Electronic Journal of Biotechnology 64 (2023) 34–41

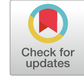
Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Research Article

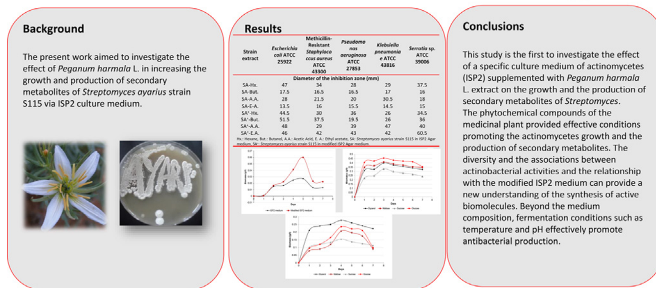
Effect of *Peganum harmala* L. extract supplemented ISP2 medium on growth and production of secondary metabolites of *Streptomyces ayarius* S115



Amel Nait Marzoug [a], Adel Ayari [b,*], Fadila Khaldi [a], Ines Guehria [c], Abdelhak Gheid [a]

[a] Laboratory of Sciences and Technology of Water and Environment, University of Mohamed Cherif Messaadia, Souk-Ahras, Algeria
[b] Laboratory of Aquatic and Terrestrial Ecosystems, University of Mohamed Cherif Messaadia, Souk-Ahras, Algeria
[c] Laboratory of Biochemistry and Biotechnology, LR01ES05, Faculty of Sciences, University of El Manar, 2092, Tunisia

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 26 August 2022
Accepted 22 December 2022
Available online 30 March 2023

Keywords:
Actinomycetes
Antibacterial activity
Biomass
Culture medium
Glucose
Glycerol
Growth
Peganum harmala
Secondary metabolites
Streptomyces ayarius

ABSTRACT

Background: The present work aimed to investigate the effect of *Peganum harmala* L. in increasing the growth and production of secondary metabolites of *Streptomyces ayarius* strain S115 via ISP2 culture medium. *Peganum harmala* L. was dried and added to ISP2 medium. The morphological properties and the antibacterial activity of *S. ayarius* strain S115 seeded in ISP2 and the modified ISP2 media was evaluated by using the agar well diffusion method against five pathogenic bacteria, *Escherichia coli Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia* sp. and Methicillin-Resistant *Staphylococcus aureus*. The biomass of *S. ayarius* strain S115 in both media was determined. The kinetics of growth and production of secondary metabolites were studied for 7 d in different carbon sources.

Results: Culture on the modified ISP2 showed an effective growth of *S. ayarius* strain S115 with changed color of the aerial mycelium from gray to white. The antibacterial activity revealed large inhibition zones against the tested pathogenic bacteria compared to those of the ISP2. The amount of *S. ayarius* strain S115 biomass was twice as high in the modified ISP2. The effect of different carbon sources on the growth and production of secondary metabolites of *S. ayarius* strain S115 revealed the highest biomass and biological activities through using glucose in the modified ISP2 on the 3rd day of culture. Of note, glycerol was found as the optimal carbon source in ISP2.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
* Corresponding author.
E-mail address: adel.ayari@univ-soukahras.dz (A. Ayari).

