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Antifungal activity of *Streptomyces canescens* MH7 isolated from mangrove sediment against some dermatophytes

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Abstract

The main objective of this study is to isolate actinobacteria has antifungal activity against some dermatophytes, *Epidermophyton floccosum*, *Trichophyton rubrum*, *Microsporum canis*, and *Candida albicans*, evaluation, and optimization of various cultural and nutritional conditions for maximum antifungal metabolite production. Total 64 actinobacteria were isolated from various localities in Egypt and screened for their antifungal activity against the tested fungi. Out of 64 isolates, the identified *Streptomyces canescens* MH7 has a good antifungal activity and inhibits the growth of the tested fungi. This isolate was capable of producing glucanase, lipase, and amylase enzymes which are important hydrolytic enzyme in the lysis of the fungal cell wall. Several growth factors were optimized to maximize the production of antifungal metabolites. *Streptomyces canescens* MH7 had the best antifungal activity in starch casein broth media supplemented with starch as a carbon source, potassium nitrate as a nitrogen source, salinity of 3% (w/v), pH8, incubation temperature at 30°C, incubation for 7 days, and shaking at 180 rpm.

Keywords: Dermatophytes, Actinobacteria, Bioactive compounds, Optimization

1.Introduction

Fungi are eukaryotic organisms with protein and nucleic acid production machinery similar to that found in higher animals. As a result, finding substances that selectively inhibit fungal metabolism without causing harm in human cells is extremely difficult [1]. Dermatomycoses are a type of external fungal infection caused by the dermatophytic fungus. It is a group of fungi that attack and consume nutrients from keratin tissues in the skin, hair, and nails [2,3].

Because of the hot and humid environment that encourages their growth, dermatophytosis is distributed worldwide, especially in tropical and sub-tropical countries [4]. *Trichophyton, Microsporum, and Epidermophyton* are the main three genera of the dermatophytic fungi [5].

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Dermatophytes are classified into three categories depending on their normal habitat, geophilic which normally inhabit the soil, zoophilic that mostly parasitized on animals and anthropophilic parasitized on human and rarely infect animals [6]

Dermatophytes colonize the keratinized outermost layer of the skin when they infect humans, and they don't penetrate living tissue. Tinea capitis (scalp infection), tinea cruris (groin infection), tinea pedis (foot infection), and tinea unguium (infection of nails) are example of these infections [7]. When dermatophytes infect host tissues, they produce keratinase and cause inflammatory reactions such as redness, swelling, heat, and alopecia (loss of hair) at the infection site. These inflammations make the pathogen moves away from the infection site to a new one. This movement of the organism produces the classical ringed lesion, that's why dermatophytes called ringworm [8,9].

Candidiasis is a fungal infection caused by *Candida* spp. It affects more than 4 billion people a year worldwide [10]. The genus *Candida* includes about 150 species however, many are endosymbionts of humans that cause infections mainly in immunocompromised hosts, also capable of secreting a variety of toxins and superantigens, depending on the species, which might cause psoriasis [11]. Approximately 80% of infections are caused by *Candida albicans* [12].

For the treatment of fungal infection, only a few antifungal drugs such as polyenes and azoles are currently available. Although these drugs are very active against wide range of fungi and recorded 35% of complete cure like terbinafine [13]. However, these antifungal agents showed some limitations, like elevated levels of nephrotoxicity and infusional poisonous quality. Azole and echinocandins also clinically reported having narrow spectrum of activity and development of fungal resistance against the available therapies [9,14].

As there is lack of effective and safe antifungal antibiotics and due to the small number of effective and safe available antifungal antibiotics, there is a need for nontoxic and effective antifungal antibiotics with a unique mechanism of action and no side effects.

The pioneering work showed that actinobacteria can produce medically useful antibiotics [15]. The compound antimycin (non-polyenic) antifungal compound was isolated from *Streptomyces albidoflavus* AS25 [16]. *Streptomyces* spp is the most predominant genera comprising about 57% of the total soil actinobacteria population

[17,18]. The genus *Streptomyces* has a good ability to produce bioactive compounds with antimicrobial activities and also has important applications in medicine [19]. The antifungal activity of *Streptomyces* spp is mostly related to the production of antifungal compounds and extracellular hydrolytic enzymes. β 1,3- glucanase and Chitinase are the most important hydrolytic enzymes in the lysis of fungal cell walls [20]

The objective of this study aims to isolate naturally occurring actinobacteria with an ability to produce bioactive compounds and/or extracellular hydrolytic enzymes having antimycotic property and optimize their production.

2.Materials and Methods

2.1. Sample collection and processing

Soil and water samples were collected from various locations in Egypt, these are: Hamata mangrove1, Hamata mangrove 2, South Lahmy mangrove, Qulaan mangrove, Abou Ghsone mangrove, Jasso's Valley (red sea), Hamata mangrove water, Edko lake water, non-rhizosphere soil sample, Kom Hamada Behera, Alf Maskan soil (Cairo), Sharm Elbahary (red sea) and 17 km South Safaga mangrove. Soil samples were taken down to a 15 cm depth into soil in sterile plastic bags then transferred to the lab and stored at 4°C until use. The samples were air dried for one week to reduce the population of gram-negative bacteria [21]. Water samples were collected beneath the surface of the water in sterile bottles.

2.2. Microorganisms, growth media and culture conditions

Actinobacteria were isolated from the collected samples by serial dilution method [22] on starch casein [23], starch nitrate [24] and/ or yeast malt extract agar media [25] at 30º C for 7 days. The purified cultures were grown on slants and maintained at 4 ˚C in a refrigerator.

The tested fungal organisms used as dermatophytes, E*pidermophyton floccosum*, *Trichophyton rubrum*, and *Microsporum canis* were obtained from Mycology Center at Assiut University- Egypt while *Candida albicans* obtained from Nephrology Department at Theodor Bilharz Research Institute Hospital, Egypt. The fungal strains were cultured on Sabouraud Dextrose agar (SDA) (Oxoid, UK) at pH 6.5 and incubated at 30°C for 5- 7 days and maintained at 4 ˚C in a refrigerator.

