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Original Research

Isolation of anti-fungal agent from a soil inhabitant *Streptomyces albaduncus*-M51 and its efficacy against osmophilic food spoilage by *Saccharomyces cerevisiae*

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ABSTRACT

Aim: This study was performed to isolate antifungal substance from actinomycetes species found in soil in order to avoid osmophilic food spoilage. **Methods:** Purified isolates of different actinomycetes collected from soil in Makkah region, Saudi Arabia were screened for their antifungal bioactivity against the unicellular fungal strain, *Saccharomyces cerevisiae*. The actinomycete isolate that showed the highest fungal inhibition potency was selected and identified. Antifungal metabolite fermented by identified isolate was extracted and minimum inhibitory concentration (MIC) was determined. Food preservative efficacy of the extracted crude fungicide was tested against osmophilic foods spoilage. **Results:** Among 59 actinomycetes strains, 10 isolates exhibited antifungal efficacy against *S. cerevisiae*. Only one isolate was the most potent fungicidal and identified as *Streptomyces albaduncus*-M51. Crude antifungal was extracted and MIC value found to be 25μg/ml against the tested yeast strain. Yeast colonies were completely undetected in treated food samples at crude extract concentration (150μl/100ml for liquid foods and 200μl/100mg for solid samples). **Conclusion:** The antifungal agent produced by *S.albaduncus*-M51 demonstrated an obvious inhibitory effect against *S. cerevisiae* that causes osmophilic foods spoilage.

KEY WORDS: Soil; Actinomycetes; Antifungal; Osmophilic Foods; Spoilage; *Saccharomyces cerevisiae*; *Streptomyces albaduncus*-M51.

INTRODUCTION

Actinomycetes are morphologically, physiologically and ecologically diverse group of bacteria. They are major producers of pharmaceuticals, agricultural pesticides and veterinary medicine due to their ability to produce antibiotics [1, 2]. Actinomycetes are always used as producers of bioactive agents such as antibacterial and antifungal. Nevertheless, their application in food biocontrol was not matched. This may regard to the toxicity of many discovered actinomycete bioactive agents against human cell. Among the genera of actinomycetes, genus *Streptomyces* is represented in nature by the largest number of species and varieties, which differ greatly in their morphology, physiology and biochemical activities [3, 4]. In addition, the majority of the antibiotic-producing actinomycetes and antifungal biocontrol agents are found belonging to those species making them economically potent [4-6].

Osmophilic microorganisms are adapted to environments with high osmotic pressures. Many osmophilic microorganisms like yeasts and other fungi can grow well at the low pH and at high sugar concentrations of cultured products. Yeasts are very important within food microbiology as they can create both positive and negative effects. The origin of the positive effects of yeasts in food production probably came about by an accidental contamination of some raw materials with environmental yeasts. Conversely the ability of some yeasts to survive and grow at low pH, low water activity, and in the presence of some common chemical preservatives, makes them potent food spoilage osmophilic organisms responsible for large economic losses of some food products [7-9]. Moreover, food-associated yeasts could be an underestimated source of infections and public health risks [10]. Osmophilic and osmoduric yeasts, osmotolerant micrococci and xerophilic fungi grown in foods have been considered as an important and attractive research area, not only for avoiding economic damages to industries but for reducing public health risks caused by food-borne pathogens. Current losses to the food industry caused by yeast spoilage are estimated at tens of millions pounds annually in the world. Among the most

osmophilic yeasts are *S. cerevisiae* which used as starter for several common food products. In spite of its importance in many different fields of industry as winemaking, baking, and brewing , it has also a hygienic significance in osmophilic foods spoilage [11-13].

Therefore, this study investigates the production of the bioactive fungicidal substances from actinomycetes isolated from soil and evaluates its inhibitory affects against microbial food spoilage by *S. cerevisiae*. Different osmophilic foods were used in this work as a suspected suitable target environment for this yeast growth.

MATERIALS AND METHODS

Study design

Actinomycetes were isolated and purified from soil samples collected from Makkah region, Saudi Arabia. The isolates were screened for their antifungal efficacy against the osmophilic yeast strain *S.cerevisiae*. The actinomycete isolate that demonstrated the highest fungal growth inhibition potency was selected and identified. Fungicidal metabolite fermented by the identified actinomycete isolate was then partially extracted and minimum inhibitory concentration (MIC) was determined. Antifungal efficacy of extracted crude compound was tested as a natural food preservative in sweetened condensed milk, jams of strawberry, apricot and fig and fresh juices of strawberry, guava and extracted (without pulp) orange.

Test organism

Fungal strain: *S. cerevisiae* CAIM. The CAIM culture was obtained from the Microbial Culture Collection Center of Cairo, in Faculty of Agriculture, Ain Shams University, Egypt.

