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Date extract as an alternative natural medium for cultivating *Streptomyces* sp. SA32 and enhancing its antifungal activity

Said Belghit[1,*], Boubekeur Badji[2], Andrea Vannini[3], El Hadj Driche[4], Zineddine Benbekhti[1,5], Abdelghani Zitouni[2] and Noureddine Bouras[1,2]

[1]*Laboratory for the Valorization and Conservation of Arid Ecosystems (LVCAE), University of Ghardaia*, 47000 Ghardaïa, Algeria

[2]*Laboratory of Microbial Systems Biology (LMSB), Higher Normal School of Kouba*, P.O. Box 92, 16050, Algiers, Algeria

[3]*Department of Pharmacy and Biotechnology (FaBiT), University of Bologna*, Via Selmi 3, 40126 Bologna, Italy

[4]*Laboratory of Molecular Biology, Genomics and Bioinformatics (LBMGB), Hassiba Ben Bouali University*, 02000 Chlef, Algeria

[5]*Laboratory Antibiotics Antifungals, Physicochemical, Synthesis and Biological Activities, University of Tlemcen*, 13000 Tlemcen, Algeria

***Corresponding author**: belghit.said@univ-ghardaia.edu.dz

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Abstract: Our study investigated the use of a natural medium, date extract, which has a low market value, to cultivate a bacterial strain that produces antifungal compounds. Strain SA32 was isolated by the method of suspension dilution using chitin medium from Algerian Saharan soil. Analysis of its 16S rRNA indicated that it belongs to the genus *Streptomyces*, showing a 99.52% identity with *Streptomyces griseoflavus* LMG 19344^T. The kinetics of antifungal activity production on three solid media, date extract, ISP-2 (International Streptomyces Project-2), and Bennett, revealed that maximal production occurred on day 4 for ISP-2 and Bennett, and on day 5 for date extract. In broth media, maximal antifungal activities were observed on the 4th day of incubation for all three media. The average inhibition zones were larger in the date extract than in ISP-2 and Bennett, regardless of whether the medium was liquid or solid. The antifungal activity was detected only in the *n*-butanol fraction. HPLC analysis of the crude butanolic extract identified one fraction displaying activity against *Aspergillus carbonarius*. These findings indicated that the date extract can serve as a natural alternative to conventional culture media to produce *Streptomyces* sp. SA32 antifungals.

Keywords: natural culture medium, date extract, *Streptomyces*, conventional media, antifungal compounds

INTRODUCTION

The development of microbiology has been greatly advanced by the discovery and continuous optimization of culture media [1]. These media are extensively used for the isolation, identification, and antimicrobial susceptibility testing of various pathogenic microorganisms [2]. Louis Pasteur was the first to successfully cultivate bacteria reproducibly in the 1860s, following the development of the first artificial culture medium [3]. Culture media are primarily composed of water and essential nutrients supplemented with specific growth factors required for the proliferation of different microorganisms [4]. The formulation of these media is tailored to the metabolic capabilities of the target microorganisms to utilize the provided components [5].

The cultivation of *Actinomycetota* is important, as these microorganisms are a valuable source of secondary metabolites with significant applications for human use [4]. They are a promising source of natural antifungal compounds [6]. Optimizing the cultivation conditions of these microorganisms is essential to maximize their metabolic potential, with the choice of cultivation medium playing a crucial role [7]. The selection of carbon and nitrogen sources determines the formation of

metabolites and affects the activity of certain compounds [8]. Moreover, the high cost of synthetic culture media for microbial production poses a significant challenge, particularly in developing countries, impacting the quality of microbiological research [9].

Conventional media are often expensive due to their specific ingredients [10]. Therefore, identifying affordable, natural media for antibiotic production is crucial [11]. Nutrient availability and physical parameters can be modulated to enhance or reduce the yield of secondary metabolites [4].

This study aims to assess the ability of a *Streptomyces* strain to produce antifungal compounds in a natural date extract medium. Their performance was compared to that in two commonly used conventional media, ISP-2 and Bennett, known to support the growth of this bacterial group and the production of secondary metabolites. Comparison was carried out in both liquid and solid culture forms.

MATERIALS AND METHODS

Isolation of the *Streptomyces* strain

Streptomyces strain SA32 was isolated utilizing the suspension dilution method from a rhizospheric soil sample collected in a southern Algerian region called Metlili Châamba (32°16\'N, 3°37\'E, Ghardaïa province). The isolation medium used was chitin-vitamin agar composed of (g/L): colloidal chitin, 2; K_2HPO_4, 0.7; KH_2PO_4, 0.3; $MgSO_4$, 0.5; $FeSO_4$, 0.01; $ZnSO_4$, 0.001; $MnCl_2$, 0.001; agar, 15; and vitamins including thiamine-HCl, riboflavin, niacin, pyridoxine-HCl, inositol, calcium pantothenate, at a rate of 0.5 mg/L for each [12], and supplemented with chloramphenicol (25 mg/L) and actidione (50 mg/L) to prevent unwanted bacteria and microfungi, respectively. The dishes were incubated at 30°C for 21 days. The colonies were transferred to ISP-2 agar medium [13] after the incubation period and subcultured until a pure isolate was obtained.

Taxonomic identification of the strain

The cultural and morphological traits of strain SA32 were observed on various culture media, including date extract agar; nutrient agar composed of (g/L): peptone 5, yeast extract 1.5, beef extract 1.5, NaCl 5, agar 15; Bennett agar composed of (g/L): yeast extract 1, beef extract 1, casein enzymic hydrolysate 2, glucose 10, agar 15 [14]; ISP-2 composed of (g/L): glucose 4, yeast extract 4, malt extract 10, agar 15; ISP-3 composed of (g/L): oatmeal 20, agar 15, trace salts solution 1 mL; ISP-4 composed of (g/L): soluble starch 10, agar 15, (NH4)2SO4 2, CaCO3 2, MgSO4 1, dipotassium phosphate 1, NaCl 1, trace salts solution 1 mL [13]. The biomass of strain SA32 was cultivated in ISP-2 broth in shake flasks at 30°C for four days to conduct chemical analyses. The isomer of diaminopimelic acid (DAP), total cell sugar composition, and phospholipid profile were determined as described previously [15-17]. The physiological properties of strain SA32 were evaluated according to [18], with a total of 57 tests performed.

