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A Potent Antifungal Activity by the Marine *Streptomyces albidoflavus* sp. ADR10 from the Caspian Sea Sediment: Optimization and Primary Purification

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ABSTRACT

Fungal infections are an evolving public health challenge due to their antimicrobial resistance and the growth of immunocompromised populations. Aquatic environments, the largest ecosystem on earth, are recently considered as a source for the production of bioactive compounds. Marine actinomycetes are considered for their potential to produce novel bioactive metabolites like antifungal compounds. In this study, strain ADR10 was obtained from the sediment sample of the Caspian Sea and its 16S rDNA gene sequence analysis suggested that the isolate belongs to *Streptomyces albidoflavus*. The preliminary cross-streak and double-layer agar screening revealed that the isolate has potent activity against pathogenic fungi, *i.e. Aspergillus niger, Candida albicans, Fusarium oxysporum, and Penicillium crustosum*. One-factor-at-a-time and Response surface methodology (RSM) was employed to evaluate the effects of six parameters (carbon source, initial pH, inoculation volume, NaCl concentration, nitrogen source, and temperature) on the production of antibiotics in the basal starch casein broth medium. The maximum antibiotic activity was achieved at the initial pH 7.05, sucrose 1.17 g l^{-1}, malt 0.2 g l^{-1}, temperature 30 ºC, inoculation size 5.0% v/v, and NaCl 1% w/v after 121.1 hours. Through the optimization experiments, antifungal activity was enhanced 2.7-fold. Ethyl acetate showed the highest antibiotic extraction capacity from the fermentation media compared with dichloromethane, hexane, and chloroform. The preliminary purified antibiotic by thin layer chromatography (ethyl acetate/ mobile petroleum phase) showed a more significant growth inhibition zone than nystatin (100 µg mL^{-1}) against *Candida albicans*. This study underlines the potential of the marine actinomycete for the identification of novel antifungal agents.

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Introduction

Fungi are among the microbiota of the gastrointestinal tract, reproductive tract, oral cavity, and skin of most healthy individuals (Nobile and Johnson, 2015). The impaired immune system in some predisposing conditions (cancer, HIV, SARS-CoV-2, organ transplantation, aging, etc.) cause these commensal species to turn into invasive pathogens to develop life-threatening Invasive Fungal Infections (IFIs) which affect multiple organs or localized chronic Superficial Fungal Infections (SFIs) (Badiee and Hashemizadeh, 2014). *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis* are among the fungal pathogens showing notable infections. Antimicrobial Resistance (AMR) is an expanding global problem but is more severe when dealing with fungi. Selective drugs targeting these eukaryotic pathogens are more limited compared to those antibiotics available to treat infections caused by

prokaryotic pathogens (Bacteria). Fungi are also among the dominant causal agents of plant diseases and cause crop losses. The increasing impacts of fungal infections not only on human health but also in the food and agricultural industries revealed the importance of investigation for novel and practical antifungal activities.

Aquatic environments, the largest ecosystem on earth, are recently considered as a source for the production of (novel) bioactive compounds. Some characteristics of marines, like the high degree of competitiveness and antagonism relationship between (microbial) inhabitants, and the environmental conditions affected by nutrients limitation, oxygen, pressure, radiation, salt, temperature, etc. allow their (micro)organisms population to harbor various sequences, structures, and metabolites far from those who live in terrestrial ecosystems (De La Hoz-Romo *et al*., 2022). Bioprospecting aquatic habitat with the majority of undiscovered microorganisms offers umpteen bioactive metabolites with numerous biological properties like antifungal activity.

Actinomycetes are (often) characterized as Gram-positive filamentous bacteria with a high GC content in their DNA. They are responsible for the production of about half of the discovered bioactive metabolites for medical, industrial, agricultural, and veterinary applications (de Simeis & Serra, 2021). Actinobacteria were preliminarily obtained from terrestrial environments. High throughput culture-independent DNA sequencing studies discovered many indigenous marine actinomycetes in aquatic systems worldwide (Ossai *et al*., 2021). The diversity of actinomycetes in marine has been shown to vary with environmental conditions, including temperature, pH, pressure, total organic carbon, and salinity. Accordingly, exploring the less considered environments, with exclusive environmental characteristics, can help to identify new strains with the potential to produce new antifungal agents.

The Caspian Sea is the largest enclosed body of water on Earth. Because freshwater is supplied to the sea by various rivers, its salinity decreased to the maximum amount of 1.3 (% w/v) (one-third of the salinity of oceans) (Leroy *et al*., 2007). Industrial, municipal, and agricultural effluents caused the accumulation of environmental pollution (high nutrients, heavy metals, organochlorinated compounds) in this environment, which can lead to a unique microbial population. Some previous studies considered its sediments and brackish water microbial diversity by the next generation (Mahmoudi *et al*., 2015; Mehrshad *et al*., 2016) and the conventional Sanger sequencing methods (Makhdoumi, 2018). Sequences related to the actinomycetes were retired from both water and sediment samples in these studies.