2.3. Screening for bioactive compound producing actinobacteria as antifungal activity

The pure cultures of actinobacterial isolates were screened for their antifungal activity against the tested fungi, using agar disc and well diffusion methods. Agar disc of actinobacterial cultures (6mm) were placed on SDA plates seeded with the fungal culture. After 7 days for dermatophytes and one day for *C. albicans* of incubation at 30ºC, the diameter of the inhibition zone was recorded [26]. In well diffusion method, 100 µl extract of the actinobacteria grown on starch casein broth was added in the well of SDA agar plates seeded with the tested fungi and incubated at 30ºC for 7 days in case of dermatophytes and one day in case of *C. albicans*. The diameter of the inhibition zone was measured, the triplicates of each were made [27].

2.4. Morphological and Taxonomical Identification of actinobacterial isolate

Based on the screening results, the active isolate that showed a good antifungal activity was chosen for further examination. Morphological, physiological characteristics and some biochemical testes (nitrate reduction, catalase test, starch hydrolysis urease, and indole test) were studied. The taxonomic identification of actinobacteria spp was based on Bergey's Manual of Systematic Bacteriology (1989) and Shirling & Gottlieb (1966). Finally, the strain was identified using 16S rRNA sequencing

2.5. Production of extracellular hydrolytic enzymes

Actinobacterial isolate MH7 was screened for its ability to produce some important lytic enzymes like glucanase, chitinase, lipase, protease, and amylase.

2.5.1. Glucanase activity.

Glucanase production was determined by inoculating the identified actinobacterial isolate into modified SCA plates containing .2% barley β-glucan. The plates were incubated for 5 days at 30°C. After incubation the colonies were rinsed off with distilled water, then stained with .03% Congo Red, a clear zone around the colony indicated positive glucanase activity [28]

2.5.2. Chitinase production

Production of chitinase was done by plate agar assay. The colloidal chitin agar (CCA) contains (g/l): colloidal chitin, 15.0; yeast extract, 0.5; $(NH_4)_2SO_4$, 1.0;

$MgSO_4.6H_2O$, 0.3; KH_2PO_4, 1.36; agar, 15.0, and sea water1000 ml.The tested actinobacterial broth culture was inoculated onto wells on the colloidial chitin agar and incubated at 30˚C for 5 days. The colonies showing clearance zones on a creamish background were considered as chitinase-producing actinobacteria [29].

2.5.3. Lipase production

Lipase activity was performed when the tested actinobacteria was inoculated in tween 20 agar composed of (g/l): peptone 10.0, NaCl 5.0, $CaCl_2 \cdot 2H_2O$ 0.1, and agar 15.0 in 1000 ml sea water and autoclaved for 20 min; 10 ml Tween-20 was separately sterilized and added into the autoclaved medium, and the pH was adjusted to 7. After incubation at 30 ºC for 7 days lipolytic activity detected by observation of a whitish halo due to the formation of calcium crystals, contrasting with the transparent medium [30] [31] .

2.5.4. Protease production

Actinobacterial isolate MH7 was streaked on the skim agar medium contains (g/l): skim milk powder, 10; peptone, 5; NaCl, 3%; and agar,15; per 1000 ml of sea water; pH 7.0 and incubated at 30˚C for 4 days. A clear zone appeared around the actinobacteria colonies if the strain is positive for protease activity [29].

2.5.5. Amylase activity

This test was performed to indicate the ability of the actinobacterial isolate MH7 to produce amylase enzyme. It was inoculated in nutrient starch agar contains (g/l); Peptone, 5.0; Beef extract, 3.0; Starch, 20.0; NaCl, 3% ; Agar, 20.0 , and sea water, 1000 ml. After incubation for 7 days, the plates were immersed with iodine solution and the formation of clearing zone around the bacterial colonies indicates starch hydrolysis [29].

2.6. Enhancement of bioactive compound for actinobacterial isolate MH7

To obtain the ideal condition for maximum bioactive metabolite production, various parameters influencing the antifungal metabolite production were studied.

2.6.1. Effect of media

About 100 ml of each three different media: starch casein broth, starch nitrate broth , and yeast malt extract broth were inoculated with the spore suspension of MH7 actinobactrial isolate and incubated at 30°C for 7 days in an orbital shaker at 150 rpm

and the broth was centrifuged at 6000 rpm for 10 min at 4^{o}C. The antifungal activity of the filtrate was measured by agar well diffusion. The factor value corresponding to the highest readings was chosen as the optimum one.

2.6.2. Cultural and nutritional conditions optimization

The effect of various parameters, including, incubation period, pH, temperature, agitation, sodium chloride concentration, nitrogen, and carbon sources on the production of bioactive metabolite were studied. All experiments were performed in 250 ml conical flasks, each one containing 100 ml starch casein medium (contains (g/100ml): soluble starch,1.0; casein, 0.03; KNO_3, .2; NaCl, .2; K_2HPO_4,.2; $MgSO_4.7H_2O$, 0.005; $CaCO_3$, 0.002; $FeSO_4.7H_2O$, 0.001 and artificial sea water, 100 ml. The pH was adjusted at 7 in 250 ml Erlenmeyer flasks. The flasks were inoculated with the spore suspension of MH7 isolate and incubated for different periods (1–10 days), different incubation temperatures (25, 30, 35, 40, and 45˚C), different pH values (6 ,6.5 ,7,7.5 ,8 ,8.5, 9, and 10) and different shaking rates (100, 120, 140, 160, and 180 rpm). At each factor, 5 ml of the culture filtrate were then taken aseptically and centrifuged at 6000 rpm for 10 min [32]. The mycelium free culture filtrate was tested for antifungal activity against the tested fungi using agar well diffusion method as mentioned before. The effect of salt (NaCl) on metabolite production was done at conc, 0, 2, 3, 5, 10,15 and 20% (w/v) by changing the concentration of NaCl in the starch casein broth.

The influence of seven different carbon sources: glucose, fructose, sucrose, lactose, starch, glycerol, and galactose by replacing the carbon source of starch casein broth (at equimolecular basis to carbon source) and six different nitrogen sources: potassium nitrate, ammonium sulphate, ammonium nitrate, peptone, sodium nitrate, and urea by replacing the nitrogen source of starch casein broth (at equimolecular basis to nitrogen source) on metabolite production were tested.