Soil sampling

For the isolation of actinomycetes, several soil samples were randomly collected from different localities in Makkah region, Saudi Arabia during March, 2015. The superficial loose soil layers were removed and the underlying soil samples were collected in sterile polythene bags using an opened soil borer (20 cm depth and 2.5 cm diameter) at depths between 10-20 cm, labeled and transported to the laboratory. Samples were then air dried, mixed thoroughly with $CaCO_3$ (10% w/w), incubated at 30°C for 14 days and screened for actinomycetes [14].

Isolation and purification of actinomycetes

Starch nitrate agar (SNA) medium (diffco) was used for actinomycete isolation, purification and sub-culturing as proceeded by Ibrahim [1]. Different weights of each soil sample (1, 0.7, 0.5, 0.3, 0.1g) were homogenously scattered using sterile spatula on the surface of sterile SNA medium (pH 7.5 before sterilization) poured in petri dishes. Cultivated media were incubated for 7-10 days at 28°C. Developed actinomycete colonies were picked up, streaked on sterile SNA for purification and incubated under the same conditions of isolation. Purified actinomycete isolates were cultured on SNA slants and preserved for successive studies. Long term preservation of isolates was achieved in soil cultures as followed by Laidi et al. [15].

Anti-*S. cerevisiae* screening assay

The antifungal efficacy of all developed actinomycete isolates culture broth was assayed by applying agar-well diffusion test based on the agar diffusion method and expressed as diameter of the inhibition zones as examined by Abd El-Salam and Ibrahim [16]. A heavy loopful of each purified isolate, 5-days culture age, was transferred into a 250 ml Erlenmeyer flask containing 50 ml of sterile starch nitrate broth (SNB) (diffco) adjusted at pH 7.5. Flasks were incubated on a rotary shaker (200 rpm) at 28°C for 7 days. At the end of incubation period, petri dishes containing 25 ml sterile yeast extract malt extract agar (YEMEA) medium (diffco) was seeded with 100 µl inoculum of broth culture of the target fungal strain *S. cerevisiae* adjusted to final density of 10^4 CFU/ml using the McFarland standard (Biomerieux Inc.). Wells of 1.0 cm diameter were aseptically punctured centrally in seeded YEMEA petri dishes and subsequently 0.5 ml of actinomycete culture filtrate was transferred into each well. Positive control was prepared using 0.5 ml nystatin (10 mg/ml) instead of isolate filtrate and 0.5 ml of sterile saline solution (0.85% NaCl) for negative control. All plates were kept in refrigerator for 2-4 hrs to allow filtrate diffusion through agar then incubated at 35°C for 24 hrs. for yeast growth. Diameters (included disc diameter) of *S. cerevisiae* growth inhibition zones formed were measured in mm and recorded. The actinomycetes isolate that recorded the largest inhibition zone diameter was selected as the most potent fungicidal actinomycete isolate and identified.

Actinomycete identification

Most potent antifungal actinomycete isolate was identified based on a great variety of morphological, cultural, physiological and biochemical characteristics as investigated and described by many researchers [1, 4, 17, 18]. Identification was carried out according to the methods mentioned in the manual of international cooperative project for description and deposition of cultures of *Streptomyces* (ISP) [19]. Genus and species level was achieved according to Bergey's Manual of Systematic Bacteriology using the obtained data in addition to total cell hydrolysate and cell wall composition as follow [20, 21]:

Morphological Characterization

Morphological studies were conducted by microscopic observations and growth characteristics in Petri dishes incubated at 28°C for 21 days. Special media such as yeast malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch agar (ISP4) and glycerol asparagine agar (ISP5) (diffco) were used. Microscopic observations were elucidated using: cover slip culture technique;

Gram and acid-fast staining; phase contrast, light and electron microscopy. Electron microscope pictures were taken in National Research Center, Giza, Egypt. Cultural characteristics were examined weekly up to 21 days for aerial mycelium, substrate mycelium as viewed from the reverse side, and diffusible soluble pigments other than melanin. A universal colour language and dictionary of names was consulted [22]. Growth density was evaluated according to Islam et al. as follow: highly growth (++++); good growth (+++); weak growth (++); growth rare/trace growth (+); no growth (-) [18]. Morphology of the spore bearing hyphae with the entire spore chain was observed microscopically. The species involved in the genus *Streptomyces* divide into sections according to the shape of spore chains observed under microscopy as follows: Rectus (R) or straight, flexible (F) or flexeous, Retinaculum-Apertum (RA) and spiral (S).

Chemotaxonomic analysis

Chemotaxonomic analysis was assayed as described by Ibrahim [1]. Cells of actinomycete strain under investigation were obtained after incubation at 28°C for 7 days in tryptone yeast extract broth ISP1. Isomers of diaminopimelic acid in the whole cell hydrolysate and whole cell sugars were determined by thin layer chromatography analysis.