The rapid development of high throughput DNA sequencing technology reveals the majority of natural product biosynthetic gene clusters (BGCs) in the actinomycetes genome. Activating these cryptic genes has been identified through various strategies. Culture media and environmental conditions (pH, salt, temperature, etc.) have the potential to activate these cryptic pathways (Schwarz *et al*., 2021). The goal of this study is to discover a novel antifungal activity by considering the less explored marine ecosystem (Caspian Sea) for isolating actinomycete strains. Assessing the effects of culture media and environmental conditions to promote fungicide activity is another purpose of this research.

Materials and Methods

Sea sampling and isolation procedure

The sediment sample was obtained from a 30 m depth of the Caspian Sea (36.46°N; 51.02°E) (Fig. 1) and kept in the dark at room temperature for a few hours before the analysis. The sediment was serially diluted up to 10^{-5} after being preheated at 80 °C for 30 min and inoculated on ISP2 medium containing (g/l): yeast extract, 4; malt extract, 10; dextrose, 4; and agar, 20. The pH was adjusted to 7.2, and plates were incubated at 30°C for two weeks. The pure isolates were obtained by successive cultivation and stored at 4 ºC and -80 ºC (supplemented by 30% v/v glycerol) for short and long preservation. The 16S rRNA gene was amplified using the bacterial universal primers 27F and 1492R (Lane *et al*., 1985). The sequencing of PCR products was conducted on ABI 3730XL DNA sequencer at Macrogen (Seoul, South Korea). Phylogenetic analysis was performed

using the software package MEGA version 7 (Kumar *et al*., 2016).

Pathogenic strains

Four pathogenic fungal strains, including *Fusarium oxysporum* (IBRC30100), *Candida albicans* (IBRC30070), *Aspergillus niger* (IBRC 30064), and *Penicillium crustosum* (IBRC30031) were purchased from Iranian Biological Resource Center (Tehran, Iran) and used as the fungal test strains in this study. All strains were cultivated on Potato Dextrose Agar (Merck, Germany) at 37 °C.

Cross-streak and double-layer agar methods

Primary determination of antifungal activities of pure strains was performed by the cross-streak method (CSM) and double-layer agar (DLA) technique (Stachurska *et al*., 2021). Briefly, for the cross-streak method, the overnight-growth bacterial cells were inoculated on nutrient agar medium by a single streak (0.5 cm width) and kept at 30 ºC for five days. Consequently, a 1 cm *x* 1 cm plug of test pathogenic fungi mycelium was cut from the previously inoculated PDA medium and transferred to a nutrient agar plate inoculated with bacteria. The distance between the test pathogen and bacterial streak was 3cm. For the overlay method, the overnight-growth bacterial cells were inoculated on ISP2 and incubated for five days at 30 ºC. Then the melted PDA agar medium was overlaid onto the ISP2 medium (3mm height) and subjected to inoculation by testing pathogenic fungi. The growth inhibition zone for both methods was determined after seven days of incubation at 37 ºC.

Optimization of antifungal activity

To identify the optimal fermentation conditions for the antifungal activity and determine the most influential factors, one-factor-at-a-time (OFAT) and response surface methodology (RSM) were used. The bacterial strain was inoculated to the broth fermentation medium from the seed culture medium (ISP2, 24 h, 30 ºC, and 150 rpm). The fermentation process was done in a 100 ml Erlenmeyer flask at 30 °C and 150 rpm. The growth cultures were centrifuged at 10,000 ×g for 10 min, and the supernatant was utilized for antifungal assay by well-diffusion method (Balouiri *et al*., 2016). Fungal test strains were seeded on the PDA agar medium. Wells with 8 mm diameter had punched aseptically with a sterile cylinder. A 20 µl of bacterial culture supernatant was transferred to the wells, kept at 37 ºC for 48 h, and the inhibition zone was measured (mm).

Basal fermentation medium

Seven different media, including nutrient broth (NB), starch casein (SC), Kenknight (KN), and the international *Streptomyces* projects (ISP 2, 4, 5, 7) 2, 4, 5, and 7 were used as the basal medium for bacterial growth and production of antifungal compounds. Antifungal activity was assessed as stated above for days 2 to 7, and the media represented the highest mean inhibition zone against all test strains selected for further optimization.

To enhance the antifungal activity, amounts of various factors, including the carbon source; 1% w/v (glucose, starch, sucrose, dextrose, and maltose), nitrogen source; 0.1% w/v (malt extract, Potassium nitrate, L-Tyrosine, and peptone), pH (6-9), NaCl, %w/v (0-2), inoculum volume % v/v (1-10), and temperature (20-37°C) were varied one at a time in a selected basal medium. Each subsequent factor was examined after taking into account the previously optimized factor(s).

Response surface method

The combinatorial effect of selected factors in antibiotic production was evaluated using the Box-Behnken response surface methodology (RSM). The model is explained by the following empirical second-order polynomial model Eq: $Y= \beta_0 + \beta_1 X_1+ \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11}X_{12}+ \beta_{22}X_{22}+ \beta_{33}X_{32}+ \beta_{44}X_{42}+ \beta_{12}X_1X_2+ \beta_{13}X_1X_3+ \beta_{14}X_1X_4+ \beta_{23}X_2X_3+ \beta_{24}X_2X_4+\beta_{34}X_3X_4$. Where Y is the predicted enzyme activity; x_i, x_j, ..., x_k are the input variables, which affect the response Y; x^2_i, x^2_j, ..., x^2_k are the square effects; x_ix_j, x_ix_k, and x_jx_k, are the interaction effects; β_0 is the intercept term; β_i (i=1, 2,..., k) is the linear effect; β_{ii} (i=1, 2,..., k) is the squared effect and β_{ij} (i=1, 2,..., k; j=1, 2,..., k) is the interaction effect. Optimization was carried out by studying the effect of four significant parameters, including carbon source, nitrogen source, pH, and temperature at three levels (Table 1).