For the molecular study, strain SA32 was grown at 30°C in a shake flask with 100 mL of ISP-2 medium for four days. Genomic DNA was extracted as described in [19], and the 16S rRNA gene was amplified by PCR. The sequencing reaction was carried out by Eurofins Genomics Eu sequencing GmbH, based in Cologne, Germany. The resulting sequence was compared for similarity using the EzBioCloud database (http://eztaxon-e.ezbiocloud.net/). Molecular evolution analyses were performed using MEGA ver. 11 software. The CLUSTAL W program was used to align the 16S rRNA gene sequence of strain SA32 [20] and compare it with matching nucleotide sequences from reference *Streptomyces* species available in GenBank. A phylogenetic tree was constructed using the neighbor-joining (NJ) method [21]. Tree topologies were assessed using bootstrap analysis [22] by 1,000 resamples of the NJ data set.

Determination of basic chemical composition analysis of the date extract

The content of the basic components of date extract (D4) was determined, including glucose, sucrose, ash content, total nitrogen, proteins, and lipids. The glucose concentration in the date extract was determined using the glucose oxidase-peroxidase (GOD-PAP) enzymatic colorimetric method with a commercial kit (Biolabo, France), following the manufacturer's instructions. For the assay, 10 µL of date extract, glucose standard, or blank were added to 1 mL of reagent containing glucose oxidase, peroxidase, 4-aminoantipyrine, and

phenol in phosphate buffer (pH 7.5). The mixtures were incubated at 37°C for 10 min, and absorbance was measured at 505 nm against the reagent blank using a UV-visible spectrophotometer. The glucose concentration in the extracts was calculated using the following formula:

glucose (mg/dL) = absorbance of sample × concentration of standard/absorbance of standard

The method is based on the glucose oxidase-peroxidase reaction principle of Trinder and has been validated for analytical applications [23]. The sucrose content of the date extract was determined according to the method of Lane and Eynon [24]. Ash content was determined using the gravimetric method [25]. Total nitrogen and proteins were determined according to the method of Kjeldahl [26]. Total lipid concentration in the extracts was determined using the Bligh and Dyer method [27].

Antifungal activity and target microorganisms

The antifungal potential of strain SA32 was assessed against various fungal species originating from the collection of the Laboratory of Microbial Systems Biology in Algiers. These included clinically important pathogens such as *Candida albicans* ATCC 10231 and *Candida albicans* IPA200; agriculturally important pathogens such as *Fusarium graminearum* Fg3, *Umbelopsis ramanniana* NRRL 1829, and *Fusarium culmorum* FC200; as well as species of both clinical and agricultural relevance, including *Aspergillus carbonarius* M333, *Saccharomyces cerevisiae* ATCC 4226, *Aspergillus flavus* NRRL 3251, *Aspergillus westerdijkiae* NRRL 3174, and *Aspergillus parasiticus* CBS 100926. The assay was performed using the streak method on a medium composed of date extract supplemented with $NaNO_3$ as the nitrogen source [28]. Dishes were first inoculated with a single streak of the SA32 culture and incubated at 30°C for 10 days. Subsequently, the target fungi were streaked perpendicularly to the *Streptomyces* strain. The antifungal effect was determined by measuring the inhibition zone between the fungal streaks and the edge of the *Streptomyces* colony after 48 hours of incubation at 30°C.

Production of antifungal compounds

Three culture media were used: one natural medium (date extract) and two commonly used complex media, ISP-2 and Bennett, for antimicrobial metabolite production by *Streptomyces*. ISP-2 contains glucose, yeast extract, malt extract, and agar, whereas Bennett medium contains glucose, peptone, meat extract, and agar. The pH of each medium was adjusted to 7.0 before autoclaving.

Preparation of date extract

After thoroughly washing the palm dates (immature, unfertilized dates of a type named Ghars), which have a lower market value (1 kg = 0.1 €), and removing the pits, water was added at a ratio of 2 L per kg of date pulp. The mixture was then heated to 80°C for 2 h on a thermo magnetic stirrer. After heating, the mixture was filtered through filter paper, and the resulting extract was centrifuged at 4000×g for 10 min. The supernatant obtained was designated as the stock solution (D0). This stock solution was subsequently diluted as follows: D1 (80% D0+20% distilled water), D2 (60% D0+40% distilled water), D3 (40% D0+60% distilled water), and D4 (20% D0+80% distilled water). These dilutions were used to prepare media for the production of antifungal compounds by the studied bacterium.

Selection of date extract dilution

To determine the optimal solid medium for growth and antifungal production, agar (20 g/L) was added to the various date extract dilutions. The pH of the media was adjusted to 7 before use. The strain was inoculated in tight streaks across the surface of Petri dishes, each containing 20 mL of the prepared medium. The cultures were then incubated at 30°C for up to 14 days.

Kinetics of antifungal production on solid media

The antifungal activity of the strain was evaluated using the agar cylinder method [28]. In this approach, the strain was inoculated in dense strips onto three different solid media (ISP-2, Bennett, and date extract) and incubated at 30°C. Over a 10-day period, 10 mm agar cylinders were removed daily and placed on the surface of Sabouraud agar previously inoculated with

Aspergillus carbonarius M333 (10^8 CFU/mL). The Petri dishes were then incubated at 30°C, and the inhibition zones were measured with a caliper after 48 h of incubation. A control test was performed by using agar cylinders from different dilutions of solid date extract (D0, D1, D2, D3, and D4) without strain SA32, which were placed on the surface of Petri dishes containing Sabouraud agar previously inoculated with *Aspergillus carbonarius* M333, *A. flavus* NRRL 3251, and *A. parasiticus* CBS 100926.