2.7. Statistical analysis

All tests were done three times and were exhibited as a mean ± standard deviation which were statistically evaluated significant when $p<0.05$, based on ANOVA followed by Tukey's multiple assessment post-test using SPSS ver. 26 (IBM, USA).

3.Results

3.1. Isolation of actinobacteria and screening their antifungal activity

Sixty-four isolates of actinobacteria (59 mesophilic and 5 thermophilic) were obtained from different samples of water and soil from various locations in Egypt. These isolates were purified and maintained onto their isolation agar slants for further study. Isolate MH7 which was obtained from Hamata mangrove sediment sample showed antifungal activity against the tested fungi by disc diffusion method. The antifungal activity of the isolate was confirmed by agar well diffusion method and showed a good inhibitory activity against the tested fungi (Table 1). Results have showed that the highest antifungal activity was observed against *Candida albicans*.

Table (1): Antifungal activity of isolates MH7 against the tested fungi using agar disc and well diffusion method.

Tested fungi	Diameter of inhibition zone (mm)	
	Agar disc diffusion	Agar well diffusion
Microsporum canis	17	18
Epidermophyton. Floccosum	16	17
Trichophyton. Rubrum	15	18
Candida. Albicans	21	20

The actinobacterial isolate MH7 was selected and tested for further studies of identification and optimization as it showed a good antifungal activity and inhibited the growth of all the tested fungi out of the obtained sixty-four isolates.

3.2. Characterization and Identification of the active isolate MH7

2.3.1. Cultural, physiological, and biochemical characteristics

The aerial mass color of the isolate MH7 was studied on different media. It was almost yellowish white in color on all the media used; therefore, it could be assigned to the yellow color group. The color of the aerial, substrate mycelia and the growth rate

of the isolate appeared variation depending on the type of the growth media. Diffused pigments were not observed within the growth media. The color of substrate mycelium was yellow to brown on most of the media used, the isolate could grow well on almost all media (data not shown).

The results in Figure (1, a and b) of scanning electron microscope (SEM) and transmission electron microscope (TEM) showed that isolate MH7 had spore chains in section rectiflexibiles, and mature spore chains generally have 10–50 spores per chain with a smooth spore surface.

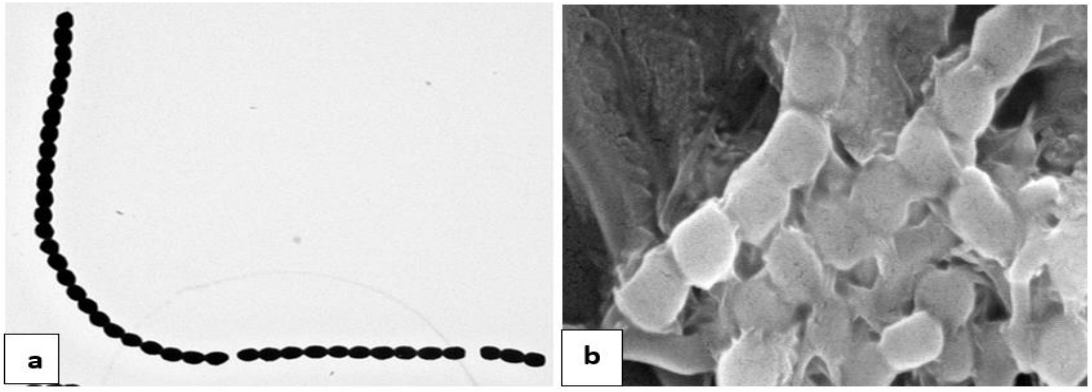

Fig (1): Scanning electron microscope for spore chain at 5000 X(a) and Transmission electron microscope for spore surface at 16000X (b) for the actinobacterial isolate MH7 grown on starch casein agar for 7 days of incubation at 30 ˚C.

Actinobacterial isolate MH7 was gram positive, can utilize all the tested carbon sources except sucrose and unable to produce melanin pigment on Tyrosine broth medium, Peptone yeast extract iron medium, and Tryptone yeast extract broth medium. It can tolerate up to 12.4% NaCl this that means the isolate is halotolerant. The isolate can grow well at a temperature ranging from 22 to 37ºC with optimum at 30ºC and at a pH range of 5 to 11with optimum at pH 8. The MH7 isolate was positive to nitrate reduction, catalase test and starch hydrolysis and negative to urease and indole test (data not shown).

Molecular identification by 16S rRNA gene sequencing was carried out and compared with the available sequences in Gene Bank using blast tool provided by NCBI. It showed that tested isolate MH7 had 99% similarity with *Streptomyces canescens* DSM 4000 (NR_119346.1). The 16S rRNA gene sequences of the identified

isolate MH7 was submitted to Gene Bank under accession number MW898314. A multiple sequence alignment and phylogenetic analysis were carried out using the obtained related species from the NCBI database and the studied actinobacterial sequence Figure (2).