Table 1. Coded and actual values for Box-Behnken design.

Independent variables	Range and Level		
	-1	0	+1
Carbone source (% w/v, X_1)	0	1	2
Nitrogen source (% w/v, X_2)	0.1	0.2	0.3
pH (X_3)	6	7	8
Time (hours, X_4)	96	120	144

Extraction solvent optimization

To identify a suitable solvent to extract the probable antifungal compound(s) from the fermentation media, four solvents, including ethyl acetate, dichloromethane, hexane, and chloroform were applied. The fermentation supernatant (obtained as stated above) and an equal volume of solvents were mixed and kept in a shaker incubator (180 rpm) for one hour. The organic phase was removed using a separatory funnel and evaporated in a rotary evaporator (Heidolph, Germany) under reduced pressure at 40°C (Kolesnichenko *et al*., 2019). Antifungal activities of the concentrated extracts were compared against fungi strains by the well diffusion method.

Preliminary purification

Thin layer chromatography (TLC) was used for partial purification of the extract containing antifungal activity (Kagan and Flythe 2014). For that, 10 µl of the concentrated extract was loaded on TLC plates (Silica Gel, 10 × 5 cm) and developed with various ethyl acetate/petroleum ether ratios as the mobile phase. The plate was dried in the oven at 40 ºC for 30 min, and the spots were detected under UV light; by spraying with vanillin sulfuric acid. The spot was scraped from the TLC plate, eluted with ethyl acetate, and subjected to antifungal activity by well diffusion assay. Nystatin (100 µg/ml) was used as the reference antibiotic.

Statistical analysis

All experiments were performed at least three times. Statistical analysis independent t-test was performed by SPSS software ver.16 (IBM Co.) to determine the significance of the difference between variables.

Results

Bacterial Isolation and identification

The bacterial strain designated as ADR10 was isolated from the sediment sample of the Caspian Sea. Strain ADR10 was Gram-stain-positive and filamentous shape bacterium (Fig. 1a). It formed a round white-yellow pigmented colony with 2-3 diameters on ISP2 medium after one week. A neighbor-joining phylogenetic tree based on 16S rRNA gene sequences depicting the phylogenetic relatedness of strain ADR10 (gene bank accession number, OQ788251) and other species of the genus *Streptomyces* (Fig. 1f). The closest relative of strain ADR10 is *Streptomyces albidoflavus* with a gene sequence similarities of 99.4%.

Antifungal activity

The preliminary screening by cross-streak and double-layer agar revealed that strain *Streptomyces* ADR10 had antifungal activity against all pathogenic fungi (Fig1 b and c). Well diffusion agar (Fig. 1d and e) showed the quantitative determination of antifungal activity by measuring the clear inhibition zone around the wells (Fig. 2).

The antifungal activity of *Streptomyces* ADR10 after 2-7 days of fermentation in seven different culture media against *Candida albicans* is presented in Figure 2a. The optimum fermentation time was varied between fermentation media. The highest antifungal activity after two days of incubation was achieved when the *Streptomyces* strain was cultured in (mm of the inhibition zone): ISP4 (8.2± 0.10), ISP5 (8.1± 0.15), ISP7 (9.1± 0.09), KN (6.9± 0.04), and NB (9.0± 0.06) media. Regarding the ISP2 medium, the maximum antifungal activity was seen after three days of incubation (10.1± 0.05 mm). However, the largest zone of inhibition against *Candida albicans* was observed by SC medium and five days incubation time (12.2± 0.04 mm). The later media/incubation time was selected for further optimization.