Kinetics of antifungal production on liquid media

Production of antifungal compounds was monitored over 10 days in three broth media: date extract, Bennett, and ISP-2. The media were prepared in 500-mL Erlenmeyer flasks containing 100 mL of medium. The pH of each medium was adjusted to 7.2 before autoclaving. Each flask containing 100 mL of medium was inoculated with 3% (v/v) of the strain culture grown in the same medium for 72 h at 30°C. The cultures were incubated on a rotary shaker (250 rpm) at 30°C for 10 days. Two-milliliter aliquots were collected every day to estimate antifungal activity using the well diffusion method against *A. carbonarius* M333 [28]. A volume of 20 mL of Sabouraud agar (containing 10 g/L agar) was inoculated with 80 µL of fungal suspension containing 10^8 CFU/mL of the target organism and then poured onto a Petri dish and allowed to solidify at room temperature for 30 min. Wells, 10 mm in diameter, were aseptically bored into the culture medium, and 200 µL of each culture filtrate was added to each well. The inhibition zones were measured using a vernier caliper. To assess pH and biomass, 2 mL of culture was collected in pre-weighed Eppendorf tubes and centrifuged at 11,000×g for 10 min. The supernatant was used to measure pH, while the mycelium pellet was washed twice with distilled water. After drying the tubes for 24 h at 100°C, the dry weight was determined by weighing the tubes again. Tests were conducted in triplicate.

Extraction of antifungal metabolites

The culture broth of the isolate SA32, incubated for 4 days in the three different media, was centrifuged to separate the biomass. To evaluate the extraction efficiency of various organic solvents, the cell-free supernatant was extracted with equal volumes of *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol. Each extract was then evaporated to dryness. The resulting dry residues were reconstituted in 1 mL of methanol and tested for antifungal activity against the *Aspergillus carbonarius* strain using the diffusion method described by [29].

Purification by HPLC

To detect the active antifungal compounds produced by the strain SA32 in the date extract liquid medium, purification was carried out on the crude butanolic extract of the isolate using a reversed-phase C18 column (5 µm, 250 mm, Teknokroma) equipped with a guard column (5 µm, 10×10 mm). The separation protocol was adapted according to [30]. Elution was carried out at a flow rate of 1 mL/min using a linear gradient ranging from 20% to 100% methanol in water for 50 min. Detection of analytes was conducted via UV absorbance at 220 nm. The collected peaks were individually pooled, concentrated, and evaluated for antifungal activity against *A. carbonarius* M333 using the disk diffusion assay.

Statistical analysis

Statistical analyses were conducted using Python (v3.10) with the scipy, statsmodels, and scikit-posthocs libraries. Due to the non-parametric distribution of the data, non-parametric methods were consistently employed. Differences in antifungal activity among the culture media, considering multiple time points and biological replicates, were evaluated using the Friedman test in conjunction with the Nemenyi post hoc test for pairwise comparisons. Data were visualized using the seaborn and matplotlib libraries. These visualizations include dot plots with annotated median values and P values indicating pairwise differences. All statistical tests were two-tailed, with significance set at $P<0.05$.

RESULTS

This study investigates the feasibility of using a low-cost, date-based natural culture medium for the production of secondary metabolites by *Streptomyces* sp. SA32, proposing it as a sustainable alternative to conventional media.

Table 1. *Streptomyces* sp. SA32 characteristics on different culture media

Medium	Growth[a]	Aerial mycelium	Substrate mycelium
Yeast extract-malt extract agar (ISP-2)	+++	Whitish gray	Dark gray
Oatmeal agar (ISP-3)	+++	Whitish gray	Grayish yellow
Inorganic salts-starch agar (ISP-4)	+++	Whitish gray	Grayish yellow
Bennett (BN)	++	Whitish gray	Grayish yellow
Date extract (D4 + $NaNO_3$)	+++	Whitish gray	Light yellow
Nutrient agar	++	Whitish gray	Light yellow

+++: abundant; ++: moderate

Table 2. Physiological characteristics of strain SA32

Test	Result[a]	Test	Result[a]
Utilization of sugars (1% w/v)		Acetate	+
Glucose	+	Benzoate	—
Fructose	+	Succinate	+
Galactose	+	Tartrate	+
Sucrose	+	**Hydrolysis of organic compounds**	
Lactose	+	Xanthine	+
Xylose	+	Guanine	+
Ribose	+	Adenine	+
Maltose	+	Tyrosine	+
Sorbitol	—	Gelatine	+
Trehalose	+	Esculin	—
Mannitol	+	Hypoxanthine	+
Mannose	+	Starch	+
Melibiose	+	**Resistance to antibiotics (µg/mL)**	
Raffinose	+	Streptomycin (10)	—
Rhamnose	+	Penicillin (25)	—
Arabinose	—	Chloramphenicol (25)	+
Adonitol	—	Kanamycin (25)	—
Cellobiose	+	Rifampicin (5)	+
Erythritol	—	Vancomycin (5)	+
myo-Inositol	+	Erythromycin (10)	+
Utilization of amino acids (0.1% w/v)		**Growth with (% w/v)**	
L-Serine	+	Phenol (0.001)	—
L-Proline	+	Crystal violet (0.05)	—
L-Alanine	+	**Growth with/at**	
L-Arginine	+	NaCl (10%)	+
L-Histidine	+	NaCl (15%)	—
L-Phenylalanine	+	30 and 40°C	+
Decarboxylation of sodium salts		45°C	—
Oxalate	+	pH 5 and 7	+
Citrate	—	pH 9	—
Pyruvate	+	Production of melanoid pigments	—

[a]: + positive test; – negative test

Selection of date extract dilution

Before producing antifungal compounds with strain SA32, we assessed its growth on solid media consisting of date extract and agar at various dilutions (D0, D1, D2, D3, and D4). The D0, D1, D2, and D3 dilutions did not support any growth of strain SA32, while moderate growth was observed in the D4 dilution. To enhance this growth, we added a nitrogen source (D4+$NaNO_3$). The results indicated significantly improved growth in the D4 medium supplemented with $NaNO_3$.