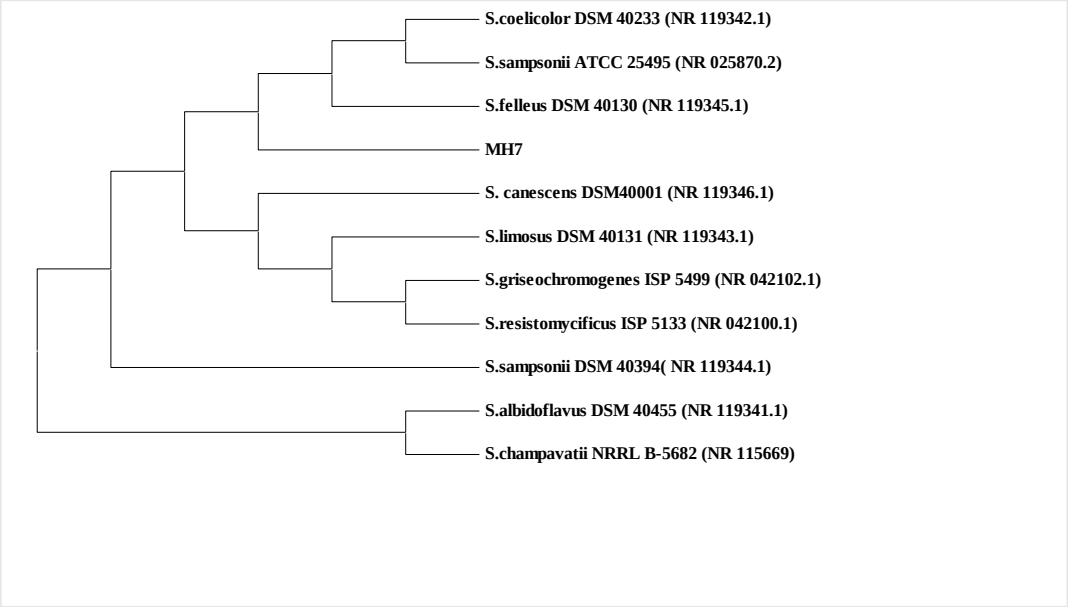

Fig (2): the phylogenetic tree constructed using 16S rRNA sequences, where the isolates MH7 obtained form was *Streptomyces canescens.*

3.3. Enzymatic bioassay of a *Streptomyces canescens* MH7:

- Glucanase activity

The results indicated, a clear zone around the colonies of *S. canescens* MH7 when incubated at 30 ºC for 5 days and it considered as glucanase positive (data not shown).

-Chitinase activity:

S. *canescens* MH7 supernatant appeared no clear zone on colloidal chitin agar, and it was considered as chitinase negative (data not shown).

-Lipase activity:

S. canescens MH7 exhibited lipase activity and was able to hydrolyze Tween 20 by the formation of a sedimentary halo around the colonies of due to the formation of calcium crystals, contrasting with the transparent medium (data not shown).

-Proteolytic Activity:

The results indicated that the identified S. *canescens* MH7 isolate was weakly able to produce protease enzyme and exhibited weak hydrolysis halo (data not shown).

-Amylase activity:

S. *canescens* MH7 was able to hydrolyze the starch polymer and produced colorless zone around the colonies after adding the substrate (data not shown)

3.4. Enhancement of bioactive compound for *Streptomyces canescens* MH7

The growth medium is essential for the production of secondary metabolites. The results in Figure (3) indicated that the maximum antifungal activity of *S. canescens* MH7 was observed in starch casein broth. The results also showed that initial antibiotic production of the strain *S. canescens* MH7 appeared after three days, and the maximum activity was observed at the seventh day of incubation time against all the tested fungi Figure (4). *Streptomyces canescens* MH7 showed an optimal antifungal production at 30ºC, pH 8 under shaking conditions at 180 rpm and at 3% NaCl Figure (5), (6), (7) and (8) respectively.

The result also appeared that *Candida albicans* was the most sensitive against the antifungal produced by the tested isolate S. canescens MH7 followed by *Trichophyton rubrum.*

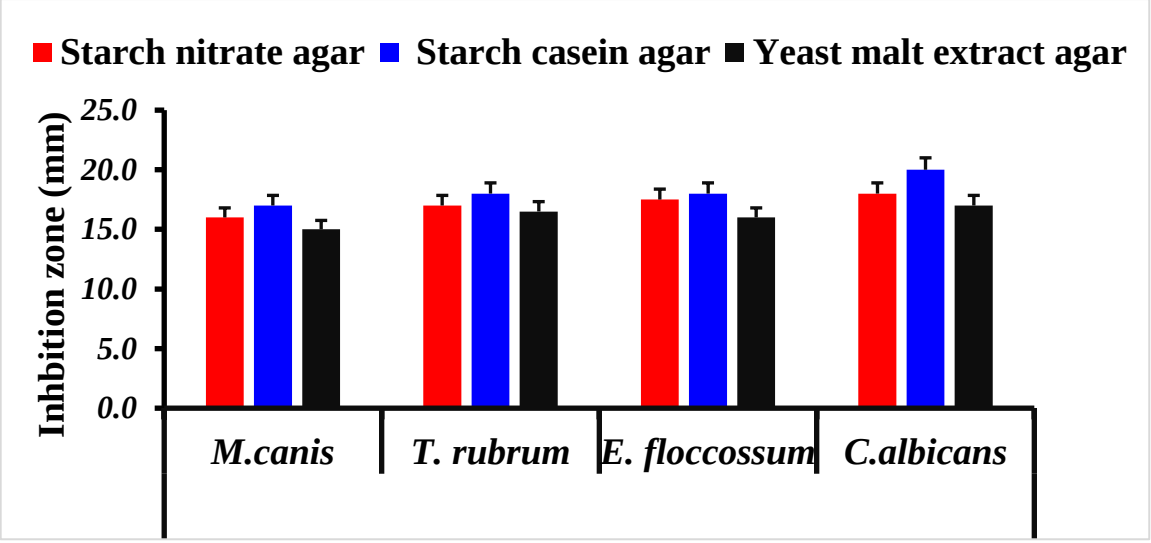

Fig (3): Effect of different media on antifungal production by *Streptomyces canescens* MH*7* against the tested fungi.

