https://doi.org/10.48047/AFJBS.6.15.2024.13304-13318

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Isolation and screening of marine Actinomycetes as a source of bioactive substances against bacteria and fungi.

Chemlal-Kherraz Djazia*, Khelil Fatima, Maatallah-Boutiba Amaria and Bouderbala Mohamed.

Environmental Monitoring Network, Department of Biology, Faculty of Science, University Ahmed Ben Bella Oran 1, Algeria.

Corresponding author: Chemlal-kherraz djazia

e-mail : kherraz_djazia@yahoo.fr

Tel: 213 555577176

Volume 6, Issue 15, Oct 2024

Received: 15 Aug 2024

Accepted: 25 Sep 2024

Published: 21 Oct 2024

doi: 10.48047/AFJBS.6.15.2024.13304-13318

ABSTRACT

Driven by the growing demand for new bioactive molecules effective against multi-resistant bacteria, the research and development of new products has been propelled to the rank of the most important challenges for humanity.

This present work allowed us the isolation and purification of seven strains of actinobacteria from marine water and sediment as well as the study of the inhibitory power of these isolates on some test bacteria and fungi.

Antimicrobial activity was evaluated by the agar cylinder technique against two gram-positive bacteria (*Bacillus subtilis and Staphylococcus aureus*), two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*), the yeast *Candida albicans* and two filamentous fungi *Fusarium oxysporum f. sp. albidinis* and *Aspergillus niger*.

The screening of antimicrobial activities demonstrated the production of antibacterial and/or antifungal metabolites of all our isolates. The largest inhibition diameters are recorded with the S1 and E6 strain against *Pseudomonas aeruginosa*.

The majority of isolates have an antimicrobial effect against the yeast *Candida albicans* with the largest zone of inhibition (18mm) noted with the E6 strain. Activity of strains S4, E5, E6 is recorded against *Fusarium oxysporum f. sp. albidinis* with a very large diameter of 23.5 for E6.

Key words: Actinobacteria, seawater, sediment, antimicrobial activity, bioactive molecules.

Introduction

Antibiotics have had an immense impact in the treatment of infections caused by pathogenic microorganisms. However, abusive antibiotic therapy, self-medication as well as the intensive

use of antibiotics in agriculture have led to the emergence of new bacterial strains that are multi-resistant to them (Zeng et *al.*, 2018). As a result, there is currently no class of antibiotics; both of natural and synthetic origin; escapes this phenomenon. The resistance of bacteria to antibiotics remains a major public health problem today and one that is evolving over time (Luepke et *al*., 2017).

Actinomycetes are the largest producers of antibiotics (Sanglier et *al.,* 1993; Takahashi et Omura, 2003; Parra et *al*., 2023). The Streptomyces genus is the most exploited for its numerous antagonistic properties and continues to attract the interest of several laboratories around the world.

Marine actinobacteriology has become one of the major emerging areas of research worldwide (Sivakumar et *al.*, 2007). Marine actinobacteria occur on sediments and in water and also on other biomasses and substrates (Gargi et Suthindhiran, 2022). However, it has not yet been resolved whether these microorganisms are part of the indigenous marine microbial community, native to land as habitat or they are simply carried to the sea in the form of resistant spores (Larpent et Sanglier, 1989; Goodfellow et Haynes, 1984; Bull et *al.*, 2000).

The diversity of the marine biotope has exerted a driving force on the selection of bacteria leading to new adaptation strategies and the synthesis of new active bio-metabolites (Jensen et Fenical, 1996; De Carvalho et Pedro, 2010). Studies have made it possible to isolate and identify a very large number of new molecules of great structural originality, many of which have interesting biological activity. Indeed, several studies confirm that actinomycetes are capable of emitting inhibitory substances such as the work of Rose et *al.* (1980); Turhan et Grossmann (1986); Crawford et *al. (*1993); Yan et Crawford (1995); El Tarabily et *al.* (1997); Barakate et *al.* (2002); Gretha et *al.* (2005); Aftab (2017). *Salinispora tropica* is an actinomycete isolated from marine sediments, secreting Salinosporamid A with anticancer activity (Ma et *al.*, 2011; Ashwini et Charushila, 2023).

The isolation of actinomycetes from little or no exploited ecosystems surely offers possibilities for discovering bacteria with a new genetic heritage allowing the discovery of new or rare strains other than Streptomyces with unexploited potential and this It is in this context that our work takes place, the objectives of which are the isolation and purification of actinobacteria from samples of marine water and sediment and the study of the antagonism of these isolates towards germs. pathogenic for humans and plants.

Materials and methods

1. Sampling technique

Water and sediment samples were taken at Madagh 1 beach, from Algerian west coast. Water sampling was carried out manually, by introducing a 5liter bottle (previously washed and rinsed several times) into the water. After filling the bottle is hermetically closed. For the sediment, 100 to 150 g of sand is collected in a sterile container. The samples are transported in a cooler to the laboratory for analysis.