Morphological characteristics

Isolate SA32 exhibited robust growth on ISP-2, ISP-3, ISP-4, and date extract (D4+$NaNO_3$) media, while showing moderate growth on Bennett medium and nutrient agar (Table 1). The aerial mycelium appeared whitish gray across all media, characterized by branched structures that fragmented into straight to flexuous spores, with 10 to 50 spores per chain. The substrate mycelium presented as dark gray on ISP-2, grayish yellow on ISP-3, ISP-4, and Bennett, and light yellow on date extract (D4+$NaNO_3$) and nutrient agar. Diffuse pigments were produced in brown on both ISP-2 and Bennett media, and with only minimal production observed on date extract (D4+$NaNO_3$).

Chemical and physiological properties

Chemical analysis revealed that the cell wall of strain SA32 contained glycine and the LL-diaminopimelic acid isomer. The presence of uncharacteristic sugars in whole-cell hydrolysates indicated that the cell wall was of type IC. The phospholipid profile corresponded to type PII. These findings support the classification of strain SA32 within the genus *Streptomyces* [26]. The physiological characteristics of strain SA32 are summarized in Table 2. Optimal growth

was observed at pH 7 and 30°C, while the strain was capable of growth at pH 5, 40°C, and in 10% NaCl. However, it did not grow at pH 9, 45°C, or in the presence of 15% NaCl, phenol (0.001%), or crystal violet (0.05%). Strain SA32 hydrolyzed all tested organic compounds except sorbitol, arabinose, adonitol, erythritol, oxalate, benzoate, and esculin. Antibiotic susceptibility tests showed resistance to chloramphenicol (25 µg/mL), rifampicin (5 µg/mL), vancomycin (5 µg/mL), and erythromycin (10 µg/mL), while sensitivity was observed towards streptomycin (10 µg/mL), penicillin (25 µg/mL), and kanamycin (25 µg/mL).

Table 3. Chemical composition of the date extract

Chemical composition	D4 (g/L)	D3 (g/L)	D2 (g/L)	D1 (g/L)	D0 (g/L)
Glucose	7.39	15	22.3	29.5	36.9
Sucrose	0.2	0.4	0.59	0.78	1
Ash	41	81	122	160	205
Total nitrogen (NTK)	0.12	0.23	0.35	0.47	0.6
Proteins	0.8	1.5	2.4	3.1	4
Lipids	1.1	2.1	3.2	4.3	5.4

Phylogenetic analysis

The complete 16S rRNA sequence of strain SA32, consisting of 1,472 nucleotides, was sequenced and uploaded to GenBank with accession number KM999987. The phylogenetic relationship of strain SA32 with other species within the genus *Streptomyces* is shown in the neighbor-joining dendrogram in Fig. 1. Phylogenetic analysis based on the 16S rRNA sequence confirmed that isolate SA32 belongs to the genus *Streptomyces* and shares 99.52% similarity with *Streptomyces griseoflavus* LMG 19344^T (AJ781322), the most closely related species.

Chemical composition analysis of the date extract

The results of the chemical analyses of the date extract composition at different dilutions (D0, D1, D2, D3, and D4), expressed in grams per liter of extract, are presented in Table 3. The composition of dilution D0 was as follows: glucose 3.69%, sucrose 0.1%, ash 20.5%, total nitrogen 0.06%, proteins 0.4%, and total lipids 0.54%. Overall, the date variety showed a high content of glucose and ash but low levels of sucrose, proteins, and lipids.