https://doi.org/10.1016/j.ejbt.2022.12.006
0717-3458/© 2023 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Conclusions: The discovery of new bioactive molecules from actinomycetes seeded in specific culture media may contribute to the development of therapeutic solutions
How to cite: Nait Marzoug A, Ayari A, Khaldi F, et al. Effect of *Peganum harmala* L. extract supplemented ISP2 medium on growth and production of secondary metabolites of *Streptomyces ayarius* S115. Electron J Biotechnol 2023;64. https://doi.org/10.1016/j.ejbt.2022.12.006.
© 2023 Pontificia Universidad Católica de Valparaíso. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Antibacterial resistance is a major-human problem [1,2,3,4]. Additionally, the pathogenic bacteria such as Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Pseudomonas aeruginosa* resistant to several antibiotics lead to serious health threats [5,6]. As a result, several approaches have been explored to control the pathogenesis of these microorganisms, in addition to the recent great interest in the use of the natural antibiotic sources, owing to their potential therapeutic efficiency [3]. In this regard, the pharmaceutical industries in the world are interested in producing antibiotics from actinomycetes due to their ability to synthesize the bioactive secondary metabolites [7,8]. These molecules are diverse chemical organic compounds that indirectly contribute to the ordinary growth, development, or reproduction of the producing organisms. Also, they can be synthesized under optimal growth conditions in the stationary phase or at the end of the exponential growth phase [9], and are considered antifungal, antiviral, anticancer, antiparasitic, and immunosuppressant agents whose enzymes are commonly used in the chemical pharmaceutical industries [9,10,11]. Furthermore, *Streptomyces* is a major producer of a wide range of secondary metabolites and is the ideal source of several antibiotics, anticancer compounds, enzymes and enzyme inhibitors, etc. [3,12,13,14]. Noteworthy, the antibiotics are primarily produced on a large scale by the fermentation process, by which the increasing production yields require improvement of the producing strains and their culture conditions, and consequently, the bacterial metabolism can be effectively regulated. Thus it seems to be valuable to provide the stimulators for increasing the growth and production of secondary metabolites that being used against pathogenic microorganisms [15]. Here in, the use of medicinal plant extracts in culture media is a worthy tool for the design and the development of fermentation processes. As far as known, the specific medium of International *Streptomyces* Project-2 (ISP2) actinobacteria supplemented with a medicinal plant extract to increase the bacterial potential growth and production of bioactive secondary metabolites has not been investigated. In this study, *Peganum harmala* L., of the family Zygophyllaceae, widely found in the arid and semi-arid regions of Algeria, was selected as an effective medicinal plant commonly used in traditional Algerian medicine for its various virtues [16,17]. *P. harmala* is an alkaloid-rich plant, and thus a hallucinogenic plant with psychotropic effects, and various biological properties such as analgesics, diuretics, antiproliferative, antihelmintics, abortifacients, and antimicrobials [18]. Moreover, *Streptomyces ayarius* strain S115 is a new strain belonging to the actinobacteria and was isolated from the sediments of Lake Oubeira (north-eastern Algeria) and registered at GenBank under the accession number JQ965757. A series of approaches used to enhance the production of the bioactive compounds were adopted as the application of culture media using associations with medicinal plants and classical methods. Based on the Algerian natural sources of antimicrobial metabolites, this work is a representative example of the possibility of closely performing a chemical-biological study of the natural products.

2. Materials and methods

2.1. Strain isolation

The *S. ayarius* strain S115 strain used throughout this study was kindly provided by Dr. Adel Ayari, from the Culture Collection of the Active Biomolecules Valorization Team of the Aquatic and Terrestrial Ecosystems Laboratory of Mohamed Cherif Messaadia University of Souk Ahras, Algeria [19]. The strain *S. ayarius* strain S115 was isolated from a sediment sample collected in Oubeira's lake of the El-Kala region (east-north of Algeria), on casein-starch medium (starch, 10 g; casein, 0.3 g; K_2HPO_4, 2 g; KNO_3, 2 g; $CaCO_3$, 0.02 g; $FeSO_4$ $7H_2O$, 0.01 g; $MgSO_4$ $7H_2O$, 0.05 g; agar, 20 g; H_2O, 1000 ml, pH 7.2) as recommended by Shirling and Gottlieb [20], by the dilution method. The strain was stored on International *Streptomyces* Project-2 (ISP2) agar slant medium at 4°C.

2.2. Plant material

The seeds of *P. harmala* were collected in July 2019 during the flowering period from the region of Sidi Fredj (South-East of Souk Ahras, Algeria) in the geographic coordinates: 36°0′58.94″ N; 8°13′54.03″ E; 544 m (Latitude/longitude/altitude). The seeds were air-dried in the shade and ground into a fine powder using an electric blinder.

2.3. Preparation of modified ISP2 medium

The plant powder of *Peganum harmala* was suspended in sterile distilled water, filtered through Whatman No. 1 paper, and then sterilized. The filtrate was afterward added to the medium containing the International *Streptomyces* Project-2 agar (yeast extract, 4 g; malt extract, 10 g; glucose, 4 g; agar, 20 g; H_2O, 1000 ml, pH 7.2).