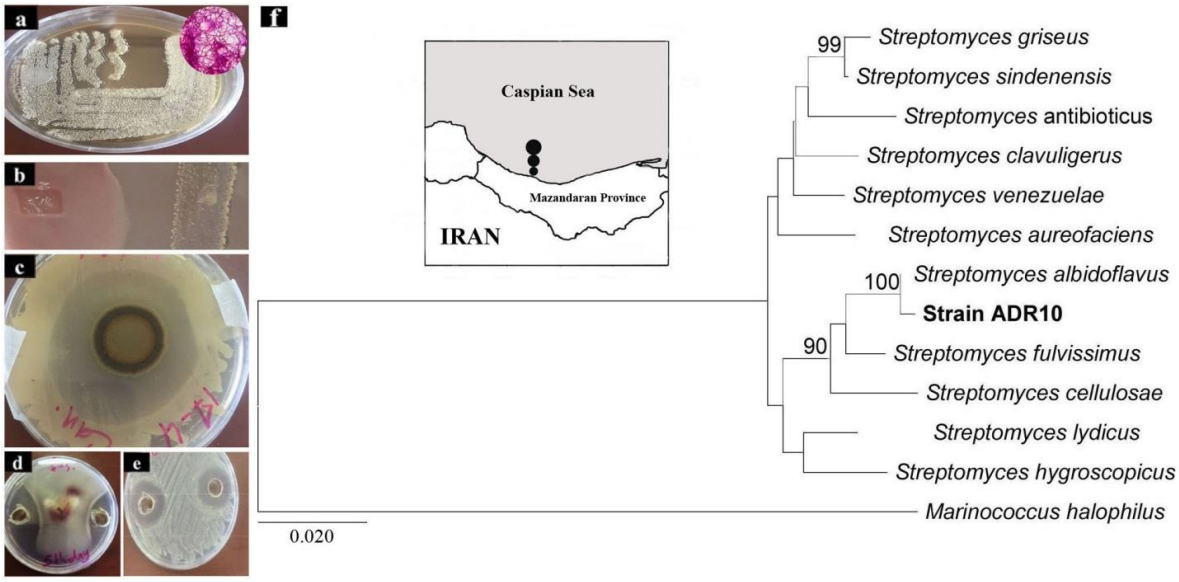

Fig. 1. Culturing and phylogenetic tree of *Streptomyces albidoflavus* ADR10: a) *S. albidoflavus* ADR10 pure culture and microscopic image (a, inset); b) Antifungal activities: cross streak against *F. oxysporum*; c) Overlay against *C. albicans*; d) Well diffusion against *F. oxysporum*; e) Well diffusion against *C. albicans*; f) Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strain ADR10^T with other members of the genus *Streptomyces*. Bootstrap values (%) are based on 1000 replicates. Bar, 0.02 substitutions per nucleotide position. The sequences of *Marinococcus halophilus* (X90835) were used as an outgroup. Inset: Location map of the sampling site used in this study.

OFAT and RSM optimization

In order to identify the most relevant factors to antifungal activity with *Streptomyces* sp. ADR10, factors were varied one at a time in the SC basal media (Fig. 2 b-g). Regarding all factors/levels, the highest antifungal activity was observed against *C. albicans* and then followed by *F. oxysporum*, *A. niger*, and *P. crustosum*. The most relevant factors for the antifungal compounds production were sucrose (1 % w/v), malt extract (0.2 % w/v), pH 7, NaCl (1.0 % w/v), inoculums size (5.0 % v/v), and temperature 30 °C. The resulting inhibition zones for *C. albicans*, *F. oxysporum*, *P. crustosum*, *A. niger* were equal to (mm): 31.1± 0.23, 21.2± 0.16, 16.2± 0.18, and 19.4± 0.19, respectively. The four key variables (carbon source, nitrogen source, incubation time, and pH) were selected based on the results of the OFAT design and were optimized using the Box-Behnken response surface methodology (Box and Behnken 1960). The experimental design and the antifungal activity (zone of inhibition) obtained in the experiments are given in Table 2 and the ANOVA results of RSM optimization are demonstrated in Table 3. Among the four interactions, CD (pH/time) is significant, as evidenced by the low P value (P< 0.05). The response surfaces from the interactions are illustrated in Figure 3.

According to the analysis of results, the predicted optimized culture condition was at sucrose (1.17% w/v), malt extract (2.05% w/v), pH 7.1, and incubation time 121.23 h. The fermentation process at the optimal conditions predicted by this approach resulted in a 32.9± 0.31 mm inhibition zone, which was 2.7 times more than that of the initial fermentation in the SC broth medium.

Preliminary purification

The mean diameters of the inhibition zones for various solvents against pathogenic fungi are shown in Figure 2h. The largest zones of inhibition for fungal strains were observed by the extract obtained by ethyl acetate. A photograph of the chromatogram obtained with mobile phase (ethyl acetate/petroleum ether 100:0) in UV light at 254 is shown in Figure 4. Using this mobile phase, a single spot appeared in the silica gel. The antifungal activity of the TLC purified fraction (100 µg/ mL) in comparison to nystatin

(100 µg/ mL) is presented in Figure 4. The inhibition zone of the purified extract against *C. albicans* (38 mm) was 1.8-folds greater than the inhibition zone obtained by nystatin (21 mm). The novel compound showed antifungal activity against *C. albicans* with a MIC value equal to 4 µg/mL (6.25 µg/mL for nystatin).

Table 2. Experimental design matrix and results of Box-Behnken.

Run	Sucrose (% w/v)	Malt extract (% w/v)	pH	Time (hours)	Inhibition zone (mm)
1	0	0.2	7	96	16.0 ± 0.04
2	1	0.3	7	144	20.0± 0.11
3	2	0.2	6	120	21.0 ± 0.08
4	2	0.2	7	144	17.0± 0.03
5	1	0.2	7	120	29.0± 0.17
6	2	0.1	7	120	24.0± 0.15
7	1	0.2	8	144	16.0 ± 0.25
8	1	0.3	6	120	20.0± 0.02
9	0	0.3	7	120	17.0 ± 0.07
10	1	0.2	6	96	11.0± 0.07
11	1	0.1	8	120	21.0 ± 0.21
12	1	0.3	8	120	22.0± 0.07
13	1	0.1	7	144	18.0± 0.06
14	0	0.2	8	120	10.0 ± 0.12
15	1	0.2	7	120	30.0± 0.04
16	2	0.3	7	120	23.0 ± 0.06
17	1	0.2	8	96	23.0± 0.10
18	1	0.3	7	96	15.0± 0.11
19	1	0.1	6	120	17.0± 0.16
20	2	0.2	7	96	14.0± 0. 06
21	0	0.2	6	120	18.0 ± 0.10
22	1	0.1	7	96	15.0± 0. 07
23	0	0.1	7	120	19.0 ± 0.08
24	0	0.2	7	144	14.0± 0.03
25	1	0.2	6	144	19.0± 0.11
26	1	0.2	7	120	29.0± 0.13
27	2	0.2	8	120	19.0± 0.08