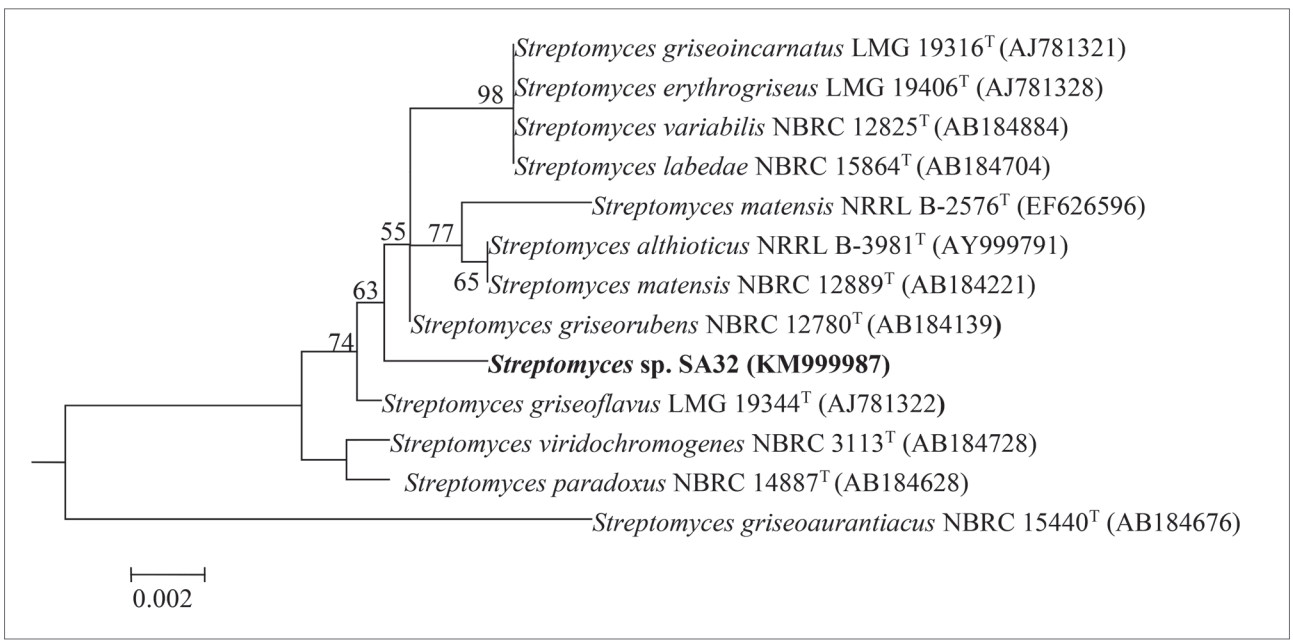

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences illustrates the relationship between strain SA32 and related type species within the genus *Streptomyces*. Bootstrap values (≥50%) shown at the nodes are derived from 1,000 replicate neighbor-joining analyses. Scale bar represents 0.002 nucleotide substitutions per nucleotide position.

Antifungal activity

Strain SA32 demonstrated broad-spectrum antifungal activity on the date extract + $NaNO_3$ medium (Table 3). It showed significant effectiveness against *A. carbonarius* M333 and *U. ramanniana* NRRL 1829, as well as strong to moderate activity against *S. cerevisiae* ATCC 4226, *C. albicans* ATCC10231, *C. albicans* IPA200, *F. graminearum* Fg3, *F. culmorum* FC200, but low activity was found against *A. flavus* NRRL 3251, *A. westerdijkiae* NRRL 3174, and *A. parasiticus* CBS 100926.

Kinetics of antifungal production on solid media

The antifungal production kinetics for strain SA32 on solid media are illustrated in Fig. 2 and Fig. 4A. The activity against *A. carbonarius* M333 was first observed on the second day of incubation across all three media (ISP-2, Bennett, and date extract). The highest activity was noted in the date extract medium, peaking at 30 mm on the fifth day. In contrast, the maximum inhibition diameters for the ISP-2 and Bennett media were achieved on the fourth day, measuring 27 mm and 25 mm, respectively.

The results of the control test (Fig. 4C) showed that none of the agar disk dilutions produced an inhibition zone against the three fungal strains: *A. carbonarius* M333, *A. flavus* NRRL 3251, and *A. parasiticus* CBS 100926.

Kinetics of antifungal production on liquid media

The data on cell growth, pH changes, and antifungal activity are presented in Figs. 3 and 4B. The cell growth curve displayed two distinct phases: an initial rapid growth phase across all media until the fourth day of incubation, followed by a stationary phase lasting until the tenth day. The pH measurements indicated an initial decrease in pH across all media, followed by a subsequent increase. Antifungal activity in the culture supernatant was first observed on the second day of incubation in all media and continued to rise until the fourth day, reaching peaks of 27 mm in ISP-2, 25 mm in Bennett, and 28 mm in date extract (D4 + $NaNO_3$). After this peak, antifungal activity gradually decreased.

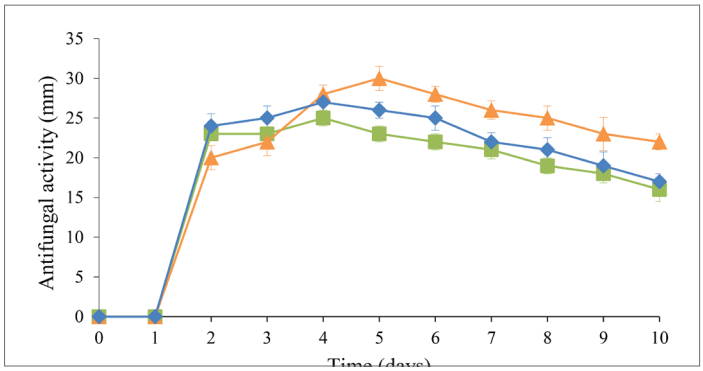

Fig. 2. Time course of activity against *Aspergillus carbonarius* M333 on ISP-2 (◆), Bennett (■), and date extract (▲) solid media. Activity against yeast was measured by the size of the clear zone around the agar disc (10 mm). The values shown are the average ± standard deviation from three repeated tests for each treatment.