Physiological and biochemical Characterization:

Physiological and biochemical ⁋ criteria of the examined isolate were studied. Growth on Czapek's agar medium (diffco), sensitivity to streptomycin sulphate, nitrate reduction, milk coagulation, gelatin liquefaction, starch hydrolysis and production of catalase and melanoid pigments as well as utilization of different carbon sources as substrates by the *Streptomyces* strain were examined. All tests were performed at incubation temperature 28°C. Melanin reactions were detected by growing the isolate on the ISP media; tryptone-yeast extract broth (ISP1), peptone- yeast extract-iron agar (ISP6) and tyrosine agar (ISP7) (diffco). Detection of deep brown to black diffusible pigment on at least one of these ISP media indicated positive result (+) [23]. Absence of the color was recorded as negative (-). In carbon utilization test, the following sugars were tested: D- glucose (as a positive control), L-arabinose, D-xylose, I-inositol, D-mannitol, D-fructose, rhamnose, raffinose, sucrose, cellulose and galactose. Basal agar medium were prepared without a carbon source and used as negative control.

Physical properties

Temperature, NaCl and pH tolerance of the selected actinomycete species were screened by growing the isolate at different growth temperature such as 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60°C, at NaCl concentrations ranging between 2 and 7% using ISP2 medium and over a wide range of pH adjusted by using phosphate buffer from 4.0 to 9.0 on SNA medium. Growth density in plates was evaluated visually according to Islam et al. [18].

Large Scale Antifungal Fermentation

Actinomycete secondary metabolite was fermented into 20 liters of broth culture as follow: 20 liters of SNB with some supplements as applied by Ibrahim [1]. SNB additives were glucose (diffco) (3%), yeast extract (diffco) (250 ppm) and L- asparagine (BDH) (at equimolecular amount with $NaNO_3$). Medium was adjusted to pH 7.5 using phosphate buffer. The 20 liters was distributed in one liter Erlenmeyer flasks, 500 ml in each. Sterile flasks were inoculated by 10 ml actinomycete isolate SNB culture (10^6 CFU/ml) of 5 days old. All flasks were incubated on a rotary shaker (200 rpm) at 28°C for 7 days. Twenty-liter total volume was filtered through Whatman No.1 filter paper, followed by centrifugation at 5000 rpm for 20 minutes. The clear filtrates were collected for antifungal extraction.

Extraction of antifungal product

Fermented antifungal crude compound was extracted according to Atta et al. [24]. At first 250 ml out of 20 l clear filtrate previously prepared was adjusted at different pH values (4 to 9) to select the best pH at which extraction process produce the highest amount of the active antifungal compound. Extraction process was carried out using seven different solvents; Methanol, Diethyl ether, Chloroform, Ether, Butanol, Ethanol and Benzene (Sphinx). Each solvent was tested separately at the level of 1:1 (v/v) using separation funnel technique. The seven organic phases formed were concentrated to dryness under vacuum using a rotary evaporator at 45°C to obtain 7 dried residues. Each of the 7 residues was dissolved in 3 ml of its corresponding solvent. Then residue-solvent solution was filtered through Whatman No.1 filter paper.

Agar disc diffusion test

The 7 residue-solvent filtrates were tested for their antifungal activity according to agar disc diffusion method to select the best extracting solvent [25]. Sterile Whatman No.1 filter paper discs of 1.0 cm were saturated with the tested filtrates and dried. Each disc was then placed under aseptic conditions at the center of YEMEA medium surface seeded with *S. cerevisiae* and poured in petri dishes. All plates were kept in refrigerator for 2-4 hours to allow filtrate diffusion through agar. Plates were subsequently incubated for 24 and 48 hours at 35°C for yeast growth. After the incubation period, diameters of formed growth inhibition zones were measured in mm including discs diameters. Extractant solvent whose corresponding residue exhibited the largest inhibition zones was selected for antifungal large scale isolation.

The un-extracted remaining 18.750 L filtrate out of the 20 liter broth culture was then washed three consecutive times with the selected extractant using same separation funnel technique. The organic phase was collected and subjected to same steps mentioned above to obtain the residue containing crude antifungal. This extracted actinomycete antifungal residue filtrate was preserved in refrigerator as a

stock for further studies.

Kinetics of antifungal product

Kinetics of the antifungal production was followed for 28 days incubation of actinomycete isolate [4]. A set of flasks each containing 500 ml fermentation medium was prepared as mentioned above. Then 50 ml of this fermentation medium was taken daily to be extracted, as explained before, by previously selected organic solvent. Actinomycete antifungal activity was tested for the daily extracted crude by the method of the agar disc diffusion.

