Original Article

Taxonomic Characterizations of Soil *Streptomyces cavourensis* DW102 and Its Activity against Fungal Pathogens

Gouse Basha Sheik¹, Aroob Abdullah Alhumaidy², Ali Ismail Ali Abdel Raheim³, Zeyad Abdullah Alzeyadi¹, Mohammed Ibrahim AlGhonaim³

¹Department of Clinical Laboratory Sciences, College of Applied Medical Sciences (Male), Shaqra University, Ad-Dawadmi, Saudi Arabia, ²Department of Infectious Diseases, Saudi Center for Disease Prevention and Control (SCDC), Riyadh, Saudi Arabia, ³Department of Microbiology, College of Biological Sciences, Imam Muhammed Ibn Saud Islamic University, Riyadh, Saudi Arabia

**ABSTRACT**

**Background:** *Streptomyces* spp. are soil bacteria that have commercial value from which numerous secondary metabolites such as antifungal compounds have been derived. There is growing concern that antifungal resistance is on the rise, and soil *Streptomyces* from various geographical places might produce novel antifungal molecules. The aim of this study was to characterize and identify the actinomycetes strain namely *Streptomyces* isolate DW102, and to evaluate its antimicrobial activity against nosocomial fungal pathogens. **Materials and Methods:** *Streptomyces* isolate DW102 was identified based on morphological, cultural, physiological, and biochemical properties, together with 16S rRNA sequence. Its antifungal activity was determined by agar well-diffusion assays. **Results:** The isolate DW102 phenotypic and molecular characterization was identified as *Streptomyces cavourensis* DW102 and sequencing results were deposited in GenBank under accession number MK508855. Furthermore, the fermented broth of *Streptomyces* isolate DW102 inhibited the growth of *Aspergillus niger* and *Candida albicans* in vitro. **Conclusions:** Phenotypic, molecular, and phylogenetic analysis of DW102 identified the strain to be *S. cavourensis*. The antifungal assay showed that DW102 fermentation broth was active against both *C. albicans* and *A. niger* in vitro. Further studies are required to use the *Streptomyces* isolate DW102 as a promising source for the development of antifungal drugs.

**Keywords:** 16S rRNA, antifungal, *Aspergillus niger*, *Candida albicans*, *Streptomyces cavourensis*

Introduction

During the past few decades, fungal infections among immunosuppressed and immunocompromised patients had increased, which are of major concern in the medical field.[1,2] At present, the clinical efficacy of some antifungal agents that are used to treat invasive fungal infections has plateaued because of emerging drug-resistant fungi, and there is a need to biosynthesize new antifungal compounds.[3] Among the opportunistic fungal infections, aspergillosis and candidiasis are the two most frequently occurring nosocomial infections.[2,4,5] The virulence of *Aspergillus* spp. depends on the host immune response. It produces certain mycotoxins that are known to be carcinogenic,[6] and it is correlated with a high mortality rate in intensive care units (ICUs).[7,8] Furthermore, candidiasis and candidemia are conditions of the overgrowth of *Candida* spp., which represent major problem for immunocompromised and elderly people.[3] *Candida* spp. are the third leading cause

**Address for correspondence:** Dr. Gouse Basha Sheik, Department of Clinical Laboratory Sciences, College of Applied Medical Sciences (Male), Shaqra University, Ad-Dawadmi, Saudi Arabia. E-mail: gbasha@su.edu.sa

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Sheik GB, Alhumaidy AA, Abed Raheim AI, Alzeyadi ZA, AlGhonaim MI. Taxonomic characterizations of soil *Streptomyces cavourensis* DW102 and its activity against fungal pathogens. J Pharm Bioall Sci 2020;12:462-7.
of infections in the ICU and cause more than 90% of fungal infections in hospitals.[9] Currently, amphotericin B and azole are the antifungal agents used as first-line drugs. However, a number of species belonging to *Aspergillus* and *Candida* show resistant to these drugs which require aggressive patient management and careful identification of species.[10]