2. Preparation of culture media:

ISP2 medium (International Streptomyces Project 2) was used for the isolation and purification of actinobacteria species. After autoclaving (121°C, 15 min) and cooling to around 50°C, a solution of nystatin (antifungal) (50 µg ml-1) and 10 µl ml-1 of nalidixic acid (antibacterial inhibiting Gram-negative bacteria) are added in the middle (Cavala et Eberlin, 1994; Nooshin et Janardhana 2015).

3. Isolation of actinomycetes:

From the seawater sample, decimal dilutions were prepared. For the sediment, 10g were weighed then suspended in 90ml of sterile physiological water. After a good mixing of this suspension which corresponds to 10-1, a series of decimal dilutions is carried out (Kumar et *al.*, 2010). The ISP2 medium is inoculated with 0.1ml of each dilution (water and sediment). The plates are incubated at 28°C for 10 to 14 days (Collins et *al.*, 1995).

3.1 Purification of strains:

To obtain pure strains, the different colonies which are similar in their macroscopic and microscopic aspects to actinomycetes, are transplanted and inoculated by the streak method in Petri dishes containing the same isolation medium, then incubated for two weeks at 28°C.

4. Antimicrobial activity

The antimicrobial activity of actinomycete isolates is demonstrated by the agar cylinder technique.

4.1 Preparation of inocula of test bacteria:

4.1.1 Test bacteria:

The strains used in microbiological tests belong to two groups of microorganisms: *Pseudomonas aeruginosa* (ATCC 27853); *Escherichia coli* (ATCC 25921); *Bacillus subtilis* (ATCC 6633); *Staphylococcus aureus* (ATCC 25923) and fungi: *Candida albicans*, *Fusarium oxysporum f.sp.albedinis* and *Aspergillus niger.*

The bacterial strains were preserved at 4°C in nutrient agar slant while the fungal strains in Sabouraud medium.

4.1.2 Bacterial pre-cultures:

In order to stimulate the growth of the preserved bacterial strains, they are inoculated in test tubes containing 5ml of nutrient broth then incubated at 37°C for 24 hours. They are then inoculated by exhaustion streaks on nutrient agar, in Petri dishes and incubated at 37°C for 24 hours.

4.1.3 Fungal pre-cultures:

A mycelial implant of 0.5 cm side is taken from a culture of filamentous fungus, it is seeded on Sabouraud medium in a Petri dish and incubated at 25°C for 3 to 7 days. For yeast, a unicellular fungus, seeding is carried out by exhaustion streaks on Sabouraud medium.

5. Confrontation using the agar cylinder technique:

The search for antibacterial metabolites is carried out using the agar cylinder technique. The actinomycete isolates are seeded in tight streaks on the surface of the ISP2 medium and incubated at a temperature of 28°C for 14 days. Agar cylinders 6 mm in diameter are then taken and placed on the surface of the Muller-Hinton medium previously seeded with the test bacteria (after measuring their optical density). The boxes are incubated at a temperature of 37°C for 24 hours. The inhibition zones formed around the cylinders are then measured (Pazhanimurugan et *al.*, 2012).

For antifungal activity, the filamentous fungi are subcultured on PDA medium and incubated at 25°C for 7 days. A dense suspension of spores is prepared to obtain a spore concentration of 10^7 spores/ml (Hwang et *al*., 2001). The agar cylinders with the actinomycetes are cut out and placed on the surface of the dishes with the PDA seeded with the fungal sporal solution. After incubation of the boxes at 25°C for 5 days, the inhibition zones are noted.

6. Study of antibiotic resistance:

The method used to perform the antibiogram is the standardized technique according to WHO (world Health Organization) recommendations. The antimicrobial effect was tested by different

antibiotics commonly used on the 4 bacteria, in order to compare their therapeutic effectiveness with that of actinomycetes.

6.1 Carrying out the antibiogram:

Antibiotic resistance was determined by the diffusion method of antibiotic-impregnated disks and the measurement of inhibition diameters. After adjusting the density of the inoculum to that of the 0.5 Mac Farland standard (corresponds to 10^6CFU/mL) the Muller-Hinton medium is inoculated, allowed to dry, then 10 different discs of commercially available antibiotics (Ceftazidime, Amoxicillin, Clindamycin, Penicillin, Spiramycin, Gentamicin, Cefoxitin, Trimethoprim + Sulfamethoxazole, Chloramphenicol and Erythromycin are deposited. After incubation at 37°C for 24 hours, inhibition zones can be observed around the discs, the diameter of the inhibition zone is then measured.

All analyzes were carried-out in triplicates and the experimental data were expressed as means ± standard deviation using Microsoft Office Excel 2007.