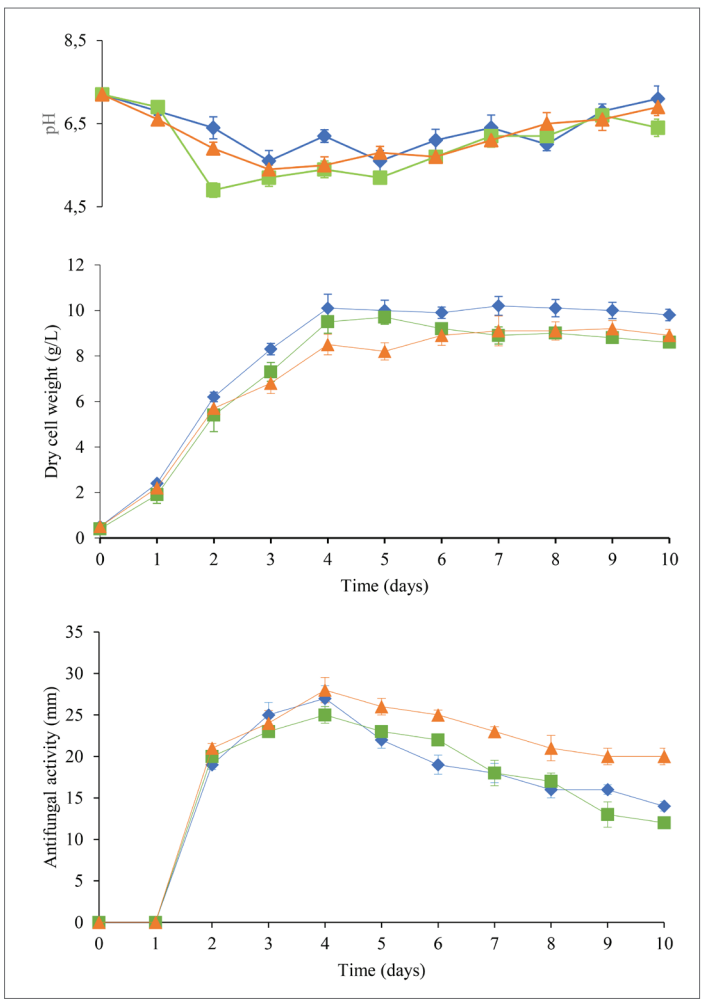

Fig. 3. Time course of pH, growth, and activity against *Aspergillus carbonarius* M333 in ISP-2 (◆), Bennett (■), and date extract (▲) broth media. Measurements of activity against fungus represent the diameters of inhibition and the well diameter (10 mm). Each measure represents the mean ± standard deviation from three replicates per treatment.

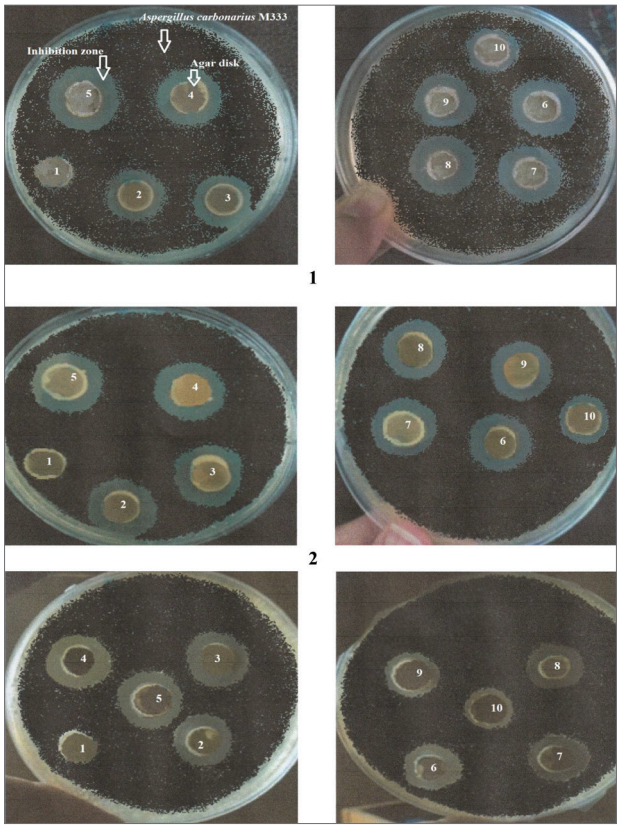

Fig. 4A. Antifungal activities of strain SA32 in different solid media evaluated using the agar disk diffusion method for 10 days. Agar disks were taken from solid cultures of strain SA32 grown on (**1**) date extract, (**2**) ISP-2, or (**3**) Bennett medium.

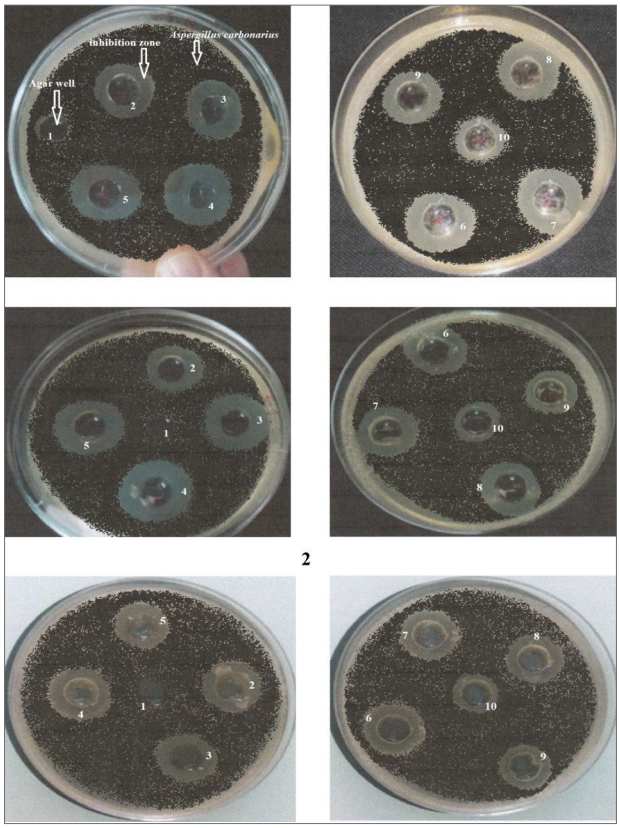

Fig. 4B. Antifungal activities of strain SA32 in different liquid media evaluated using the agar well diffusion method for 10 days. Wells were filled with 200 µL of culture filtrate collected daily from the liquid culture of strain SA32 grown in (**1**) date extract, (**2**) ISP-2, or (**3**) Bennett medium.

Extraction of antifungal metabolites and purification by HPLC

Out of the four solvents utilized for antifungal extraction and evaluated using the disk diffusion method, only the butanolic extract exhibited antifungal activity, with an inhibition diameter of 30 mm. The HPLC profile of the crude butanolic extract (Fig. 6) displayed several peaks, with one peak demonstrating antifungal activity (retention time: t_R 41.50 min, corresponding to 92% methanol). This peak was isolated and re-injected into the HPLC system until complete purification and tested against *A. carbonarius* M333.