Actinomycetes are a group of bacteria, which are responsible for the production of various bioactive molecules, including antifungals.[11-13] Among actinomycetes, the *Streptomyces* spp. are mostly soil bacteria which have commercial value from which numerous secondary metabolites have been derived.[14] Moreover, the species of *Streptomyces* and *Micromonospora* are responsible for the production of 80% of antimicrobials, which are of human pharmaceutical use,[14,15] and many reports in the literature have shown that the extracellular metabolites obtained from *Streptomyces* spp. have antifungal properties.[16-18]

The objectives of this study were to characterize and identify the soil *Streptomyces* isolate DW102 based on morphological, cultural, physiological, and biochemical properties, together with 16S rRNA sequence, and to evaluate its antimicrobial activities against selected fungal pathogens.

### Materials and Methods

#### Microorganisms and culture conditions

The strain DW102 was isolated from a soil sample of Dawadmi region, Saudi Arabia, and deposited at the Culture Collection of The Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Dawadmi, Shaqra University, Saudi Arabia.[13] The isolate was maintained on slopes containing starch-casein agar medium of the following composition (g/L): Starch 20; Casein 0.3; KNO₃ 2; K₂HPO₄ 2; MgSO₄·7H₂O 0.05; NaCl 2; CaCO₃ 0.02; FeSO₄·7H₂O 0.01; Agar 20; Distilled water 1L. *Aspergillus niger* inoculum is prepared by suspending the colonies of *A. niger* grown on potato dextrose agar slant, by covering the slant with 1 mL of sterile distilled water supplemented with 0.1% Tween 20. Then the suspension was adjusted to 3 × 10⁶ conidia/mL, using a hemocytometer. Then it was further diluted to obtain final working inoculums 3 × 10⁵ conidia/mL. *Candida albicans* inoculum was prepared by suspending colonies of *C. albicans* in sterile distilled water. The suspension was adjusted at 0.5 McFarland turbidity standards. Then, the inoculum size was further adjusted to 2.5×10⁵ CFU/mL. Finally, 50 μL of both the inoculums were inoculated on separate PDA plates. Wells were bored in the center of the plates with the insertion back of the 1000 μL micropipette tip and loaded with 100 μL of fermented broth to evaluate its antifungal activity. The plates containing *A. niger* were incubated at 28°C for 72 h and the plates containing *C. albicans* were incubated at 35°C for 48 h. Then they were observed for zone of inhibition.

#### Identification of isolate DW102

**Phenotyping**

The phenotypic characteristics of the isolate DW102 were done based on the standard procedure of Bergey’s Manual of Determinative Bacteriology.[21] Cultural characteristics of pure isolates of DW102 were studied in various media (SCA [starch casein agar], ISP2 [yeast malt extract agar], ISP3 [oatmeal agar], ISP4 [inorganic salt starch agar]) after incubation at 30°C for 7–14 days. Gram staining and acid fast staining was performed. Mycelium morphology and spore chain morphology of DW102 were observed under a light microscope. After preliminary studies, the isolates were identified by biochemical analysis, that is, catalase test, coagulation of milk, nitrate reduction, gelatin liquefaction, lecithin degradation, hydrolysis of starch, and casein. Carbohydrate fermentation tests such as -glucose, fructose, galactose, L-arabinose, cellulose, maltose, xylose, inositol, sorbitol, trehalose, and rhamnose were studied.