2.4. Cultivation and extraction of Streptomyces ayarius strain S115 in ISP2 and modified ISP2 medium

The *Streptomyces ayarius* strain S115 strain was simultaneously inoculated by tight streaks on two media (ISP2 agar and modified ISP2 agar) and incubated at 28°C for 14 d. Then, small blocks of matured *S. ayarius* strain S115 agar were added to 100 ml of ISP2 broth and modified ISP2 broth medium in baffled Erlenmeyer flasks. The broths were incubated for 7 d at 28°C in a shaker incubator (Edmund Bühler, Germany), and the bacterial strain media were centrifuged to remove biomass. An equal volume of organic solvent was used to extract the cell-free supernatant, since the bacterial effectiveness was tested in four extraction solvents, including n-hexane, ethyl acetate, acetic acid, and n-butanol. The solvent phases containing a crude extract of secondary metabolites were dried using a rotary evaporator (Buchi, Switzerland) and were afterward recovered in 1 ml of methanol [19,21].

2.5. Assay of antibacterial activity in ISP2 and modified ISP2 medium

The antibacterial activity of *Streptomyces ayarius* strain S115 was tested by using the agar well diffusion method [8]. The tested

microorganisms, namely *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 43816, *Pseudomonas aeruginosa* ATCC 27853, *Serratia* sp. ATCC 39006 and Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300 were obtained from the culture collection of the Active Biomolecules Valorization Team of the aquatic and Terrestrial Ecosystems Laboratory of Mohamed Cherif Messaadia, University of Souk Ahras, Algeria. These strains were inoculated on Muller Hinton agar medium, and the agar wells were made using a sterile well driller. A volume of 150 µl of the methanolic extract was deposited on the wells and incubated at 37°C for 24 h. After incubation, the clear inhibition zones were measured using a caliper.

2.6. Determination of biomass in ISP2 and modified ISP2 medium

The evolution of the biomass during the fermentation process in two media was estimated according to the method of Pfefferle et al. [22]. In brief, 2 ml of the bacterial samples were centrifuged at 5000 rpm for 15 min, the resulting supernatants were discarded, and the cell pellet was washed twice with distilled water and dried at room temperature. The dry weight was measured and recorded as previously reported [23].

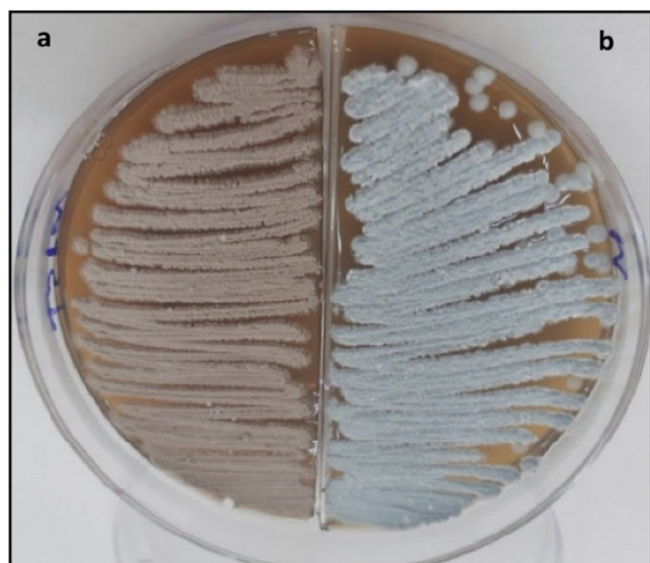


Fig. 1. Morphological characteristics of *Streptomyces ayarius* strain S115 in ISP2 Agar (a) and modified ISP2 Agar medium (b).

2.7. Growth kinetics and secondary metabolites production in ISP2 and modified ISP2 medium

The growth Kinetics and secondary metabolites production in the two culture media, as well as the evolution of the antibacterial activity against the pathogenic bacteria, were monitored daily for 7 d.

2.8. Effect of carbon sources

Different carbon sources were used to replace the carbon sources in ISP2 and modified ISP2 media, and all other components were held constant. Carbon sources including maltose, sucrose, and glycerol were sterilized separately and added just before inoculation, as well as the strain biomass and production of secondary metabolites, were respectively recorded.