Data analysis

Analysis of the data from solid media revealed a significant difference in antifungal activity produced by strain SA32 (χ^2=21.06, P<0.001). The Nemenyi post hoc test showed that Bennett agar supported significantly lower antifungal activity than both date extract agar

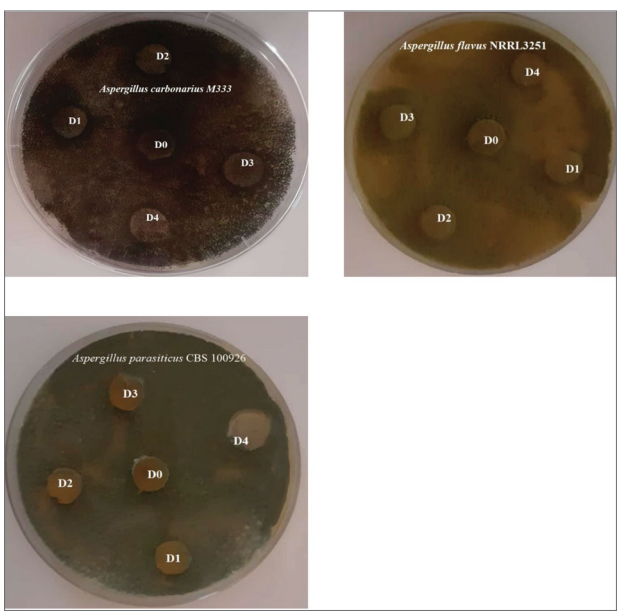

Fig. 4C. The control test included agar disks containing date extract dilutions without inoculation of *Streptomyces* sp. SA32. No inhibition zones were observed against *Aspergillus carbonarius* M333, *Aspergillus flavus* NRRL3251, or *Aspergillus parasiticus* CBS100926.

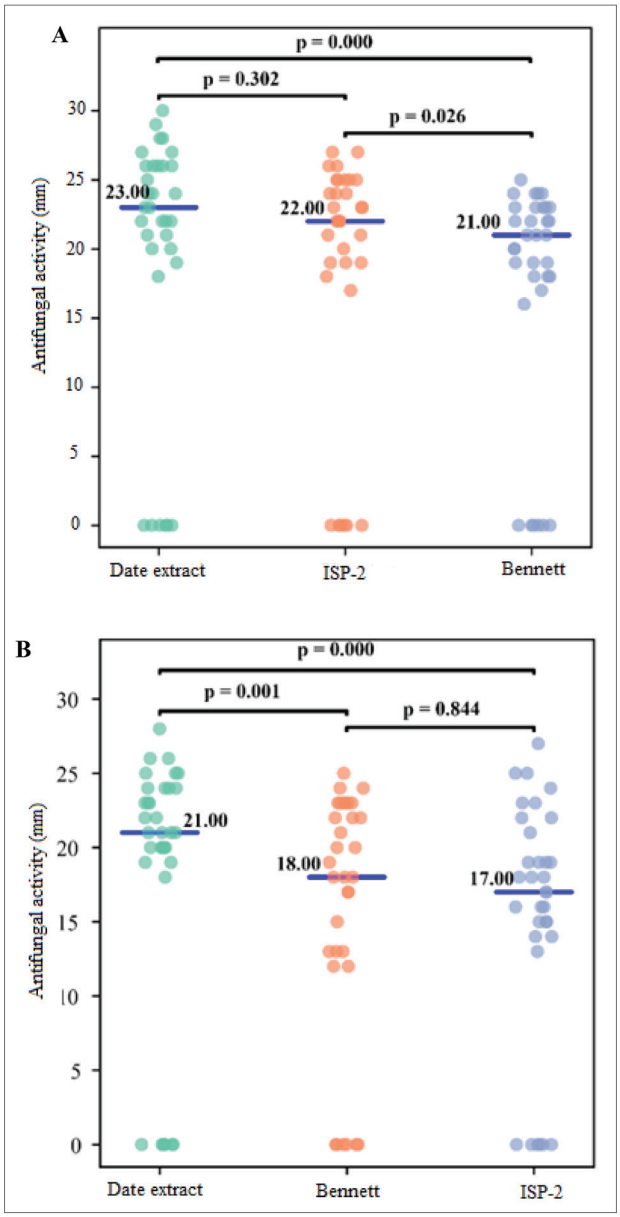

Fig. 5. Antifungal activity of the strain SA32 on: **A** – ISP-2, Bennett and date extract + $NaNO_3$ in solid states. **B** – ISP-2, Bennett and date extract + $NaNO_3$ in liquid states. The blue line indicates median values.

(P = 0.0001) and ISP-2 agar (P = 0.0263), whereas no significant difference was observed between the date extract and ISP-2 agar media (P = 0.3019). Median antifungal activity values for the solid media were 23 for date extract, 21 for Bennett, and 22 for ISP-2. The distribution of antifungal activity values for the solid media is illustrated in Fig. 5A.

Similarly, data from broth media: date extract broth, Bennett broth, and ISP-2 broth, revealed a

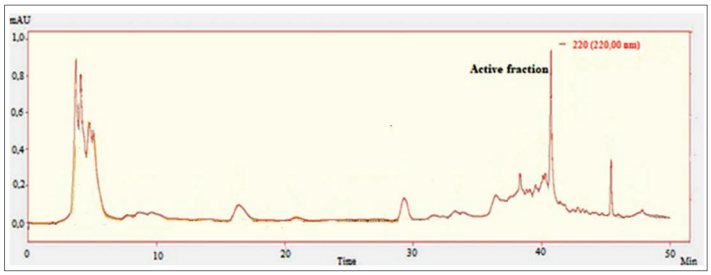

Fig. 6. HPLC profile of the crude butanolic extract obtained from the filtrate of a *Streptomyces* strain SA32 culture in a liquid date extract medium for 4 days. One active antifungal fraction was detected at 220 nm with a retention time of tR 41.50 min.

Table 4. Antifungal activities of strain SA32 on date extract supplemented with nitrogen using the cross-streak method.