Minimum inhibitory concentration

MIC assay was performed for the previously extracted residue containing antifungal crude obtained in the large scale fermentation section. Agar disc diffusion method was applied under the same conditions proceeded before using YEMEA medium seeded with *S. cerevisiae*. Extracted residue stock was dissolved in the selected solvent to get final concentrations 200, 100, 50, 25 and 12.5 μg/ml and the filter paper discs were saturated with 50 μl tested concentrations. Saturated discs were let to dry. Triplicates of petri dishes were prepared for each concentration. Positive and negative controls were performed using nystatin (10 mg/ml) and saline solution respectively. All Petri dishes were incubated at 35°C for 24 hrs. Diameters of yeast inhibition zones formed surrounding discs together with discs diameters were measured in mm. Diameters mean values were calculated and MIC value was specified. The MIC was determined as the lowest concentration of antifungal showing a zone of inhibition. MIC was expressed in μg/ml.

Osmophilic food preservative efficacy

The extracted fungicidal crude compound was tested for its food preservative efficacy against *S. cerevisiae*. Six variable osmophilic food products were tested. 100 ml canned sweeten condensed milk; home-made jams of strawberry, apricot and fig, 100 g of each; 100 ml of each of strawberry, guava and extracted orange fresh juices (clear juice without pulp). All fresh juices were supplemented with 30% sucrose (diffco). A total of 7 food sets were prepared and sterilized. Each set was formed of the same food sample. Jams were not provided with any additives during cooking except sugar. Clear orange juice was sterilized by filtration using Seitz bacterial filter (Seitz FA Series FA 05). Juice samples with pulp of strawberry and guava and jam samples were sterilized by autoclaving at 121°C and steam at a pressure 1.5 atm. for 10 minutes. The seven sets were tested for their sterility using sterile YEMEA medium. Each food set was supplemented with the actinomycete product (residue filtrate stock) to conduct six concentrations plus control. The six tested concentrations were 300, 250, 200, 150, 100 and 50μl stock/food sample (v/v for liquid and v/g for solid samples). All the seven sets were then inoculated under aseptic conditions with 100µl inoculum/food sample and mixed well. Broth culture of fungal strain *S. cerevisiae* adjusted to final density of 10^4 CFU/ml was used as the target inoculum. All inoculated food samples were incubated at 35°C then inspected daily for a month. Yeast growth was followed via sample monitoring for physical food distortion, yeasty and fermented off-flavors and gassy appearance, and *S. cerevisiae* total count expressed in CFU/ml on YEMEA medium.

RESULTS

Actinomycetes survey for anti-*Saccharomyces cerevisiae*

Purified 59 actinomycete isolates were collected from Makkah soil samples and among them only 10 isolates including M7, M16, M20, M24, M27, M38, M43, M49, M50 and M51 showed fungal growth inhibition zones with diameters more than 15 mm against *S. cerevisiae* (Table 1). Isolate M51 exhibited the strongest antagonistic activity, with an inhibition zone of 30 mm.

Table 1. Growth Inhibition zones (including disc diameter) produced by 10 different actinomycete filtrates of (5 days old) against *Saccharomyces cerevisiae*

Isolate number	Inhibition zone (mm)*
M7	20±0.7
M16	18±1.2
M20	19±1.0
M24	17±1.1
M27	16±1.2
M38	18±1.4
M43	17±1.4
M49	16±1.3
M50	19±0.9
M51	30±0.5

*Means of three replications

Actinomycetes isolate M51 identification

Due to the strongest antagonistic activity against osmophilic *S. cerevisiae*, M51 isolate was selected to be identified. Microscopic investigation and staining properties of isolate M51 showed that it is Gram positive and non-acid fast. Also, it has filamentous, extensively branched aerial and substrate mycelia. No fragmentation was observed on either aerial or substrate mycelium. Further observations by transmission and scanning electron microscope detected formation of short closed spirals of elongated spiny spores on short conidiophore (Figure 1). Sclerotia, pycnidial sporangia or synnemata-like structures were not found. M51 growth patterns, amount of growth, aerial mass colour, reverse colour and soluble pigment colour on four ISP and SNA media for 21 days were recorded in Table 2. Aerial mycelium mass colour of strain M51 was found belonging to grey series based on universal colour language and dictionary of names. In addition it possessed an earthy odour. On the other hand, chemotaxonomic analysis denoted that LL-diaminopimelic acid and glycine was chemotype cell wall. No sugars were detected in the total cell hydrolysate.