Table 3. Analysis of variance for response surface quadratic model.

Source	Sum of square	Degree of freedom	Mean square	F	P
Model	0.081	14	0.0058	4.46	< 0.05
A-Sucrose	0.0104	1	0.0104	54.76	< 0.05
B-Time	0.0011	1	0.0011	5.88	0.581
C-pH	0.0106	1	0.0106	55.69	0.432
D-Time	0.0003	1	0.0003	2.08	0.343
AB	0.0008	1	0.0008	0.26	0.160
AC	0.0013	1	0.0013	0.18	0.345
AD	0.0048	1	0.0048	1.33	0.505
BC	0.0001	1	0.0001	0.89	0.362
BD	0.0055	1	0.0055	0.12	0.335
CD	0.0101	1	0.0101	6.96	< 0.05
A^2	0.0026	1	0.0102	22.94	< 0.05
B^2	0.0297	1	0.0327	8.96	< 0.05
C^2	0.0017	1	0.0028	18.85	< 0.05
D^2	0.0016	1	0.0016	37.85	< 0.05
Residual	0.0022	12	0.00019		
Lack-of-fit	0.0022	10	0.00022	14.77	0.078
Pure error	0. 069	2	0.0033		
Total	0.073	26			

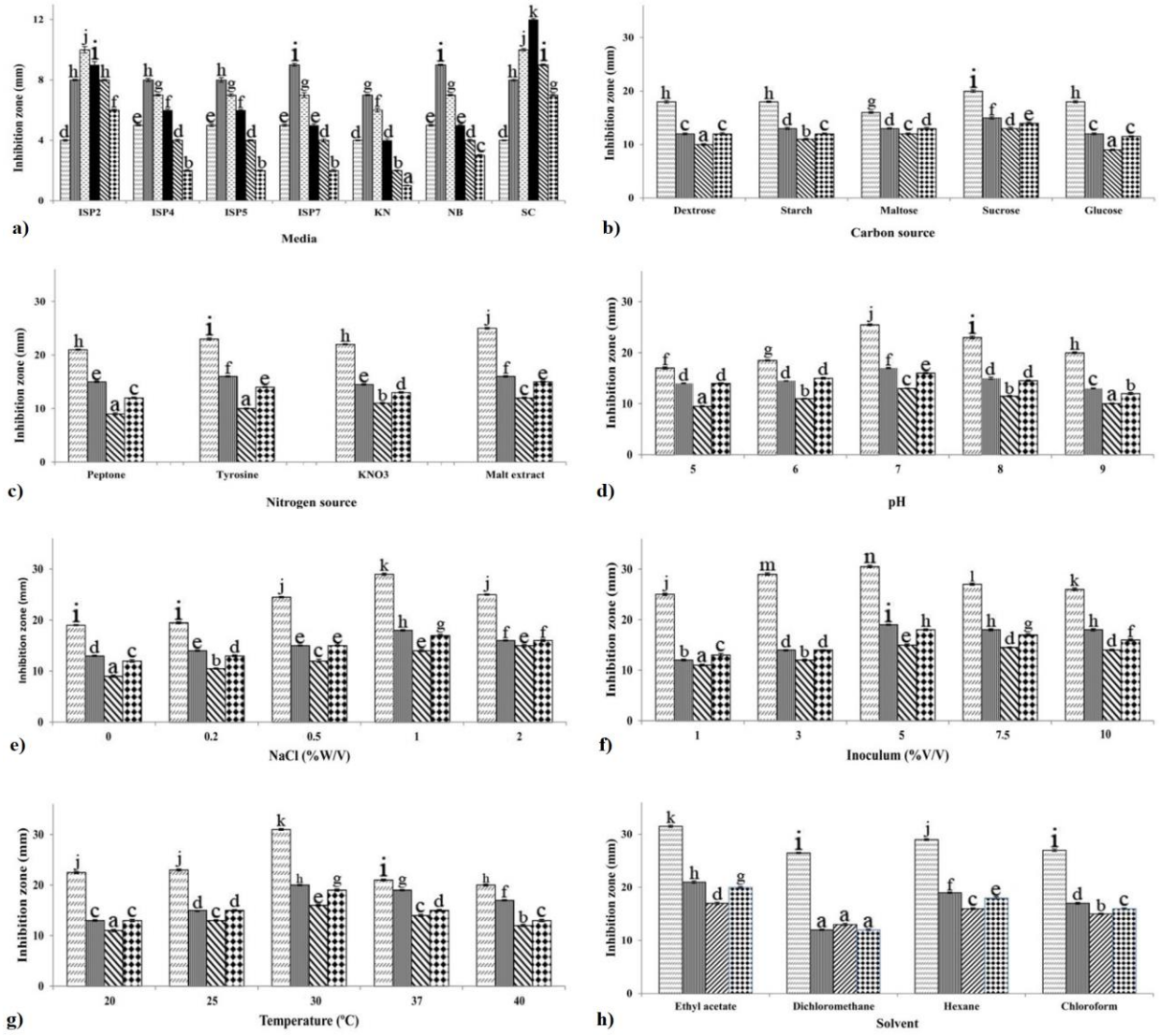

Fig. 2. Antifungal activity under various conditions: a) effects of different basal media on the antifungal activity of *Streptomyces* ADR10 against *Candida albicans* measured by well diffusion method. Dash, day 2; vertical, day 3; vertical dash, day 4; black, day 5; diagonal, day 6, and diamond, day 7; b-h) Factors affecting antifungal activity against four fungal pathogens measured by well diffusion method. Dash, *C. albicans*; vertical, *F. oxysporum*; diagonal, *P. crustosum*, and diamond, *A. niger*.

Discussion

The Caspian Sea is the largest inland body of water on the planet. The previous culture-independent studies revealed that its southern region has high microbial diversity (Mehrshad *et al*., 2016; Makhdoumi, 2018). However, there is little knowledge about the potential of its bacterial residence to produce antifungal compounds, which has been addressed in the current study. Strain ADR10 was obtained from the Caspian Sea sediment and phylogenetically related to *Streptomyces albidoflavus*. Members of this species were previously isolated from soil (Islam *et al*., 2009), wastewater (Souagui *et al*., 2019), and marine (Prakasham *et al*., 2012) environments. Marin actinobacteria are associated mainly with sediments and evolved with unique cellular features that enable them to survive in stressful ecosystems. The predominance of *Streptomyces* species in the sediments was also common in other reports (Dalisay *et al*., 2013).

They have a remarkable ability to produce bioactive secondary metabolites with exceptional biological activity for medical applications.