Results

1. Isolation of actinomycetes:

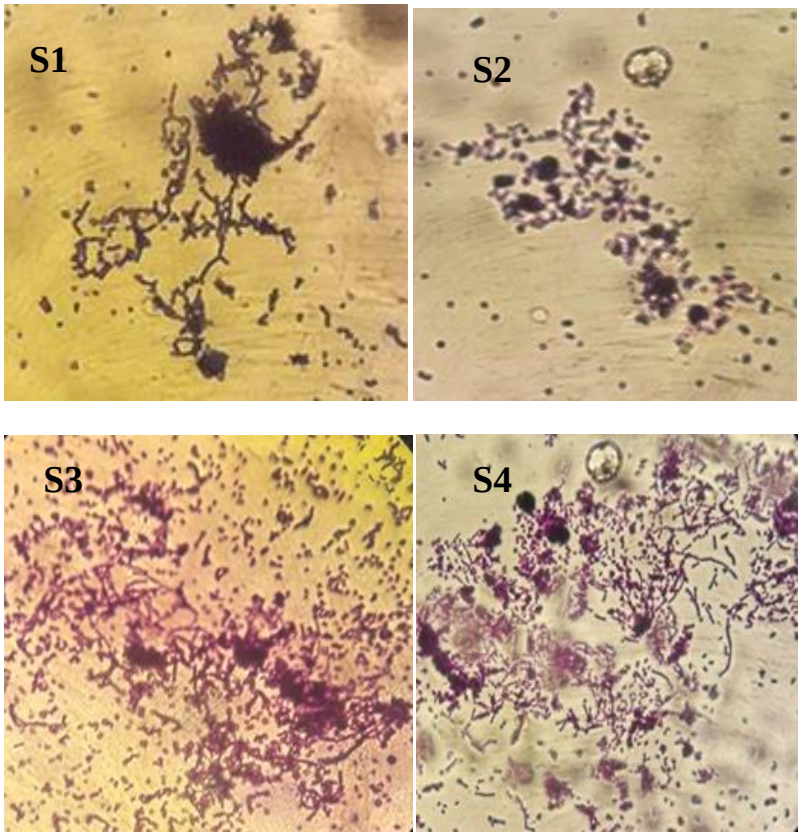
Actinomycete colonies isolated from seawater and sediment and seeded on ISP2 supplemented with the antibiotic and antifungal appear after 10 to 21 days of incubation at 28ºC. The colonies are recognized by their macroscopic appearance (hard colonies embedded in the medium) and microscopic (branched filamentous appearance).

Among the strains obtained, seven could be purified and subcultured on the medium, these are the strains coded S1, S2, S3, S4, S7 isolated from the sediment and E5, E6 isolated from sea water. The colonies have different sizes (small, medium, large) of variable shape (smooth, rounded, flattened), are all embedded in the agar, having a vegetative mycelium surmounted by an aerial mycelium of different colors (whitish or grayish) (*Figure 1*).

Figure 1: *Macroscopic appearance of the S7 strain isolated on ISP2*

1.1 Microscopic observation:

According to Gram staining, microscopic observation shows that all strains of isolated and purified actinomycetes present a filamentous appearance with the presence of spores isolated or in clusters which are sometimes short or long chains. This staining confirms the belonging of these isolates to the groups of Gram-positive bacteria *(Figure 2).*



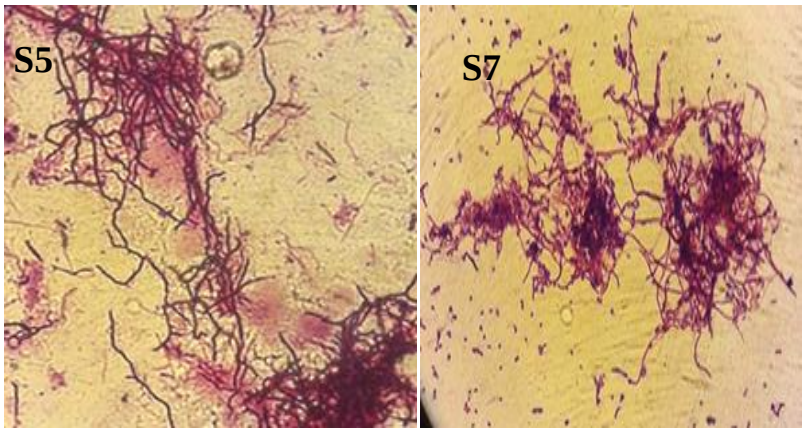


Figure 2: *Microscopic appearance of some isolated strains after Gram staining Grx100*

2. Result of antimicrobial activity:

According to the results obtained *(Figure 3),* the antibacterial activity differs from one actinomycetal bacteria to another, however this activity remains very moderate for all the isolates.