Target fungi	Distance of inhibition (in mm)[a]
Aspergillus carbonarius M333	28.0±1.0
Umbelopsis ramanniana NRRL 1829	24.0±1.1
Saccharomyces cerevisiae ATCC 4226	19.0±1.1
Candida albicans ATCC10231	17.0±1.0
Candida albicans IPA200	15.5±0.7
Fusarium graminearum Fg3	15.0±0.7
Fusarium culmorum FC200	13.0±0.5
Aspergillus flavus NRRL 3251	9.5±0.7
Aspergillus westerdijkiae NRRL 3174	8.0±1.0
Aspergillus parasiticus CBS 100926	7.0±0.5

[a] mean±standard deviation from three replicates per treatment

statistically significant difference in antifungal activity produced by strain SA32 (χ^2=26.14, P<0.001). Post hoc comparisons showed that date extract broth enabled strain SA32 to produce significantly higher antifungal activity compared to both Bennett broth (P=0.0013) and ISP-2 broth (P=0.0001). No significant difference was found between Bennett and ISP-2 (P=0.8445). Median antifungal activity values for the broth media were 21 for date extract, 18 for Bennett, and 17 for ISP-2 (Fig. 5B). These results indicate that the date extract broth medium substantially enhances the antifungal production capacity of strain SA32 relative to the standard media.

These findings demonstrate that the culture medium significantly influenced the production of antifungal compounds by strain SA32. The date extract medium consistently supported stronger antifungal activity in broth and solid conditions, while Bennett's medium showed the lowest production performance across the tested formulations.

DISCUSSION

Based on the previously described morphological and chemical characteristics, strain SA32 has been classified within the genus *Streptomyces* [31], which currently includes 1,341 species and 78 subspecies (https://lpsn.dsmz.de/search?word=*Streptomyces*. Accessed on July 22, 2025). Molecular analyses have corroborated earlier findings confirming that strain SA32 belongs to the genus *Streptomyces*. Comparisons of the 16S rRNA gene sequence with those of other *Streptomyces* species revealed a 99.52% similarity with *Streptomyces griseoflavus* LMG 19344^T, the closest related species. However, SA32 exhibits some phenotypic differences from *S. griseoflavus*, including the whitish-gray color of its aerial mycelium on the tested media, the production of soluble pigments, its capacity to utilize sucrose and myo-inositol for growth, and its inability to metabolize arabinose. Phylogenetic analysis further indicates that strain SA32 represents a distinct phyletic line, suggesting it may be a new genomic species.

Results of the chemical composition analysis indicated that the date extract contained high levels of glucose and ash but low amounts of sucrose, proteins, and lipids. The reduced growth of strain SA32 observed in dilutions D0, D1, D2, and D3 of the date extract may be attributed to their elevated glucose concentrations, which were 3.69%, 2.95%, 2.23%, and 1.5%, respectively. *Streptomyces* typically metabolize glucose efficiently at concentrations ranging from 2% to 20% (w/v); however, higher glucose levels can inhibit normal growth and induce carbon catabolite repression (CCR) [32,33]. In addition, osmotic pressure can also be detrimental to microbial cells. For example, high sugar concentrations in foods create a hypertonic environment that dehydrates microorganisms (https://www.britannica.com/science/sulfur. Accessed October 2, 2025).

The optimal antifungal production by strain SA32 was recorded at dilution D4, which contained 0.73% glucose. This concentration was suitable when compared with the glucose levels in ISP-2 medium (0.4%) and Bennett medium (1%). It should be noted that the optimal glucose concentration varies depending on the *Streptomyces* strain [34]. For instance, a glucose concentration of 1% enhanced growth and antibiotic production in *S. tanashiensis* strain A2D [35], while the optimal concentrations for *Streptomyces* sp. 891-B6 [36] and *S. rimosus* AG-P1441 [37] were 0.4% and 3%, respectively. In general, glucose concentrations above 2-3% may trigger catabolite repression [38], although certain strains can tolerate concentrations as high as 5-10%, albeit with reduced growth and metabolic imbalance [39].

The addition of the mineral source $NaNO_3$ to the D4 dilution of the date extract medium promoted good growth and antifungal production for strain SA32. In particular, analyses indicated that date extract is deficient in nitrogen and proteins. Literature suggests that the type and concentration of nitrogen are crucial in regulating antibiotic biosynthesis in bacteria such as *Streptomyces* [40]. Overall, the results indicate that both solid and liquid date extract supplemented with $NaNO_3$ were the most effective media for antifungal production. All complex culture media used (ISP-2, Bennett, and date extract supplemented with $NaNO_3$) provided both carbon and nitrogen sources. Selecting appropriate carbon and nitrogen sources significantly influences the production levels of secondary metabolites [41]. Date extract is rich in minerals (Zn, Fe, Co, Ni, Cu, Mo, Ca, K, Mg, P), which are important for the production of secondary metabolites [42,43]. Additionally, date extract serves as an excellent sugar source and can facilitate a cost-effective fermentation process without requiring special treatments such as acid hydrolysis, steam explosion, or enzymatic processes to make sugars fermentable.

The ingredients of conventional media are expensive in Algeria: glucose costs 111 €/kg; yeast extract is estimated at 303 €/kg; malt extract around 290 €/kg; and peptone costs 280 €/kg (https://www.sigmaaldrich.com/DZ/fr. Accessed on July 03, 2025). Given this, date extract has been successfully used to produce citric and lactic acids [44,45], as well as antibiotics like oxytetracycline and pristinamycin [46,47]. Therefore, it can be concluded that natural date extract could serve as a cultural medium for producing secondary metabolites, providing a viable alternative to conventional media.

In conclusion, the bacterial strain exhibited robust growth in both solid and liquid forms of the diluted natural date medium supplemented with nitrogen, demonstrating higher antifungal activity than conventional ISP-2 and Bennett media. Since inexpensive, lower-quality dates supply the necessary nutrients for bacterial growth, the natural medium could serve for

the production of active metabolites by *Streptomyces* sp. SA32.

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