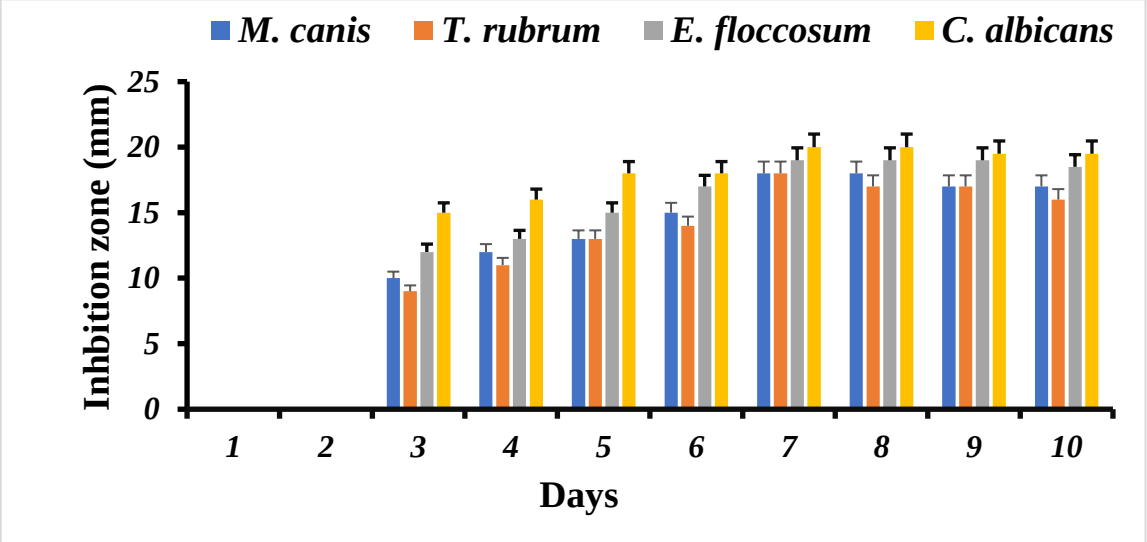

Fig (4): Effect of incubation period on antifungal production by *Streptomyces canescens* MH7 against the tested fungi.

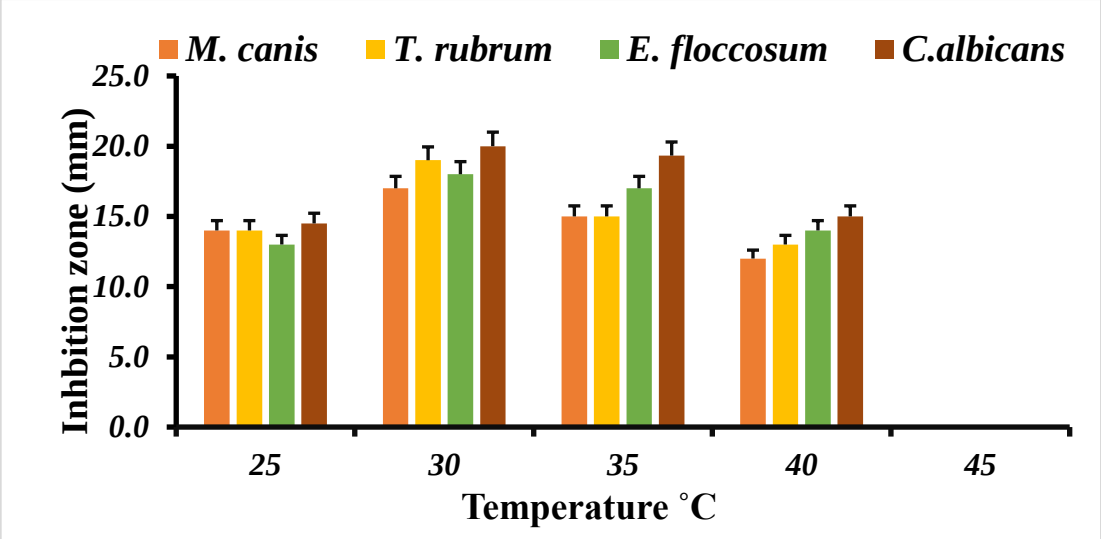

Fig (5): Effect of temperature on antifungal production by *Streptomyces canescens* MH*7* against the tested fungi.

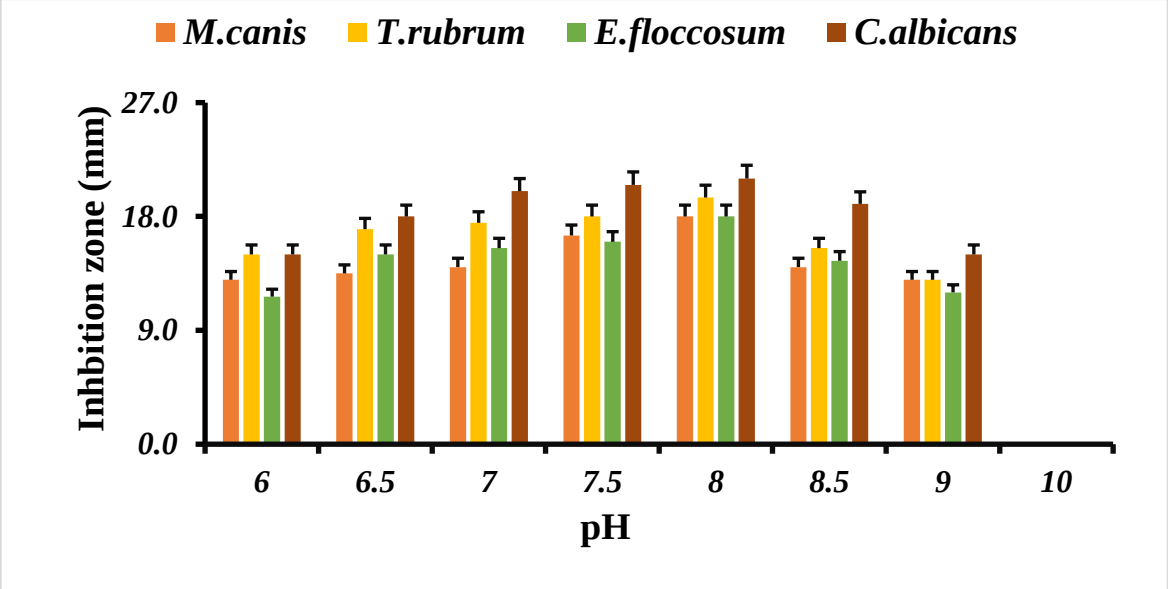

Fig (6): Effect of pH on antifungal production by *Streptomyces canescens* MH*7* against the tested fungi.