**Analysis of 16S rRNA sequence**

**Genomic deoxyribonucleic acid extraction**

The *Streptomyces* strain DW102 was grown in 20 mL of SCB in a 100-mL conical flask at 37°C in a rotary
shaker (200 rpm) overnight. Aliquots of 1.5 mL of culture was transferred to Eppendorf tubes and centrifuged at 8,000 rpm for 2 min. The supernatant was discarded and drained well onto a tissue paper and the pellet was re-suspended in 400 µL of TE buffer, then 32 µL of lysozyme (10 mg/mL) was added and the tube was incubated at 37°C for 30 min. Following incubation 100 µL of 0.5 M ethylenediaminetetraacetic acid (pH 8) and 60 µL of 10% sodium dodecyl sulfate were added. To this, 1.5 µL of proteinase K (20 mg/mL) was added and the tube was incubated at 50°C (water bath) for 12 h. The tube was brought to room temperature and 250 µL of equilibrated phenol (equilibrated with Tris hydrochloride [Tris-HCl]) was added and mixed well. Then 250 µL of chloroform was added and the solution was centrifuged at 10,000 rpm for 10 min and extracted twice with phenol-chloroform (1:1 ratio). The aqueous phase was extracted once again with chloroform: isoamyl alcohol (24:1 ratio), and the supernatant was collected and precipitated with 2 volume of absolute ethanol. The precipitate was centrifuged at 10,000 rpm for 10 min and the supernatant was discarded. The pellet was air dried completely and washed with 70% ethanol and allowed it to dry at room temperature. After complete drying, the pellet was dissolved in 20–50 µL of sterile distilled water and stored at −20°C.

Polymerase chain reaction amplification of 16S rRNA

The 16S rRNA gene was amplified from genomic deoxyribonucleic acid (DNA) obtained from isolate DW102 using the thermal cycler Biorad T100 (Bio-Rad, Hercules, California) with universal forward primer-16S rRNA AGAGTTTGATCCTGGCTCAG and universal reverse primer-16S rRNA ATTACCGCGGCTGCTGG. The cyclic conditions were as follows: initial denaturation at 95°C for 2 min, 45 cycles of 95°C for 40 s, 51°C for 30 s, and 72°C for 1 min, and final extension of 7 min and hold at 4°C. The polymerase chain reaction (PCR) products were confirmed by 2% agarose gel electrophoresis and purified using PureLink PCR Purification Kit (Invitrogen).

Sequencing of 16S rRNA genes

In this study, cycle sequencing (sequencing PCR) was done by using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) with specific primers. After that, second purification made using BigDye XTerminator Purification Kit (Applied Biosystems), then sequencing by 3130 Genetic Analyzer (Applied Biosystems). The acquired DNA sequence was analyzed by using Sequencer software, version 4.9 (Gene Codes Corporation, Ann Arbor, Michigan), and SeqA6 (Sequencing Analysis Software, Applied Biosystems).

Phylogenetic analysis

Nucleotide sequences were compared to those in the GenBank database with the Basic Local Alignment Search Tool NCBI BLAST algorithm to identify known closely related sequences. Followed by multiple sequences alignment, which was generated for all matched sequences using MUSCLE tool. Then, to determine the genetic relationship between Streptomyces cavourensis strains, a maximum-likelihood phylogenetic tree was created based on the probabilistic model using MEGA 7 tool.

RESULTS

Identification of Streptomyces isolate DW102

Microscopic observations and staining properties of isolate DW102 showed Gram-positive and nonacid fast filamentous branching bacteria, which is one of the important criteria for Streptomyces sp. under microscope. Mycelium’s were filamentous and branched; they have rectiflexible spore chain morphology. Maximum growth was obtained on SCA and ISP2 followed by ISP3 and ISP4 media. Cultural properties of the strain DW102 are shown in Table 1. Morphological and biochemical properties of the strain DW102 are shown in Table 2. Here, biochemical characteristics of the isolate DW102 were found to be positive for catalase test, hydrolysis of starch and casein, coagulation of milk, and nitrate reduction tests, whereas negative results were obtained for gelatin liquefaction and lecithin degradation. In our study, carbohydrate fermentation tests showed that isolate DW102 fermented D-glucose, fructose, galactose, L-arabinose, and cellulose. Our strain was able to ferment maltose and xylose moderately but negative for inositol, sorbitol, trehalose, and rhamnose. The results showed that DW102 was

| Table 1: Cultural characteristics of Streptomyces cavourensis DW102 on different media |
| Media | Aerial mycelium | Reverse side pigmentation | Soluble pigments | Growth |
|-------|----------------|--------------------------|------------------|--------|
| SCA   | White          | Light brown              | Faint brown      | +++    |
| ISP2  | White          | Dark brown               | NIL              | +++    |
| ISP3  | White          | Gray                     | NIL              | ++     |
| ISP4  | White          | Gray                     | NIL              | ++     |
Sheik, et al.: Soil Streptomyces cavourensis DW102 and its activity

capable of fermenting sugar without producing gas. The Morphological and biochemical properties of isolate DW102 strongly suggested that the isolate belongs to *Streptomyces* sp.