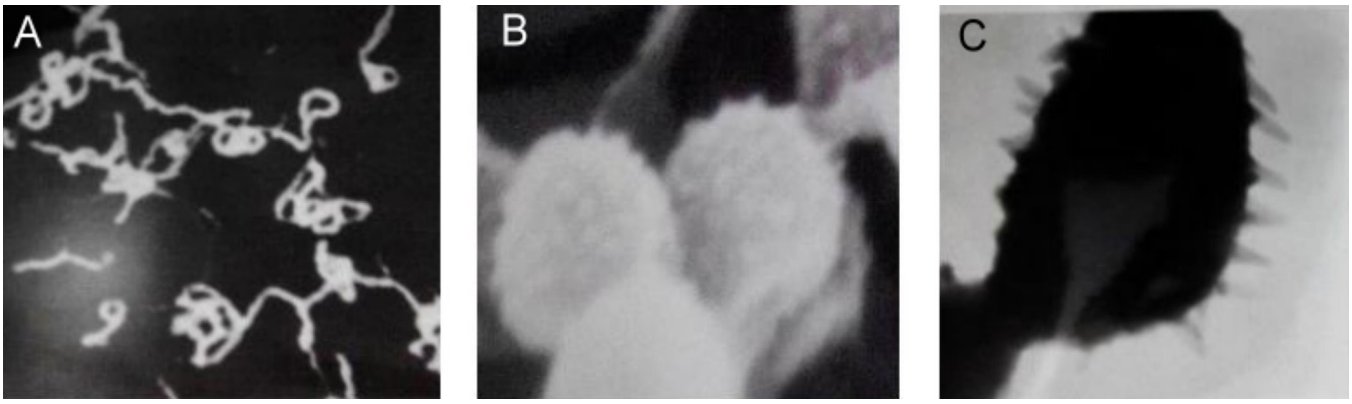

Figure 1. Scanning and transmission electron microscopy of *Streptomyces albaduncus*-M51

a- Closed spiral spore chains with short conidiophores on aerial mycelia. (scanning electron microscope, x 2000)
b- Spiny spores (scanning electron microscope, x 10000)
c- Elongated spiny spores (transmission electron microscope, x 63000)

Table 2. Cultural characteristics of the selected M51 isolate on different media after incubation period of 21 days at 28°C

Media	Growth density	Colony properties	Aerial mycelium	Reverse colour	Soluble pigment
Yeast extract malt extract agar	+++ [b]	Powder	Dark grey	Dark brown	Yellowish green
Oatmeal agar	+++ [b]	Velvet	Grey	Beige	Yellowish green
Inorganic salts-starch agar	++++ [a]	Powder	Dark greenish grey	Brown	Yellowish green
Glycerol asparagine agar	+++ [b]	Velvet	Dark grey	Dark brown	Yellowish green
Starch Nitrate agar	++++ [a]	Powder	Dark greenish grey	Brown	Yellowish green
Czapek's agar	++++ [a]	Powder	Dark grey	Brown	Negative

[a] Highly growth (++++); [b] good growth (+++)

Results of studied physiological, biochemical and physical characteristics of isolate M51 were displayed in Tables 3 and 4. It is demonstrated from Table 3 that this isolate is catalase positive, sensitive to streptomycin sulphate and able to hydrolyze starch, liquefy gelatin, coagulate milk, reduce nitrate, produce melanoid pigments and grow on Czapek's agar. Isolate M51 could also fully utilize D-glucose, D-xylose, maltose, galactose, and D-fructose as the carbon source. It could partially utilize L-arabinose, sucrose, L-inositol, D-mannitol, rhamnose, raffinose, while not able to utilize cellulose as presented in Table 4.

The selected M51 isolate proved to be a thermotolerant organism. It is able to grow at 40 and 50°C and highly growth was detected at 30-35°C. Weak growth was appeared at 4-7% NaCl while highly and good growth was obtained at 2 and 3% NaCl respectively. Optimum pH for growth was 7.5, it can also grow between 4.0 and 9.0 pH. This isolate is fast grower actinomycete; good growth appeared after 48 hrs. It is also resistant to many antibiotics as cefadroxil, neomycin, penicillin, rifampicin, gentamycin and streptomycin sulphate.

In accordance with Bergey's Manual of Systematic Bacteriology (1989 and 1994), results of morphological, physiological, biochemical and physical characteristics of M51 isolate indicated that it shared the characteristic features of genus *Streptomyces* and closely related to *Streptomyces albaduncus*. So it was named *S.albaduncus*-M51.

Table 3. Physiological, biochemical and physical properties of the selected M51 isolate

Test parameter	Result
Production of melanoid pigments	
Tryptone-yeast extract broth ISP1	Negative
Peptone- yeast extract-iron agar ISP6	Black
Tyrosine agar ISP7	Brown
Liquefaction of gelatin	Positive
Coagulation of milk	Positive
Hydrolysis of starch	Positive
Reduction of nitrate	Positive
Production of catalase	Positive
Antimicrobial activity	Positive
Streptomycin sulphate sensitivity	Sensitive
Tolerance to NaCl	
2%	[a] ++++
3%	[b] +++
4-7%	[c] ++
Growth at 10 – 15 °C	- [e]
Growth at 20 °C	++ [d]
Growth at 25°C	+++ [c]
Growth at 30 – 35°C	++++ [a]
Growth at 40°C	+++ [b]
Growth at 45 and 50 °C	++ [d]
Growth at 55 - 60 °C	- [e]
Acid fast stain	Non

[a] Highly growth (++++); [b] good growth (+++); [c] Weak growth (++); [d] growth Rare/Trace growth (+); [e] No growth (-)

