolates were *B. subtilis, G. stearothermophilus*and*B.amyloliquefaciens*when identified by PCR amplification of 16S rDNA;Table (4) showed the strains with their homology percent and Gene Bank Accession Number.

Moreover, the nucleotide sequences was deposited in the GeneBank sequence database, and given the accession numbers KP243195 for *B. subtilis* clone NS4182-01**,** KP243197 for *B. amyloliquefaciens* clone NS4182-03, KP243198 for *B.subtilis* clone NS4182-04 and KP243199 for*G. stearothermophilus*cloneNS4182-05**,** KP273194 for*B. subtilis* clone NS4182-06 and KP273195 for*B. amyloliquefaciens* clone NS4182-07.

Hsieh *et al.*, (2008) compared different methods for identifying *Bacillus* strains capable of producing the antifungal lipopeptides Iturin A and they concluded that the consistency between results of PCR, HPLC and growth inhibition

assay suggest that the PCR method could be used as an alternative tool for fast screening of Iturin A- producing *Bacillus* strains from the environment. Also **Sihem *et al.*, 2011** used sequencing of 16S rDNA gene and Blast sequence comparisonin the GeneBank database to identify two *Bacillus* isolates B27 and B29 as *B. subtilis* and *B. amyloliquefaciens*, respectively.

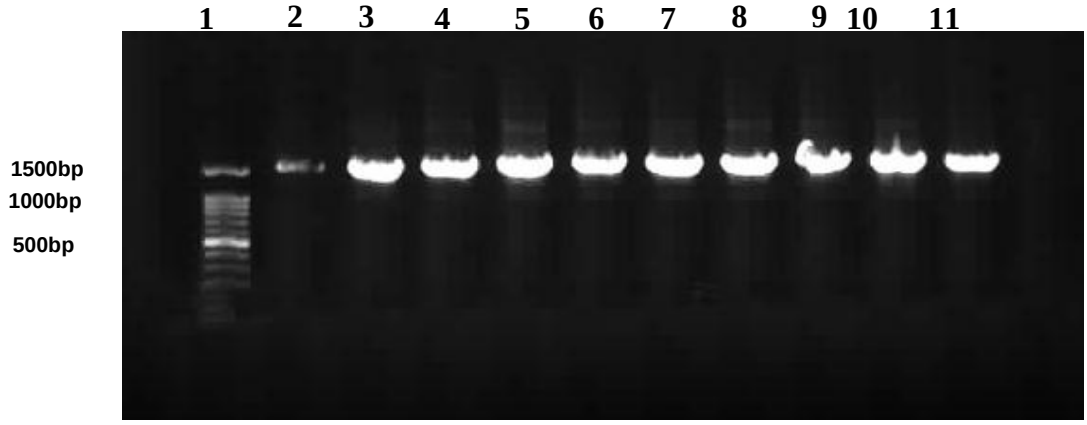

Figure (3): DNA banding pattern obtained from PCR of the *Bacilli* isolates which inhibit *S. cerevisiae*; Lane 1, molecular weight marker; Lane (2,3) strain 1, Lane (4,5) strain 2, Lane (6,7) strain 3 , Lane (8,9)strain4**,** Lane (10) strain 5 and Lane (11) strain 6.

Table (4): Molecular identification of bacterial isolates that inhibit S. cerevisiae growth

Strain No.	Bacterial Isolate Code	Percent hemology with GeneBank database	GeneBank Accession Number of identified isolates	Identiified species with clone in GeneBank	S. cerevisiae inhibition zone (mm) (Mean± SE)
1	NS-00	98%	KP243195	*B. subtilis* NS4182-01	22.8± 2.3
2	NS-21	99%	KP273194	*B. subtilis*NS4182-06	15.5±1.3
3	NS-57	99%	KP243197	*B. amyloliquefaciens*NS4182-03	21.3± 1.5
4	NS-63	99%	KP273195	*B. amyloliquefaciens*NS4182-07	13.5± 0.65
5	NS-135	99%	KP243198	*B. subtilis*NS4182-04	18.3± 1.03
6	NS-138	98%	KP243199	*G. stearothermophilus* NS4182-05	22.8± 2.3

Conclusion

The results of this work revealed that there is a high level of contamination in compressed baker's yeast to the extent of considering bacteria is an important constituent in it. Some of these contaminants showed adverse effect against *S. cerevisiae* and were identified as *B. subtilis, B. amyloliquefaciens* and *G. stearothermophilus*. The results recommend to take more measures to keep the level of bacterial contamination of baker's yeast as low as possible to improve the product quality.

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