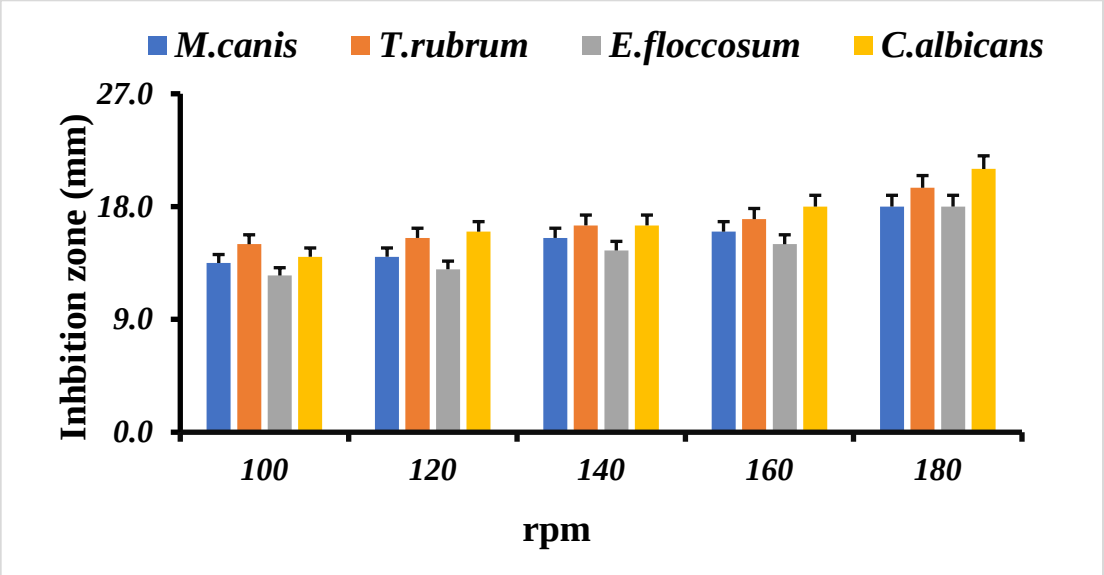

Fig (7): Effect of shaking on antifungal production by *Streptomyces canescens* MH7 against the tested fungi.

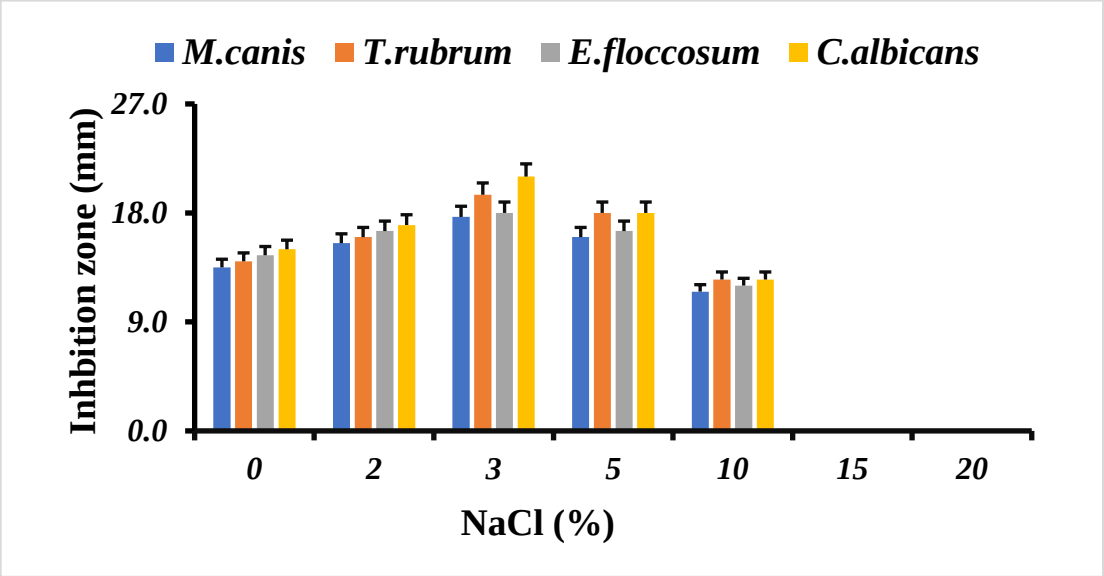

Fig (8): Effect of NaCl concentrations on antifungal production by *Streptomyces canescens* MH7 against the tested fungi.

3.4.2. Effect of carbon and nitrogen sources

Antimicrobial metabolite production was greatly influenced by the nature and type of the carbon source. *Streptomyces canescens* MH7 produced the highest antifungal compound in medium supplemented with starch followed by fructose then glucose respectively Figure (9).

Metabolite production was also greatly influenced by the nature and type of the nitrogen source. The maximal antifungal productivity of *Streptomyces canescens* MH7 was obtained using potassium nitrate as nitrogen source followed by ammonium nitrate against the tested fungi as recorded in Figure (10).