Table 4. Carbon sources utilization by the selected M51 isolate

C-source	Result	C-source	Result
D-glucose	++++ [a]	D-mannitol	++ [c]
L-arabinose	++ [c]	D-fructose	++++ [a]
Sucrose	++ [c]	Rhamnose	++ [c]
D-xylose	++++ [a]	Raffinose	++ [c]
I-inositol	++ [c]	Galactose	++++ [a]
Maltose	++++ [a]	Cellulose	- [e]

[a] Highly growth (++++); [b] good growth (+++); [c] Weak growth (++); [d] growth Rare/Trace growth (+); [e] No growth (-)

Fermentation, Extraction and kinetics of antifungal product

Seven different solvents were tested for best extraction result. They were Methanol, Diethyl ether, Chloroform, Ether, Butanol, Ethanol and Benzene. Among these solvents, diethyl ether (1:1, v/v) at filtrate of pH 7.0 registered the best conditions for antifungal crude extraction process. The produced residue of isolate M51 exhibited the largest yeast inhibition zone. Isolated antifungal crude was yellowish green liquid. Figure 2 demonstrated the kinetics of antifungal production. It is clear from this figure that the antagonistic activity of M51 isolate was appeared at the third day of incubation. It increased gradually to reach its maximum with the 5th day of *S.albaduncus*-M51 incubation and remain more or less stable thereafter.

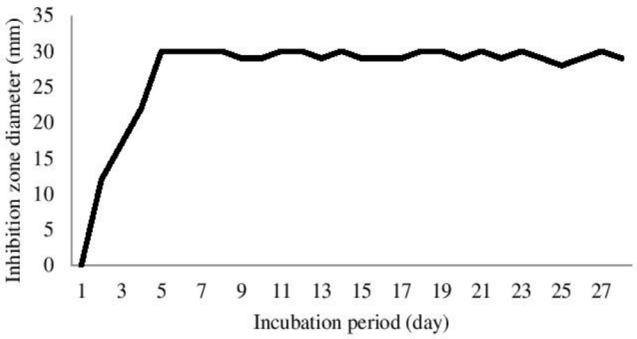

Figure 2. Time course of antifungal production of *Streptomyces albaduncus*-M51 on SNA medium

Minimum inhibitory concentration

Extracted crude MIC value against the tested yeast strain was determined by agar dilution method. On comparing documented data in the dose response study, it was found that high fungal growth inhibition noticed with increasing antifungal crude concentration. In contrast, low extracted crude concentration (25 µg/ml) weakly inhibited the growth of *S. cerevisiae*. On the other hand, at a highly concentration (50 µg/ml), antifungal crude exhibited a marked fungicidal efficacy against yeast strain. A 200µg/ml crude concentration indicated the highest ever toxicity as completely yeast growth inhibition was established. As a result of achieved data, MIC value of crude antifungal was registered at 25µg/ml against *S. cerevisiae*. Deduced diameters mean values of *S. cerevisiae* growth inhibition zones at concentration 25µg/ml was 14 mm and at 50µg/ml it was 21 mm, while at concentration 100µg/ml inhibition zone was 36 mm.