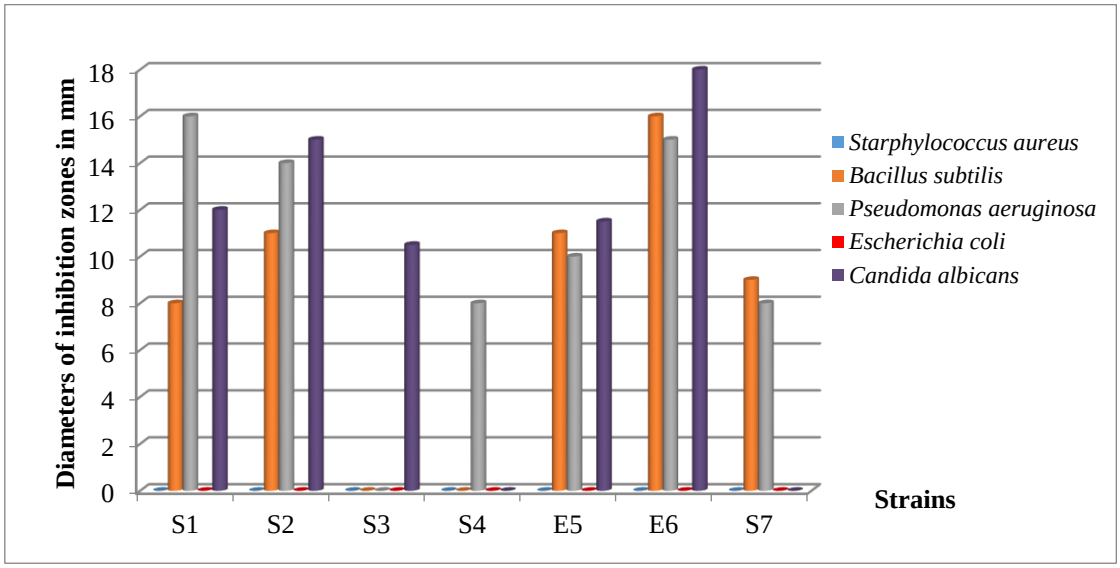


Figure 3: *Results of antimicrobial activity tests*

3. Antifungal test results:

The antifungal test results are shown in *Figure 4.*

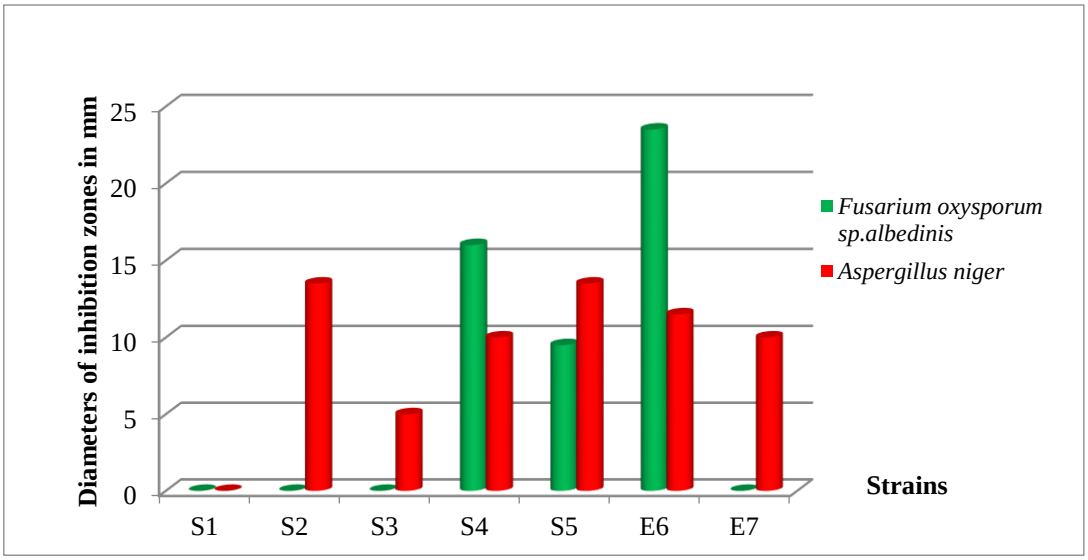


Figure 4: *Antifungal test results*

4. Antibiogram result:

The sensitivity of the test bacteria to the 10 antibiotics used gave the results presented in *Table 1*.