### Genotyping of isolate DW102

In our study, the molecular characteristic of *Streptomyces* strain DW102 was evaluated by PCR amplification of 16S rRNA gene. The 16S rRNA gene of *Streptomyces* strain DW102 was partially sequenced using universal forward primer- 16S rRNA AGAGTTTGATCCTGGCTCAG and universal reverse primer- 16S rRNA ATTACCGCGGCTGCTGG for genotypic identification of the organisms. The results of our study showed that the 16S rRNA sequencing analysis of the isolate yielded 493 base pairs with quality score (Qscore) \( > 50 \). The NCBI BLAST search analysis based on the topology of phylogenetic analysis using maximum-likelihood tree method showed that the sequence was related to several sequences of *Streptomyces* species. The sequence was closely related to NR112345.1 *S. cavourensis* strain NBRC 13026 with an identity of 100\% [Figure 1].

### Antifungal assay of *Streptomyces* isolate DW102

The results of agar well diffusion assays of this study has shown that the filtered supernatant of fermented broth of *S. cavourensis* DW102 showed good antifungal activity against *A. niger* and *C. albicans*. Filtered supernatant of fermented broth of *S. cavourensis* DW102 showed maximum zone of inhibitions (15 and 17 mm) against *A. niger* and *C. albicans*, respectively.

### DISCUSSION

In this study, the strain DW102 which was isolated from a soil sample of Dawadmi region, Saudi Arabia was

---

**Table 2: Morphological and biochemical characteristics of *Streptomyces cavourensis* DW102**

| Characteristics          | Results       |
|--------------------------|---------------|
| Gram stain               | Gram-positive |
| Acid-fast stain          | Nonacid fast  |
| Nature of mycelium       | Filamentous, branched |
| Spore chain morphology   | Rectiflexible |
| Optimum temperature      | 30°C          |
| Optimum pH               | 7             |
| Catalase test            | Positive      |
| Starch hydrolysis        | Positive      |
| Casein hydrolysis        | Positive      |
| Milk coagulation         | Positive      |
| Nitrate reduction        | Positive      |
| Gelatin liquefaction     | Negative      |
| Lecithin degradation     | Negative      |
| D-Glucose                | Positive      |
| Fructose                 | Positive      |
| Galactose                | Positive      |
| Maltose                  | Moderate      |
| Xylose                   | Moderate      |
| L-Arabinose              | Positive      |
| Cellulose                | Positive      |
| Inositol                 | Negative      |
| Sorbitol                 | Negative      |
| Trehalose                | Negative      |
| Rhamnose                 | Negative      |

---

**Figure 1:** Phylogenetic tree of MK508855 *Streptomyces cavourensis* DW102
characterized and identified based on morphological, cultural, physiological, and biochemical properties, together with 16S rRNA sequence, and its antifungal activity was determined. Isolate DW102 showed Gram-positive and nonacid fast filamentous branching bacteria, which distinguish from other actinomycetales such as nocardia, actinomyces, and rhodococcus which are partially acid fast. In our study, the optimal culture temperature was 30°C, and the optimal media pH for Streptomyces strain DW102 was found to be 7 which both falls in the findings of previous work done by Sholkamy et al.[26] In our study various biochemical parameters and carbohydrate fermentation tests were used for identification of Streptomyces. Previous studies also considered Catalase test, starch hydrolysis, urea hydrolysis, nitrate reduction, gelatin hydrolyses characterizing Streptomyces.[27,28] It is important to taxonomically place isolated actinomycetes based upon their genus and species levels. The 16S rRNA sequence analysis has become an important tool in bacterial identification, as it provides information about the phylogenetic placement of the species,[28] and it has been used by previous researchers for Streptomyces sp. identification.[26,30-32] In concordance with previous studies, phylogenetic 16s RNA helped to identify the isolate to the species level.[29,30,31] In our study, the phylogenetic analysis results showed that the isolate occupies a distinct phylogenetic position within the radiation including the representatives of Streptomyces family. Hence, the morphological, cultural, biochemical, and 16S rRNA analysis of isolate DW102 strongly suggested that the isolate belongs to S. cavourensis. The gene sequence was deposited to the database of NCBI gene bank with accession number MK508855.