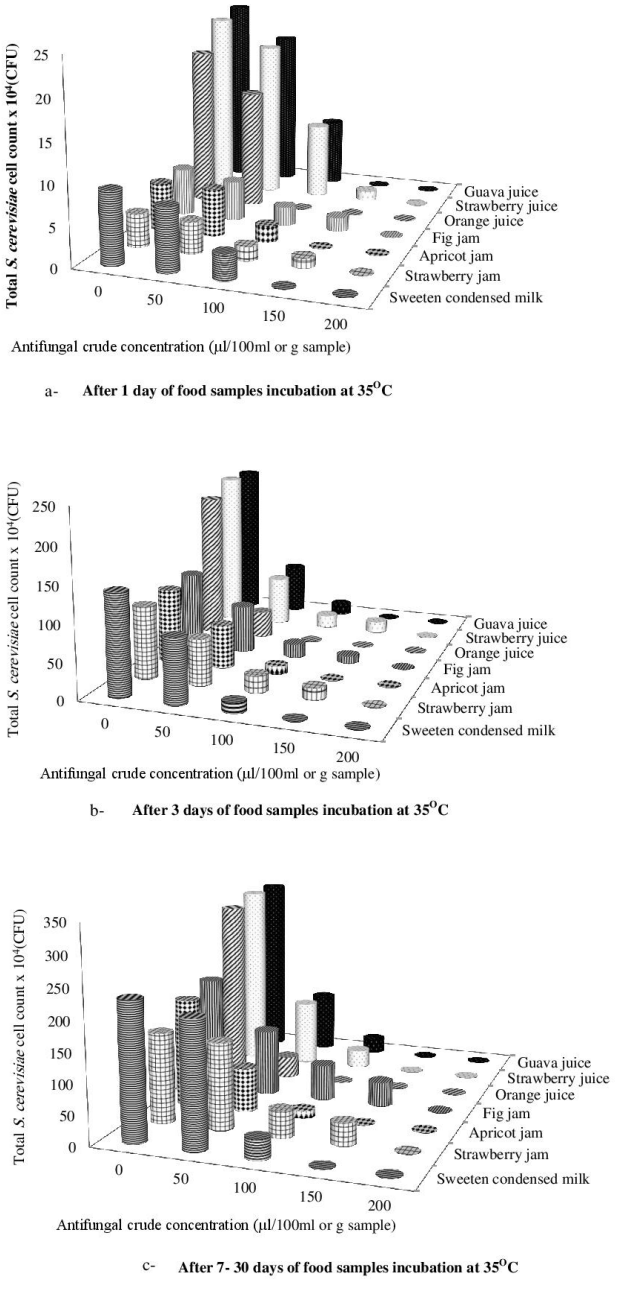

Figure 3. (a,b,c). Total yeast cell count (CFU/ml) in 100 ml liquid and l00g solid osmophilic food samples inoculated with 100µl *Saccharomyces cerevisiae* / sample in relation to different concentrations of isolate M51 antifungal crude product at different incubation period (1,3,7-30 days)

Osmophilic food preservative efficacy

Testing of isolate M51 fungicidal efficacy against osmophilic food preservation was performed by monitoring yeast counting and physical distortion in six sets of osmophilic food products; each was adjusted at six residue concentrations. Recorded data illustrated in Table 5 and Figure 3 demonstrated total yeast cell count and food spoilage symptoms in relation to incubation period and crude concentration. Observed results showed that neither yeast count nor physical food distortion was recorded in all treated food sets after a month of incubation at antifungal crude concentrations 300, 250, and 200 µl/100 (ml or g) food samples and at 150µl/100ml for liquid foods and 200µl/100mg for solid samples. Yeasty, fermented off-flavors and gassy appearance were totally undetected. Similar observations were also noticed with orange juice 100µl/100ml and apricot jam at 150µl/100g after 7 days of samples incubation. All food sets under investigation did not exhibit any of food spoilage symptoms after one day of incubation

Table 5. Food spoilage symptoms in 100 ml liquid and 100g solid osmophilic food samples inoculated with 100µl *Saccharomyces cerevisiae*/ sample in relation to different concentrations of isolate M51 antifungal crude product at different incubation period (day)

Antifungal crude concentration (µl/sample)	Osmophilic food samples (100ml or g)	Food spoilage symptoms	
		Incubation period (day)	
		3	7 - 30
Control (0)	Sweeten condensed milk	Fermented off-flavors	Fermented off-flavors and gassy appearance
	Strawberry jam	Yeastyappearance	Yeastyappearance
	Apricot jam	Yeastyappearance	Yeastyappearance
	Fig jam	Yeastyappearance	Yeastyappearance
	Extracted fresh orange juice + 30% sucrose	Fermented off-flavors	Fermented off-flavors and gassy appearance
	Fresh strawberry juice + 30% sucrose	Fermented off-flavors	Fermented off-flavors and gassy appearance
	Fresh guava juice + 30% sucrose	Fermented off-flavors	Fermented off-flavors and gassy appearance
50	Sweeten condensed milk	Moderate fermented off-flavors	Fermented off-flavors and gassy appearance
	Strawberry jam	Slight yeasty appearance	Yeastyappearance
	Apricot jam	Slight yeasty appearance	Slight yeasty appearance
	Fig jam	Slight yeasty appearance	Yeastyappearance
	Extracted fresh orange juice + 30% sucrose	Weak fermented off-flavors	Weak fermented off-flavors
	Fresh strawberry juice + 30% sucrose	Moderate fermented off-flavors	Fermented off-flavors and gassy appearance
	Fresh guavajuice+30% sucrose	Moderate fermented off-flavors	Fermented off-flavors and gassy appearance
100	Sweeten condensed milk	No change	Weak fermented off-flavors
	Strawberry jam	No change	Very slight yeasty appearance
	Apricot jam	No change	No change
	Fig jam	No change	Slight yeasty appearance
	Extracted fresh orange juice + 30% sucrose	No change	No change
	Fresh strawberry juice + 30% sucrose	No change	Weak fermented off-flavors
	Fresh guava juice + juice + 30% sucrose	No change	Weak fermented off-flavors
150	Sweeten condensed milk	No change	No change
	Strawberry jam	No change	Very slight yeasty appearance
	Apricot jam	No change	No change
	Fig jam	No change	Very slight yeasty appearance
	Extracted fresh orange juice + 30% sucrose	No change	No change
	Fresh strawberry juice + 30% sucrose	No change	No change
	Fresh guava juice + juice +30% sucrose	No change	No change