Table 1: Antibiotic susceptibility and resistance (mm)

Bacteria / Antibiotics	***S. aureus***	***B. subtilis***	***P. aeruginosa***	***E. coli***
Cetazidine	R	R	12.00 ± 1.00	12.00 ± 1.52
Amoxicillin	R	R	15.00 ± 0.75	R
Clindamycin	R	20.00 ± 1.52	10.00 ± 2 .08	R
Penicillin (G)	R	40.00 ±0.57	R	R
Spiramycin	R	22.00 ± 1.52	32.00 ± 1.00	12.00 ± 1.73
Gentamicin	R	20.00 ± 1.52	10.00 ± 0.75	20.00 ± 2.64
Cefoxilin	R	15.00 ± 1.15	R	17.00 ± 4.34
Trimethoprim+Sulphamethoxazole	R	15.00 ± 1.00	26.00 ± 0.00	26.00 ± 2.00
Chloramphenicol	37.00 ± 1.15	27 .00 ± 0.75	32.00 ± 1.52	35.00 ± 1.73
Erythromycin	R	24.00 ± 2.00	17.00 ± 1.00	R

Discussion

The sampling carried out at the water and sediment level of Madagh beach initially allowed us to isolate and purify seven strains presenting cultural and morphological characteristics similar to those known for the Actinomycetes group.

Several sample pretreatment techniques are cited in the literature such as the use of antibiotics and antifungals (Zhang, 2011) and the pretreatment of soil by physical methods such as heating

(Baskaran et *al.,* 2011) and drying (Yamamura et *al.,* 2012, Benita et Krishnan, 2014). These pretreatment methods aim to reduce the microflora and allow slow-growing actinobacteria to colonize the environment.

For our work, despite the use of an antifungal and an antibiotic in the isolation medium (ISP2) which is the reference medium for the culture of actinomycetes and which allows good sporulation, the number of the latter has been reduced this can be explained by the choice of antimicrobials and the absence of pretreatment of samples such as the addition of CaCO3, followed by incubation for 7 to 9 days at room temperature in an atmosphere saturated with humidity. This pretreatment has the effect of reducing the fungal flora as well as increasing the number of actinomycetes contained in each sample (Ullah et *al.,* 2012). According to some authors, a universal technique for isolating actinomycetes does not exist. It is always necessary to vary the methods and isolation environments in the same screening, in order to successfully isolate the actinomycetal flora which makes up the sample studied (Boudemagh, 2007).

However, numerous studies tend to confirm the presence of actinobacteria in aquatic environments (Alqaraawee et *al.,* 2023). Terkina et *al.* (2002) revealed the predominance of the genera *Steptomyces* and *Micromonospora* in Lake Baikal (Russia); they observed that the lake waters were dominated by Streptomyces (66% of isolates) and the sediments by Micromonospora (59% of isolates). The same findings were also reported by Rifaat (2003) at the Nile (Egypt). As for the work carried out on the sediment of Loktak Lake (India), of the 172 isolates, the majority belonged to the genus Streptomyces and nocardioform actinomycetes (Sanasam et *al.,* 2011). These results are different from those of Terkina et *al.*, (2002) and Rifaat (2003), but can be explained by the trophic character of the lakes. Indeed, Lake Baikal has an oligotrophic character while Lake Loktak is eutrophic and heavily polluted by waste-laden currents and agricultural runoff.

The search for antimicrobial activities on the solid ISP2 medium in the water and sediments of Madagh beach also allowed us to demonstrate that all the actinomycetes isolated during our work had antimicrobial activity against at least one microorganism test used (bacteria or fungi). Indeed, several studies have corroborated the remarkable antimicrobial power of these bacteria (McKenzie et *al.*, 2010; Mythili et Ayyappa, 2011; Ng et Amsaveni, 2012; De La Hoz-Romo et *al.,* 2022). Valli et *al.* (2012) isolated 21 strains of actinomycetes from a sample collected from the seashore where all isolates were capable of producing antimicrobial substances.

Microbial production of secondary metabolites is generally influenced and connected to the primary metabolism of the producing strain. Intermediate metabolites at the end of primary

metabolism serve as precursors for the biosynthesis of these bioactive secondary metabolites. The composition of the culture medium influences the metabolic capacities of the producing organism as well as the biosynthesis of secondary metabolites (Smaoui, 2010) in addition to nutritional factors, other factors, influencing the production of antimicrobial molecules, in particular the pH, temperature, and inoculum (Muiru et *al*., 2007; Atta et *al.*, 2010; Alqaraawee et *al.*, 2023).

The two test bacteria *Staphylococcus aureus* and *Escherichia coli* are not sensitive to any isolated strain; on the other hand, the majority of our actinomycetal strains exert an inhibitory power against *B. subtilis* and *P. aeruginosa*. We noted the activity of most strains against *C. albicans*, this is in agreement with the work of Souagui et *al.* (2019) and Alqarrawee et *al.* (2023). The antifungal activity was more evident against *Aspergillus niger* with 5 active strains (S2, S3, S4, S5, E6, and E7) compared to only three active ones (S5, E6 and E7) on *F. oxysporum sp. albedinis*. Noting that the largest area recorded was that noted against the latter by the E6 strain (23.5mm). Other antifungal activities of actinomycetes have been reported against *A. niger* (Augustine, 2005) and *F. oxysporum* (Carlucci et *al.*, 2022). These variations in antimicrobial activity results can be explained by the fact that an actinomycetal bacteria can produce several types of antibacterial molecules whose nature depends on the composition and concentration of the components of the culture medium (Boughachiche et *al.*, 2005; Alqaraawee et *al.*, 2023).