Due to the global increase in fungal diseases especially in immunocompromised patients and the rapid development of antifungal drug resistance, finding new antifungal molecules has become a major challenge to the medical and pharmaceutical industry today.[10,33] Our study has shown that the filtered supernatant of fermented broth of S. cavourensis DW102 has good antifungal activity against A. niger and C. albicans. Species of Streptomyces have been reported to produce antifungal activities against major pathogens such as C. albicans, Aspergillus spp., Fusarium spp., Penicillium spp., and Rhizopus spp.[16,19,30] The inhibitory effect may be due to the activity of the secondary metabolites produced by the Streptomyces spp. on fungal hyphae.[16,19,34] However, further assessment is needed to investigate the antifungal cytotoxicity, mode of action, and minimum inhibitory concentration. Also, DW102 growth optimization is needed to increase antifungal production and purification which will allow for further work on the compound.

**Conclusion**

Here we report that the *Streptomyces* isolate DW102 isolated from the soil sample of Dawadmi region was identified as *S. cavourensis* based on the morphological, cultural, biochemical, and 16S rRNA analysis. Moreover, BLAST search analysis showed that the sequence was closely related to NR112345.1 *S. cavourensis* strain NBRC 13026 with an identity of 100%. The strain was deposited to GenBank under the accession number MK508855. The DW102 antifungal assay showed that it was active against both *C. albicans* and *A. niger in vitro*. Further studies are required to use *Streptomyces* isolate DW102 as a promising source for the development of antifungal drugs.

**Financial support and sponsorship**

Deanship of Scientific Research at Shaqra University, Saudi Arabia (D180402/G01/N009).