DISCUSSION

Soil is the best natural environment source for *Streptomyces* isolation, in which they compete with naturally colonized microbiota among which are also plant pathogens or spoilage microorganisms. For that reason we systematically screened for soil inhabitant antagonistic *Streptomyces* as antifungal-producing agents. We evaluated 59 actinomycetes strains for their antifungal biocontrol activities against food- and public health-associated yeasts. For this purpose, the osmophilic yeast species, *S. cerevisiae*, was used which is a potent food spoilage organism responsible for large economic losses of some food products and a source of infections and public health risks. The results of this study supported by other investigations which registered that genus *Streptomyces* is economically potent antifungal biocontrol microbiota [4-6, 26]. In the present research, Saudi Arabia soil (Makkah region) was the source of actinomycetes isolation and one isolate proved the highest antifungal potency. This finding was in consistent with results obtained by Atta who used Saudi Arabia soil (Taif City in Makkah region) as the source of actinomycetes isolation and found only one isolate exhibited a wide spectrum of antimicrobial activities including *S. cerevisiae* [27].

The present work describes soil isolated actinomycete strain *S.albaduncus*-M51. According to Bergey's Manual of Systematic Bacteriology (1989 and 1994), our isolate is belonging to *S. albaduncus*. It shares most of the characteristic features of genus *Streptomyces* and species *albaduncus*. Both *S.albaduncus* and *S.albaduncus*-M51 have elongated spiny spores, spiral spore chains, same cell wall chemotype, the ability to produce antibiotics and almost same responses to physiological, biochemical and physical reactions. While, *S.albaduncus*-M51 produces soluble pigments and *S.albaduncus* does not. Some carbon and nitrogen sources were also utilized in different manners between them.

SNB medium with supplements were used for large scale extraction test in this work because Ibrahim registered that it was the best for the production of antibacterial and antifungal agents by *Streptomyces sp*. He examined condition optimization for antimicrobial agents' production [1].

The results of this work announced that the best solvent for antifungal extraction is diethyl ether (1:1, v/v) at filtrate of pH 7.0. Ibrahim examined different solvent and solvent systems to isolate a *Streptomyces sp*. antibiotic metabolite and found that diethyl ether was the best [1]. While Atta et al. and Atta used ethyl acetate and *n*-butanol respectively to extract actinomycete active metabolites (1:1, v/v) at pH 7.0 [24, 27]. Results also indicated that maximum antagonistic activity of *S. albaduncus*-M51 reached its maximum with the 5th day of incubation which agreed with Atta who tested the effect of incubation period on actinomycete antagonistic activity against different bacteria and yeasts. He found that the level of antibiotic yield increased gradually with increasing the incubation period up to the end of 5 days [27]. Otherwise, Nanjwade et al. recorded 84 incubation hrs. for maximum antagonistic activity of actinomycete A4 against *Klebsiella pneumoniae* [26].

The isolated crude metabolites showed significant antifungal activity against yeast strain at concentration 50μg/ml and the MIC was 25μg/ml. Results recorded by Atta et al. showed that the MIC of the compound fermented by *Streptomyces albidoflavus*143 against unicellular fungi *S. cervisiae* ATCC 9763 was 31.25 μg/ml and *Candida albicans* RIMU 3669 was 25.25 μg/ml [24]. While Atta registered the MIC value for antifungal metabolite produced by *Streptomyces* strain No T-4 against *S. cerevisiae* ATCC 9763 was 46.9 μg/ml [27].

Fungi can be particularly troublesome in food and food products, because they can adapt to the environment of the food. Spoilage of osmophilic foods is managed by using biocontrol agents as an alternative safe method to the use of synthetic fungicides. Today in food production, yeasts are more usually linked with food spoilage. ⁋ For this reason we tried to manage a technique to biocontrol yeast food spoilage. This is the first study of applying a fermented product isolated by *Streptomyces* in osmophilic food samples as antagonist of *S. cerevisiae*. Some published researches investigated the biocontrol of food spoilage by natural products for example plant products as inspected by Abd El-Salam and Ibrahim and Ibrahim and Abd El-Salam who tested food biocontrol by essential plant oils [16, 25]. Other studies managed the effect of fermented actinomycetes products on harmful microorganisms using only lab microbiological media [5, 27-29]. The spoilage caused by yeasts may alter both the physical and sensorial properties of foods as a result of their activity as registered by Loureiro and Querol [30]. That was also confirmed in this investigation when food products were inoculated with *S.cervisiae*. The results of this study emphasized that the extracted actinomycete metabolite prevented the growth of *S.cervisiae* in all tested osmophilic food samples and no food spoilage was detected at concentration 200μl/100mg for long period. That means a long shelf life period for foods treated with that compound.

RECOMMENDATION

In recommendation, this compound should be not commercially applied until it is completely purified, identified and tested for human hygiene safety in further toxicological study.

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