Indeed, it is well established that culture conditions enormously influence the production of antibiotics by actinomycetes (Schwarz et *al.*, 2021). For example, Cheraiti et Gacemi, (2012) showed that the antifungal activity of certain strains of actinomycetes was greater in GYEA medium compared to ISP1, ISP2 and Bennett media. However, Boussaber et *al.* (2013), found that Bennett medium was the best medium for antifungal production since 86.2% of the actinomycetes studied presented inhibitory activity against at least one of the fungi tested on this medium. Furthermore, Augustine (2005) showed that antifungal production can be stimulated or inhibited by variations in pH.

By comparing the results of the antimicrobial activity of the isolates with those of the antibiograms we noted that the resistance of *Staphylococcus aureus* to the antibacterial molecules produced by our actinomycetal strains is probably due to its natural or acquired resistance since it is resistant to 9 out of 10 antibiotics used. The sensitivity of *Bacillus and Pseudomonas* towards our isolates was confirmed, but it remains far inferior to the effect of some antibiotics used.

Conclusion

In view of these results, it appears that these actinomycetes species have good antimicrobial activity against Gram-negative and Gram-positive bacteria, yeasts and fungi. This is why it is necessary to deepen these studies and characterize the bioactive molecules of the isolates with the aim to possible biotechnological application.

References

Aftab, S.I. (2017). Antitumor peptides from *Streptomyces sp*. SSA 13, isolated from Arabian Sea. *The International Journal of Peptide Research and Therapeutics* 23(2) :199–211. https://doi.org/10.1007/s10989-016-9552-6

Alqaraawee, R., Afra, S., Asoodeh, A. et Makhdoumi, A. (2023). A potent antifungal activity by the marine *Streptomyces albidoflavus sp.* ADR10 from the Caspian Sea sediment: optimization and primary purification. *Journal of Genetic Resources.* 9(2): 29-38. https://doi: 10.22080/jgr.2023.25049.1345.

Ashwini, B. A. et Charushila J. B. (2023). Marine natural products and derivatives. *RPS Pharmacy and Pharmacology Reports*, Volume 2, Issue 2, April 2023, rqad008, https://doi.org/10.1093/rpsppr/rqad008

Atta, H. M., Bayoumi, R. , El-Sehrawi, M. , Aboshady, A. et **Al-Humiany, A. (2010).** Biotechnological Application for Producing Some Antimicrobial Agents by Actinomycetes Isolates from Al-khurmah Governorate. *European Journal of Applied Sciences*. Vol: 2. N°: 3. Pp: 98-107.

Augustine, S.K. (2005). Production of a growth dependent metabolite active against dermatophytes by *Streptomyces rochei* AK 39. *Indian Journal of Medical Research.,* 121: 164-170.

Barakate, M., Ouhdouch, Y., Oufdou, K.H. et **Beaulieu, C. (2002).** Characterization of rhizospheric soil Streptomyces from Moroccan habitats, and their antimicrobial activities. *World Journal of Microbiology and Biotechnology.* 18 :49-54.

Baskaran, R., Vijayakumar, R. et Mohan, P. M. (2011). Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Malaysian Journal of Microbiology*, Vol 7. N°1. Pp. 26-32.

Benita, M. R. et Krishnan, K. (2014). Extraction and Identification of Antibacterial Secondary Metabolites from Marine *Streptomyces sp*. VITBRK2. *International Journal of Molecular and Cellular Medicin.* 3(3): 130–137.

Boudemagh, A. (2007). Isolation, from Saharan soils, of actinomycetal bacteria producing antifungal molecules, molecular identification of active strains. *Doctoral thesis in applied microbiology; University Mentouri Constantine.* Pp: 144.

Boughachiche, F., Reghioua, S., Oulmi, l., Zerizer, H., Kitouni, M., Boudemagh, A. et **Boulahrouf, A. (2005).** Isolation of actinomycetales producing antimicrobial substances from the sabkha of Ain Mlila. *Sciences & Technologie*. N: 23. Pp: 5-10.

Boussaber, E., El Idrissi, S. B. S., Meftah Kadmiri, I., Hilali, L. et Hilali A. (2013). A molecular approach to the identification of actinomycetes producing antimicrobial activities strains by sequencing of the 16S rDNA. *International Journal of Innovation and Applied Studies*. Vol. 3 No. 4, pp. 1053-1065.http://www.issr-journals.org/ijias/

Bull, A.T., Ward, A.C. et Goodfellow, M. (2000). Search and discovery strategies for biotechnology: the paradigm shift. *Microbiology and Molecular Biology Reviews.* 64 :573–606.