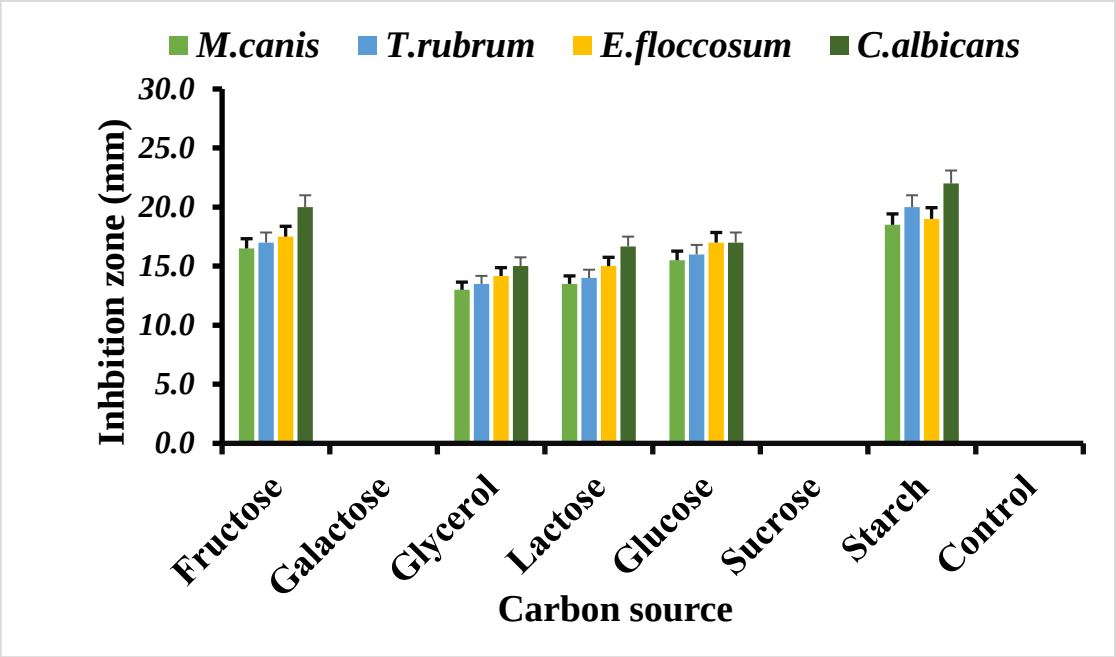

Fig (9): Effect of carbon source on antifungal production by *Streptomyces canescens* MH*7* against the tested fungi.

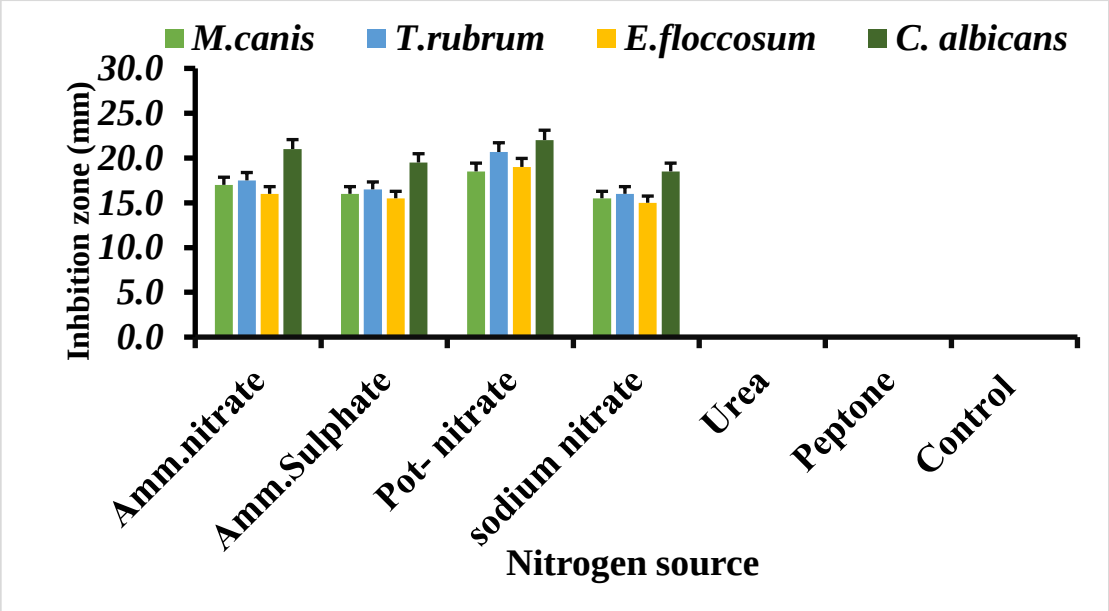

Fig (10): Effect of nitrogen source on antifungal production by *Streptomyces canescens* MH*7* against the tested fungi.

4. Discussion

Usage of commercial antibiotics for human fungal disease treatment produces undesirable side effects [33]. So, there is a need for safe antifungal compounds with no side effects from naturally accruing microorganisms.

Streptomyces provides more than half of the naturally occurring antibiotics currently used in pharmaceutical industry [34]. It has been shown that 75-80 percent of antibiotics produced and used by actinomycetes come mostly from *Streptomyces* species [35]. In this study, we screened and evaluated actinomycetes isolated from different localities in Egypt for their antagonist activity against *M. canis, T. rubrum. E. floccosum* and *C. albicans*, the causative agent of human dermatophytosis and Candidiasis.

Over 50 isolates were obtained, and then *in vitro* tests were performed and out of them an isolate MH7 which obtained from Hamata mangrove sediment sample appeared activity against the tested fungi. This deals with the fact that marine environment and ecology had a community of new and potent bioactive compounds producing organisms [36]. Actinobacteria make up 10% of the total colonizing bacteria in marine collections [37]. From the late 1990s to the present, forty novel bioactive microbial compounds have been discovered in marine species, with near 50 percent of them was found in actinobacteria [38] and [39].

Research on the biological properties of actinomycetes has shown that these bacteria are potential candidates for the production of antifungal compounds as mentioned by previous study which appeared that 20.3% (13/64) of the actinobacterial isolates studied were active against *C. albicans* [40]. Also, recently, a study observed that 33.3 % (3/9) of *Streptomyces* spp analyzed showed some degree of activity against *Candida* spp [41].

The discovery of antifungal substances that are effective against dermatophyte fungi has been fraught with challenges [42]. Out of 218 actinomycete isolates and observed only one (0.46%) exhibited activity against fungi of this group [1]. Similarly, of the 100 isolates tested, only 3% were active against dermatophyte fungus species [8].

The tested isolate MH7 was subjected to identification studies. The cultural, morphological, and biochemical characteristics of the MH7 isolate indicated that this isolate belonging to Genus *Streptomyces*. Because the morphological characteristics of the tested isolate MH7 were limited to its identification at the Genus level, the isolate's

molecular identification by 16S rRNA gene sequencing confirmed that it belongs to *S. canescens.*

The antagonistic activity of *Streptomyces* to fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes [43]. Since the structural components of the fungal cell walls are chitin, amino polysaccharides, α- and β-glucans, proteins, lipids [44]. So, the identified strains *S. canescens* MH7 was screened for the production of some important hydrolytic enzymes (chitinase, glucanase, protease, lipases and amylase). The identified isolate *S. canescens* MH7 was able to produce glucanase, protease, lipase, and amylase enzymes.

Hydrolytic enzymes can break down glycosidic bonds in chitin and glucan. Thus, they play an important role in the biological control of many fungal diseases by degrading the cell walls of these pathogens. They affect fungal growth by their lytic action on cell walls, hyphal tips, and germ tubes [45].