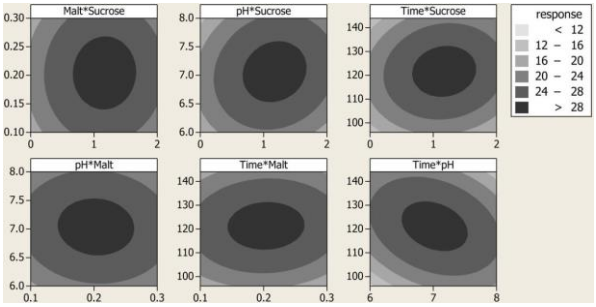

Fig. 3. Contour plots showing the individual and interactive effects of variables on the antifungal activity of Streptomyces strain ADR10.

Strain ADR10 showed remarkable antifungal activity. Other antifungal activities of previously isolated strains in this species were reported against *C. albicans* (Souagui *et al*., 2019), *Passalora fulva* (Chen *et al*., 2015), *A. niger* (Augustine, 2005), and *Fusarium oxysporum* (Carlucci *et al*., 2022). However, the strain obtained in this study showed the simultaneous inhibitory effects on four different fungal species, *i.e. A. niger*, *C. albicans*, *F. oxysporum*, and *P. crustosum*. The production of secondary metabolites is a complex process that is influenced by the expression of genes that code for enzymes involved in their biosynthesis. The expression of these biosynthetic gene clusters (BGCs) is not immutable in the microorganisms. Nevertheless, it can be significantly affected by constituents of production medium/conditions by promoting the silent genes and/or enhancing the pre-existing biochemical pathways (Schwarz *et al*., 2021). This study revealed that the SC medium with the complex and slow-digesting carbon/nitrogen sources (starch and casein) is more suitable for secondary metabolites production. Using statistical optimization, antibiotic production by strain ADR10 increased almost three times. Several researchers applied RSM as a statistical tool to increase antibiotic production. For example, Wang et al. applied the RSM approach for medium optimization and found that the temperature and inoculation volume had a significant effect ($P< 0.05$) on the production of antibiotics (Wang *et al*., 2008). In another research, Yun et al. reported a 56% increase in antibiotic production by *Streptomyces* sp. 1-14 using the RSM approach (Yun *et al*., 2018).

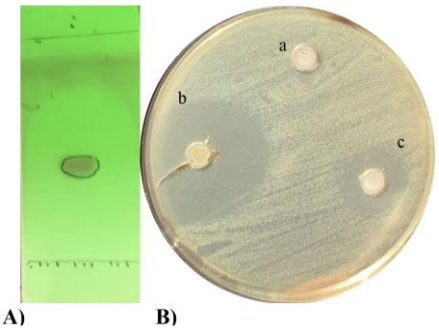

Fig. 4. TLC and antifungal activity of *S. albidoflavus* sp. ADR10: A) Fraction from silica gel column chromatography on thin layer chromatography plate eluted with Ethyl Acetate/Petroleum Ether= 100: 0 v/v., 254 nm UV light. B) Antifungal activity of 100 µg/ mL purified fraction against *C. albicans* (b) in comparison to water (a) and 100 µg/ mL nystatin (c).