Carlucci, A., Raimondo, M. L., Colucci, D. et 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

Cavala, M. et Eberlin T. (1994). Isolement des streptomycètes du sol. *L'opéron*. 4 : 7-13.

Cheraiti, N. et Gacemi, K. D. (2012). Isolation of actinomycete strains producing new antifungal molecules. *Revue de microbiologie industrielle, sanitaire, et Environnement*. Vol. 6, No. 1, pp. 18-34.

Collins, C. H., Lyne, P. M. et Granje, J. M. (1995). Morphological Methods. *7th Edn., Butterworth and Heinemann Publishers*, London. 129-131.

Crawford, D. L., Lynch, J. M. et Ousley, M.A. (1993). Isolation and characterization of actinomycetes antagonists of a fungal root pathogen. *Applied and Environmental Microbiology*. 59: 3899-3905.

De Carvalho, C.C.C.R. et Pedro, F. (2010). Production of metabolites as bacterial responses to the marine environment. *Marine Drugs.* 8 :705–727.

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

El Tarabily, K.A., Hardy, G.E., Sivasithamparam, H. K. et **Kurtboke, I.D. (1997).** The potential for the biological control of cavity spot disease of carrots caused by *Phytium coloratum* by steptomycete and non-strepyomycete actinomycetes in wastern Australia. *New pathologist*. 137: 495-507.

Gargi S. et Suthindhiran K. (2022). Diversity and Biotechnological Potential of Marine Actinomycetes from India. *Indian Journal of Microbiology*. 62(4) :475–493. https://doi.org/10.1007/s12088-022-01024-x

Getha, K., Vikineswary, S., Wong, W.H., Seki, T., Ward, A. et Goodfellow, M. (2005). Evaluation of *Streptomyces sp.* strain g10 for suppression of *Fusarium* wilt and rhizosphere colonization in pot-grown banana plantlets. *Journal of Industrial Microbiology and Biotechnology*. Vol: 32. Pp: 24-32.

Goodfellow, M. et Haynes, J.A. (1984). Actinomycetes in marine sediments. In Biological, Biochemical, and Biomedical Aspects of Actinomycetes. Ortiz-Ortiz L. Bojalil L.F. and Yakoleff V. (eds). *New York, USA: Academic Press*, pp. 453–472.

Hwang, B. K., Lim, S.W., Kim, B.S., Lee, J.Y. et Moon S.S. (2001). Isolation in Vivo and in Vitro of Antifungal Activity of Phenylacetic Acid and Sodium Phenylacetate from *Streptomyces humidis. Applied and Environmental Microbiology*. 67: 3739-3745.

Jensen, P.R. et Fenical, W. (1996). Marine bacterial diversity as a resource for novel microbial products. *Journal of Industrial Microbiology and Biotechnology.* 17:346–351.

Kumar, N., Singh, R. K., Mishra, S. K., Singh, A. K. et Pachouri, U. C. (2010). Isolation and screening of soil Actinomycetes as source of antibiotics active against bacteria. *International Journal of Microbiology Research*. Vol: 2. Pp: 12-16.

Larpent, J.P. et Sanglier, J.J. (1989). Biotechnologie des antibiotiques. *Editions Masson. Paris*, 481p.

Luepke, K.H., Suda, K.J., Boucher, H., Russo, R.L., Bonney, M.W., Hunt, T.D. et al. (2017). Past, Present, and Future of Antibacterial Economics: Increasing Bacterial Resistance, Limited Antibiotic Pipe-line, and Societal Implications. *Pharmacotherapy*. 37(1) :71-84. https://doi.org/10.1002/phar.1868

Ma, Y., Qu, L., Liu, Z., Zhang, L., Yang, Z. et Zhang, L. (2011). Synthesis of salinosporamide A and its analogs as 20S proteasome inhibitors and SAR summarization.

Current Topics in Medicinal Chemistry. Vol: 11. N°: 23. Pp: 2906-22. https://doi.org/10.2174/156802611798281302

McKenzie, N. L., Thaker, M., Koteva, K., Hughes D. W., Wright, G. D. et Nodwell, J. N. (2010). Induction of antimicrobial activities in heterologous streptomycetes using alleles of the *Streptomyces coelicolor* gene absA. *The Journal of Antibiotics.* Pp: 1– 6

Muiru, W. M., Mutitu, E. W et Mukunya D. M. (2007). Characterization of antibiotic metabolites from actinomycete isolates. *African Crop Science Conference Proceedings*. Vol: 8. Pp : 2103-2107.

Mythili, B. et Ayyappa, Das. M. A. (2011). Studies on Antimicrobial Activity of *Streptomyces spp*. Isolates from Tea Plantation Soil. *Research Journal of Agricultural Sciences*. Vol: 2. N°: 1. Pp: 104-106.