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. CDC—Centre for Disease Control and Prevention [Internet]. Antifungal resistance. 2019. Available from: https://www.cdc.gov/fungal/antifungal-resistance.html. [Last accessed on 2020 Feb 4].
2. Bassetti M, Bouza E. Invasive mould infections in the ICU setting: complexities and solutions. J Antimicrob Chemother 2017;72:i39-47.
3. Perfect JR. The antifungal pipeline: a reality check. Nat Rev Drug Discov 2017;16:603-16.
4. Beck-Sague C, Jarvis WR. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980–1990. National Nosocomial Infections Surveillance System. J Infect Dis 1993;167:124751.
5. Lamoth F, Lockhart SR, Berkow EL, Calandra T. Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother 2018;73:14-13.
6. Sheik GB, AbdeRaheem AIA, AlShehri ZS, Otaibi OB. Assessment of bacteria and fungi in air from college of applied medical sciences (Male) at AD-Dawadmi, Saudi Arabia. Int Res J Biol Sci 2015;4:48-53.
7. Dimopoulos G, Frantzeskaki F, Poulakou G, Armaganidis A. Invasive aspergillosis in the intensive care unit. Ann N Y Acad Sci 2012;1272:31-9.
8. Beck O, Topp U, Koehl E, Roilides M, Simitsopoulou M, Hanisch M, et al. Generation of highly purified and functionally active human TH1 cells against Aspergillus fumigates. Blood 2006;107:2562-69.
9. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302:2323-29.
10. Arendrup MC. Update on antifungal resistance in Aspergillus and Candida. Clin Microbiol Infect 2014;20:42-8.
11. Rotich MC, Magiri E, Bii C, Maina N. Bio-prospecting for broad spectrum antibiotic producing actinomycetes isolated from virgin soils in Kericho County, Kenya. Adv Microbiol 2017;7:56-70.
12. Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 2016;79:62961.
13. Sheik GB, Raheim AIAA, Alzeyadi ZA, AlGhonaim MI. Extracellular synthesis, characterization and antibacterial activity of silver nanoparticles by a potent isolate Streptomyces sp. DW102 Asian J Biol Life Sci 2019;8:89-96.
14. Watve MG, Tickoo R, Jog MM, Bhole BD. How many antibiotics are produced by the genus Streptomyces? Arch Microbiol 2001;176:386-90.
15. Baltz RH. Renaissance in antibacterial discovery from actinomycetes.Curr Opin Pharmacol 2008;8:557-63.
16. Ayari A, Morakchi H, Djamila KG. Identification and antifungal activity of Streptomyces sp. S72 isolated from Lake Oubeira sediments in North-East of Algeria. Afr J Biotechnol 2012;11:305-11.
17. Jain PK, Jain PC. Isolation, characterization and antifungal activity of Streptomyces sampsonii GS 1322. Indian J Exp Biol 2007;45:203-6.
18. Benouagueni S, Ranque S, Gaemir Kerane D. A non-polyenic antifungal produced by a Streptomyces yatensis strain isolated from Mellah lake in El Kala, North-East of Algeria. J Mycol Med 2015;25:2-10.
19. Alier AA, Ntulume I, Odda J, Okech MA. Production of novel antifungal compounds from actinomycetes isolated from waste dump soil in Western Uganda. Afr J Microbiol Res 2017;11:1200-10.
20. Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalun N. Antibacterial activity of actinomycetes isolated from different soil samples of sheopur (A city of central India). J Adv Pharm Technol Res 2013;4:118-23.
21. Ravikumar S, Krishnakumar S. Antagonistic activity of marine actinomycetes from the Arabian Sea coast. Arch Appl Sci Res 2010;2:273-80.
22. Farris MH, Olson JB. Detection of actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett Appl Microbiol 2007;45:376-81.
23. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004;32:1792-7.
24. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 1981;17:368-76.
25. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Bio Evol 2016;33:1870-74.
26. Shokkamy EN, Ahamed MS, Yasser MM, Eslam N. Anti-microbial activities of bio-synthesized silver nano-stars by Saccharopolyspora hirsuta. Saudi J Biol Sci 2019;26:195-200.
27. Abd-Elnaby IH, Gehan M, Abo-Elala, Usama M, Abdel-Raouf, Hamed MM. Antibacterial and anticaner activity of extracellular synthesized silver nanoparticles from marine Streptomyces rochei MHM13. Egypt J Aquat Res 2016;42:301-12.
28. Yuan XW, Yang RL, Cao X, Gao JJ. Taxonomic identification of a novel strain of Streptomyces cavourensis subsp. washingtonensis, ACMA006, exhibiting antitumor and antibacteria activity. Drug Discov Ther 2010;4:405-11.
29. Brenner D, Staley J, Krieg N. Classification of procaryotic organisms and the concept of bacterial speciation. In: Boone DR, Castenholz RW, Garrity GM, editors. Bergey's manual of systematic bacteriology. 2nd ed. New York: Springer Verlag; 2000. p. 27-38.
30. Al-Garni SM, Sabir JSM, El-Hanafy AAM, Kabli SA, Al-Twiley DA, Ahmed MM. Isolation and identification of antimicrobial actinomycetes strains from Saudi environment. J Food Agric Environ 2014;12:1072-79.
31. El-Naggar NE, El-Shweihy NM, El-Ewasy SM. Identification and statistical optimization of fermentation conditions for a newly isolated extracellular cholesterol oxidase-producing Streptomyces cavourensis strain NEAE-42. BMC Microbiol 2016;16:217.
32. Pan HQ, Yu SY, Song CF, Wang N, Hua HM, Hu JC, et al. Identification and characterization of the antifungal substances of a novel Streptomyces cavourensis NA4. J Microbiol Biotechnol 2015;25:353-7.
33. Low CY, Rotstein C. Emerging fungal infections in immunocompromised patients. F1000 Med Rep 2011;3:14.
34. Loqman S, Barka EA, Clement C, Ouldouch Y. Antagonistic actinomycetes from Moroccan soil to control the grapevine graymold. World J Microbiol Biotechnol 2009;25:81-91.