Similar to these studies, the findings of this research confirm that optimization of the fermentation conditions is an efficient and relatively simple strategy to facilitate the production of antibiotics. The antifungal compound produced by strain ADR10 was successfully extracted by ethyl acetate. The efficiency of ethyl acetate in the extraction of secondary metabolites from marine *Streptomyces* has been reported in some studies (Rajan & Kannabiran, 2014; Saraswathi *et al*., 2020). In comparison to the minimum inhibitory concentration (MIC) of some compounds obtained from the marine actinomycetes against *C. albicans*, including *Streptomyces* sp. MSTMA190 (5.0 µg/mL) (Cho *et al*., 1999), *Streptomyces* sp. CNQ-085 (62.5 µg/mL) (Asolkar *et al*., 2006), and *Streptomyces* sp. ZZ741 (20 µg/mL) (Zhang *et al*., 2019), the recently obtained marine strain showed significant antifungal activity (4.5 µg/mL).

Conclusion

Bacterial strains from the sediments of the Caspian Sea were screened for their antifungal activity. Strain ADR10 showed broad-spectrum inhibitory activity against pathogenic fungi. 16S rDNA analysis confirmed that strain ADR10 belongs to the *Streptomyces* genus. Through the optimization experiments, its antifungal activity was enhanced 2.7-fold. TLC purification results in a one-spot compound, while its antifungal activity against *C. albicans* was higher than nystatin. These results indicated the potency of

marine-derived actinomycetes for novel antifungal compound production.

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Conflict of Interest

The authors declare no conflict of interest.

References

Asolkar, R. N., Jensen, P. R., Kauffman, C. A., & Fenical, W. (2006). Daryamides A- C, weakly cytotoxic polyketides from a marine-derived actinomycete of the genus Streptomyces strain CNQ-085. *Journal of Natural Products*, *69*(12), 1756-1759. https://doi.org/10.1021/np0603828

Augustine, S. K., Bhavsar, S. P., & Kapadnis, B. P. (2005). A non-polyene antifungal antibiotic from Streptomyces albidoflavus PU 23. *Journal of Biosciences*, *30*(2), 201-211. https://doi.org/10.1007/BF02703700

Badiee, P., & Hashemizadeh, Z. (2014). Opportunistic invasive fungal infections: diagnosis & clinical management. *Indian Journal of Medical Research*, *139*(2), 195-204. https://journals.lww.com/ijmr/toc/2014/39020

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Box G. E. P., & Behnken, D. W. 1960. Some new three level designs for the study of quantitative variables. *Technometrics* 2: 455-475.https://www.tandfonline.com/doi/abs/10.1080/00401706.1960.10489912

Carlucci, A., Raimondo, M. L., Colucci, D., & Lops, F. (2022). Streptomyces albidoflavus Strain CARA17 as a biocontrol agent against fungal soil-borne pathogens of fennel plants. *Plants* 11(11), 1420. https://doi.org/10.3390/plants11111420

Chen, C., Wang, Y., Su, C., Zhao, X., Li, M., Meng, X., ... & Suh, J. W. (2015). Antifungal activity of Streptomyces albidoflavus L131 against the leaf mold pathogen Passalora fulva involves membrane leakage and oxidative damage. *Journal of the Korean Society for Applied Biological Chemistry*, *58*, 111-119. https://doi.org/10.1007/s13765-015-0012-3

Cho, K. W., Seo, Y. W., Yoon, T. M., & Shin, J. H. (1999). Purification and structure determination of antifungal phospholipids from a marine Streptomyces. *Journal of Microbiology and Biotechnology*, *9*(6), 709-715. https://sciwatch.kiost.ac.kr/handle/2020.kiost/6133

Dalisay, D. S., Williams, D. E., Wang, X. L., Centko, R., Chen, J., & Andersen, R. J. (2013). Marine sediment-derived Streptomyces bacteria from British Columbia, Canada are a promising microbiota resource for the discovery of antimicrobial natural products. *PLoS One*, *8*(10), e77078. https://doi.org/10.1371/journal.pone.0077078

De La Hoz-Romo, M. C., Díaz, L., & Villamil, L. (2022). Marine actinobacteria a new source of antibacterial metabolites to treat acne vulgaris disease- a systematic literature review. *Antibiotics*, *11*(7), 965. https://doi.org/10.3390/antibiotics11070965

de Simeis, D., & Serra, S. (2021). Actinomycetes: a never-ending source of bioactive compounds- an overview on antibiotics production. *Antibiotics*, *10*(5), 483. https://doi.org/10.3390/antibiotics10050483

Islam, M. R., Jeong, Y. T., Ryu, Y. J., Song, C. H., & Lee, Y. S. (2009). Isolation, identification and optimal culture conditions of Streptomyces albidoflavus C247 producing antifungal agents against Rhizoctonia solani AG2-2. *Mycobiology*, *37*(2), 114-120. https://doi.org/10.4489/MYCO.2009.37.2.114