Ng, Z. Y. et Amsaveni, S. (2012). Isolation, Screening and Characterization of Antibiotic-Producing Actinomycetes from Rhizosphere Region of different plants from a farm of sungai Ramal Luar, Malaysia. *Journal of Advanced Biomedical & Pathobiology* Vol: 2 N°: 3. Pp: 96-107.

Nooshin, K. D. et Janardhana, G. R. (2015). Isolation, Identification and Evaluation of Antimicrobial Activity of *Streptomyces flavogriseus*, strain ACTK2 from Soil Sample of Kodagu, Karnataka State (India). *Jundishapur Journal of Microbiology.* 8(2): e15107. https://doi: 10.5812/jjm.15107

Parra, J., Beaton A., Seipke, R. F., Wilkinson, B., Hutchings, M. I. et Duncan, K. R. (2023). Antibiotics from rare actinomycetes, beyond the genus *Streptomyces*. *Current Opinion in Microbiology*. 76 :102385. https://doi.org/10.1016/j.mib.2023.102385.

Pazhanimurugan, R., Gopikrishnan, V., Shanmuga, S. T., Radhakrishnan, M. et Balagurunathan, R. (2012). Bioactive potential of actinobacteria against drug resistant pathogens. *Journal of Applied Pharmaceutical Science*. Vol: 2. N°: 5. Pp: 167-173. https://doi: 10.7324/JAPS.2012.2542

Rifaat, H. M. (2003). The biodiversity of actinomycetes in the River Nile exhibiting antifungual activity. *Journal of Mediterranean Ecology*. 4: 5–7.

Sanasam, S., Nimaichand S. et Ningthoujam, D. (2011). Novel bioactive actinomycetes from a niche biotope, Loktak Lake, in Manipur, India. *Journal of Pharmacy Research*. 4(6),1707-1710.

Sanglier, J. J., Haag, H., Huck, T.A. et Fehr, T. (1993). Novel bioactive compounds from actinomycetes: a short review (1988-1992). *Research in Microbiology*. Vol: 144. Pp: 633-642.

Schwarz, J., Hubmann, G., Rosenthal, K. et 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.

Sivakumar, K., Kumar, S. M., Thangaradjou, T. et Kannan, L. (2007). Research on marine actinobacteria in India. *Indian Journal of Microbiology*. 47 :186–196.

Smaoui, S. (2010). Purification and characterization of biomolecules from newly isolated and identified microorganisms. *Doctoral thesis in Process and Environmental Engineering. University of Toulouse.* France. 251p

Souagui, S., Djoudi, W., Boudries, H., Béchet, M., Leclère, V. et 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

Takahashi, Y. et Omura, S. (2003). Isolation of new actinomycete strains for the screening of new bioactive compounds. *The Journal of General and Applied Microbiology*. 49:3 p. 141-154

Terkina, V., Drukker, V., Parfenova V. et Kostornova, T. Ya. (2002). The Biodiversity of Actinomycetes I. A. in Lake Baikal. *Springer*, Volume 71, Issue 3, pp 346–349.

Turhan, G. et Grossmann, F. (1986). Investigation of a great number of actinomycete isolates on their antagonistic effects against soil-borne fungal plants pathogens by an improved method. *Journal of phytopathology*. 116: 283-243

Ullah, I., Arshad, M., Chuadhry, M. J. I, Noureen, U., Jadoon, W. A. et **Jadoon, M. A. (2012).** Actinomycetes screening for bioactive potential isolated from the moist forest soils of Pakistan. *Records : Zoological Survey of Pakistan*. Vol 21. 10-13 (2012)

Valli, S., Sugasini, S. S., Aysha, O. S., Nirmala, P., Kumar, V.P. et **Reena A. (2012).** A. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pacific Journal of Tropical Biomedicine*. Pp: 469-473. https://doi.org/10.1016/S2221-1691(12)60078-1

Yamamura, H., Shimizu, A., Nakagawa, Y., Hamada, M., Otoguro, M., Tamura, T. et **Hayakawa, M. (2012).** *Actinoplanes rishiriensis sp. nov.*, a novel motile actinomycete isolated by rehydration and centrifugation method. *The Journal of Antibiotics (Tokyo).* Vol 6. N°: 5. Pp: 249-53.

Yan, W.M. et Crawford, D.L. (1995). Caracterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal roots and seed rots. *Applied and environmental Microbiology*, 61 :3119-3128.

Zhang, J. (2011). Improvement of an Isolation Medium for Actinomycetes. *Modern Applied Science.* Vol. 5 (2). Pp: 124-215. https://doi:10.5539/mas. v5n2p124

Zheng, W. Sun, W. et **Simeonov, A. (2018).** Drug repurposing screens and synergistic drug-combi-nations for infectious diseases. *British Journal of Pharmacology*.175(2), 181-191. https://doi.org/10.1111/bph.13895