β-1,3-Glucan is a polysaccharide of β-(1→3)-linked glucose, it is the most essential and abundant structural components of fungal cell walls in members of all fungal phyla along with chitin and their structures are widely variable [46] [47].

Glucanase can control and impair the fungal growth by hydrolyzing and breaking down glycosidic bonds within beta-glucan to glucose causing softening, lysis of the cell walls, direct suppression of activity and/or death [48] [49].

These enzymes can hydrolyze the substrate by two possible mechanisms: (a) endo-1,3-glucanases (EC3.2.1.39) which break linkages at random sites along the polysaccharide chain, producing smaller oligosaccharides and (b) exo-1,3-glucanases (EC 3.2.1.58) that hydrolyze the substrate by cleaving glucose residues from the nonreducing end [50] [51]. These results are in good agreement with other studies which have shown that, the inhibition of fungi by *Streptomyces* may be related to the production of glucanase and chitinase [52] [53]. *Streptomyces* is known to produce endo-β-1,3-glucanases that function as anti-fungal agents [46] [54]. Also, other *Streptomyces* spp have been studied for antifungal properties along with the production of glucanase [55].

Chitin is a polymer of unbranched chains of β-1,4-linked 2-acetamido-2-deoxy-D-glucose and is a major structure component of most fungal cell walls [56]. Chitinases [EC 3.2.1.14] are chitin-degrading enzymes playing a very important role in biological

control against fungal pathogens by lysing fungal cell walls through degradation of chitin polymer present in the cell walls of these fungi [50]. *Streptomyces aureofaciens* CMUAc130 was selected for investigation because of its very high chitinase productivity and its potential role in antifungal activity [57]. A previous study reported that out of 110 actinobacterial isolates only 18 strains appeared a strong chitinase activity [58]. *Streptomyces* chitinases have been implicated against a variety of plant pathogenic fungi [59] [60].

Proteases [E.C. 3.4.24] also play a very important role in cell wall degradation of pathogenic fungi since chitin and fibrils of β-glucan are embedded into the protein [61]. Proteases recognize the structures of certain specific amino acids and then catalyze the breaking of the peptide bonds [62]. The importance of proteases in many different biocontrol processes was shown in *Trichoderma harzianum*, which is a pathogenic fungus [63] [64] and *S. griseorubens* E44G that showed a marked inhibitory effect against the pathogenic fungus *R. solani* [65].

These results and findings illustrate that the inhibition of the tested fungi by the identified strain *S. canescens* MH7 was probably related to the production of hydrolytic enzymes in the culture media.

This study has shown that the antifungal activity was observed both on solid as well as in culture broth. Media composition and culture conditions such as agitation, pH, temperature, incubation period, and carbon and nitrogen source, which vary from organism to organism, greatly affect the production of the antifungal metabolites [1]. Cultural conditions were found to affect antifungal metabolite production by tested *Streptomyces canescens* MH7. The change in pH of the culture medium induces the production of new substances that affect antibiotic production [66] [1]. For example, helvolic acid and cerulenin production of by *Cephalosporium caeruleus* were affected by a change in the pH [67] also another study reported that there was a significant association between pH and antibacterial effect of Lactobacillus against carbapenemase-producing *Enterobacteriaceae* [68]. In *S. canescens* MH7, the optimum pH was 8 while optimism temperature for antifungal metabolite production was at 30^{o} C. Aeration and mixing of the nutrients in the fermentation medium are affected by agitation.

Our present study has been found that shaking at 180 rpm increased antifungal metabolite production, which was in good agreement with previous prior study [1] Maximum production of *S. canescens* MH7 metabolite was achieved at the seventh day. Among various carbon and nitrogen sources starch and potassium nitrate were selected as best carbon and nitrogen sources for secondary metabolite production. Previous results for optimized C source and N source corresponded with our results [69].

5. Conclusion

This paper has clearly shown that *S. canescens* MH7 isolate exhibited a good potentiality to produce secondary metabolites that inhibit the growth of tested fungi, *Microsporum canis, Trichophyton rubrum, Epidermophyton floccosum,* and *Candida albicans*. This study has been found that the antifungal activity of the tested isolate was related to the production of various lytic enzymes. Also, various parameters like media, temperature, pH, incubation time, carbon, and nitrogen source had a great effect on the bioactive metabolites production.

6. References

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الملخص العربى

النشاط المضاد للفطريات من ستريبتوميسيس كانسنس MH7 المعزولة من رواسب المانجروف ضد بعض الفطريات الجلديه

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الملخص

الهدف من هذه الدراسة هو عزل البكتيريا الشعاعية التي لها نشاط مضاد للفطريات ضد بعض الفطريات الجلدية مثل ميكروسبورم كانز ,وابيدرموفيتون فلوكوسام , وتريكوفايتون ربرم و الكانددا البكانز ،تقييم وتحسين الظروف البيئيه والتغذوية المختلفة لتحقيق أقصى إنتاج لمضاد للفطريات. تم عزل إجمالي 64 بكتيريا شعاعية من مواقع مختلفة في مصر وتم فحص نشاطها المضاد للفطريات ضد الفطريات المختبرة. من بين 64 عزلة ، فإن *Streptomyces canescens* MH7 لها نشاط مضاد جيد للفطريات وتمنع نمو الفطريات المختبرة. كان لهذه العزلة القدرة على إنتاج إنزيمات الجلوكانيز والليباز والأميليز والتي تعتبر إنزيمات مهمه في تحلل جدار الخلية الفطرية. تم اختبار تحسين العديد من معايير النمو لتحقيق أقصى إنتاج من المواد المضادة للفطريات. تم الحصول على الفعالية المثلى المضادة للفطريات لـ *S. canescens* MH7 في وسط مرق الكازين النشا المضاف إليه النشا كمصدر للكربون ، ونترات البوتاسيوم كمصدر للنيتروجين بمستوى ملوحة عند 3٪ (وزن / حجم) ، ودرجة حموضة 8 ، ودرجة حرارة تحضين 30 درجة مئوية. ، فترة الحضانة 7 أيام والرج عند 180 دورة في الدقيقة.