Kagan, I. A., & Flythe, M. D. (2014). Thin-layer chromatographic (TLC) separations and bioassays of plant extracts to identify antimicrobial compounds. *Journal of Visualized Experiments*, (*85*), e51411. https://doi.org/10.3791/51411

Kolesnichenko, I. V., Goloverda, G. Z., & Kolesnichenko, V. L. (2019). A versatile method of ambient-temperature solvent removal. *Organic Process Research and Development*, *24*(1), 25-31. https://doi.org/10.1021/acs.oprd.9b00368

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7):1870-4. https://doi.org/ 10.1093/molbev/msw054

Lane DJ, Pace B, Olsen GJ, Stahl D, Sogin M, Pace NR. 1985. Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proceedings of the National Academy of Sciences of the United States of America* 82: 6955-59. https://doi.org/10.1073/pnas.82.20.6955

Leroy, S. A., Marret, F., Gibert, E., Chalié, F., Reyss, J. L., & Arpe, K. (2007). River inflow and salinity changes in the Caspian Sea during the last 5500 years. *Quaternary Science Reviews*, *26*(25-28), 3359-3383. https://doi.org/10.1016/j.quascirev.2007.09.012

Mahmoudi, N., Robeson, M. S., Castro, H. F., Fortney, J. L., Techtmann, S. M., Joyner, D. C., ... & Hazen, T. C. (2015). Microbial community composition and diversity in Caspian Sea sediments. *FEMS Microbiology Ecology*, *91*(1), 1-11. https://doi.org/10.1093/femsec/fiu013

Makhdoumi, A. (2018). Bacterial diversity in south coast of the Caspian Sea: Culture-dependent and culture-independent survey. *Caspian Journal of Environmental Sciences*, *16*(3), 259-269. https://doi.org/10.22124/CJES.2018.3066

Mehrshad, M., Amoozegar, M. A., Ghai, R., Shahzadeh Fazeli, S. A., & Rodriguez-Valera, F. (2016). Genome reconstruction from metagenomic data sets reveals novel microbes in the brackish waters of the Caspian sea. *Applied and Environmental Microbiology*, *82*(5), 1599-1612. https://doi.org/10.1128/AEM.03381-15

Nobile, C. J., & Johnson, A. D. (2015). Candida albicans biofilms and human disease. *Annual Review of Microbiology*, *69*, 71-92. https://doi.org/10.1146/annurev-micro-091014-104330

Ossai, J., Khatabi, B., Nybo, S. E., & Kharel, M. K. (2022). Renewed interests in the discovery of bioactive actinomycete metabolites driven by emerging technologies. *Journal of Applied Microbiology*, *132*(1), 59-77. https://doi.org/10.1111/jam.15225

Rajan, B. M., & Kannabiran, K. (2014). Extraction and identification of antibacterial secondary metabolites from marine Streptomyces sp. VITBRK2. *International Journal of Molecular and Cellular Medicine*, *3*(3), 130-137. http://ijmcmed.org/article-1-179-en.html

Saraswathi, K., Mahalakshmi, S., Khusro, A., Arumugam, P., Mohammed, A. K., & Alkufeidy, R. M. (2020). In vitro biological properties of Streptomyces cangkringensis isolated from the floral rhizosphere regions. *Saudi Journal of Biological Sciences*, *27*(12), 3249-3257. https://doi.org/10.1016/j.sjbs.2020.09.035

Schwarz, J., Hubmann, G., Rosenthal, K., & Lütz, S. (2021). Triaging of culture conditions for enhanced secondary metabolite diversity from different bacteria. *Biomolecules*, *11*(2), 193. https://doi.org/10.3390/biom11020193

Stachurska, X., Roszak, M., Jabłońska, J., Mizielińska, M., & Nawrotek, P. (2021). Double-layer agar (DLA) modifications for the first step of the phage-antibiotic synergy (PAS) identification. *Antibiotics*, *10*(11), 1306.https://doi.org/10.3390/antibiotics10111306

Souagui, S., Djoudi, W., Boudries, H., Béchet, M., Leclère, V., & Kecha, M. (2019). Modeling and statistical optimization of culture conditions for improvement of antifungal compounds production by Streptomyces albidoflavus S19 strain of wastewater origin. *Anti-infective Agents*, *17*(1), 39-49. https://doi.org/10.2174/2211352516666180813102424

Wang, Y. H., Feng, J. T., Zhang, Q., & Zhang, X. (2008). Optimization of fermentation condition for antibiotic production by Xenorhabdus nematophila with response surface methodology. *Journal of Applied Microbiology*, *104*(3), 735-744. https://doi.org/10.1111/j.1365-2672.2007.03599.x

Yun, T. Y., Feng, R. J., Zhou, D. B., Pan, Y. Y., Chen, Y. F., Wang, F., ... & Xie, J. H. (2018). Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by Streptomyces sp. 1-14 from cassava rhizosphere. *PloS One*, *13*(11), e0206497. https://doi.org/10.1371/journal.pone.0206497

Zhang, D., Yi, W., Ge, H., Zhang, Z., & Wu, B. (2019). Bioactive streptoglutarimides A-J from the marine-derived Streptomyces sp. ZZ741. *Journal of Natural Products*, *82*(10), 2800-2808. https://doi.org/10.1021/acs.jnatprod.9b00481