3. Results and discussion

3.1. Description of Streptomyces ayarius strain S115

Streptomyces ayarius strain S115 is an aerobic Gram-positive strain, non-motile and filamentous actinobacteria. The fragments of the mycelia aerial have a rod-shaped morphology. The well-developed substrate mycelium varies from sulfur yellow to brown, since the color of aerial mycelium is usually grey on ISP2 agar (yeast extract, 4 g; malt extract, 10 g; glucose, 4 g; agar, 20 g; H_2O, 1000 ml, pH 7.2), ISP3 agar (meals, 20 g; $MnCl_2$ $4H_2O$, 0.1 g; $FeSO_4$ $7H_2O$, 0.1 g; $ZnSO_4$ $7H_2O$, 0.1 g; Agar, 18 g, H_2O, 1000 ml, pH 7.2), ISP4 Agar (starch soluble, 10 g; K_2HPO_4, 1 g; $MgSO_4$ $7H_2O$, 1 g; NaCl, 1 g; $(NH_4)_2SO_4$, 2 g; $CaCO_3$, 2 g; agar, 20 g; H_2O, 1000 ml, pH 7.2), and peptone yeast extract iron agar (ISP6) [20].

The optimum growth temperature is between 28 and 30°C, while pH varies between 7 and 9. The bacterial strain can metabolize starch, glucose, fructose, sucrose, arabinose, galactose, inositol, lactose, maltose, mannitol, sorbitol, and weakly metabolize rhamnose, but is unable to use cellulose and xylose. Also, it exhibits activity of β-glucosidase, lysine and ornithine decarboxylases, and arginine dihydrolase. The GenBank accession number for the 16S rRNA gene sequence is JQ965757.

3.2. Cultivation of Streptomyces ayarius strain S115 in ISP2 and modified ISP2 medium

After an incubation period of 14 d in ISP2 agar and modified ISP2 agar medium, colonies of *Streptomyces ayarius* strain S115 grew well in both media and appeared with a leathery texture, slow growth inside agar, and production of hyphae, spores, and diffusible pigment. Interestingly, the aerial mycelium exhibits gray

Table 1
Antibacterial activities of *Streptomyces ayarius* strain S115 in ISP2 Agar and modified ISP2 Agar medium.

Strain extract	*Escherichia coli* ATCC 25922	Methicillin-Resistant *Staphylococcus aureus* ATCC 43300	*Pseudomonas aeruginosa* ATCC 27853	*Klebsiella pneumoniae* ATCC 43816	*Serratia* sp. ATCC 39006
Diameter of the inhibition zone (mm)					
SA-Hx.	47	34	28	29	37.5
SA-But.	17.5	16.5	16.5	17	16
SA-A.A.	28	21.5	20	30.5	18
SA-E-A.	13.5	16	15.5	14.5	15
SA^+-Hx.	44.5	30	36	26	34.5
SA^+-But.	51.5	37.5	19.5	26	36
SA^+-A.A.	48	29	39	47	40
SA^+-E.A.	46	42	43	42	60.5

Hx.: Hexane; But.: Butanol; A.A.: Acetic Acid; E.A.: Ethyl acetate; SA: *Streptomyces ayarius* strain S115 in ISP2 Agar medium; SA^+: *Streptomyces ayarius* strain S115 in modified ISP2 Agar medium.

color (Fig. 1A), while the color of the modified ISP2 agar medium changes to white color (Fig. 1B). In this study, the IPS2 medium promoting the sporulation of actinomycetes [20] was selected to evaluate the effect of the plant powder on strain growth. This medium is composed of a carbon source, glucose, nitrogen source, malt, and yeast extracts. The supplementation of plant powder retains the ability of this medium to promote bacterial sporulation and changes the aerial mycelium color from gray to white. This color change is likely related to the flavonoids and phenolic-rich plant powder.

3.3. Assay of antibacterial activity in ISP2 and modified ISP2 medium

The results of the agar well diffusion assay showed high antibacterial activity of the supernatant of fermented modified broth ISP2 of *S. ayarius* strain S115 against all the Gram positive

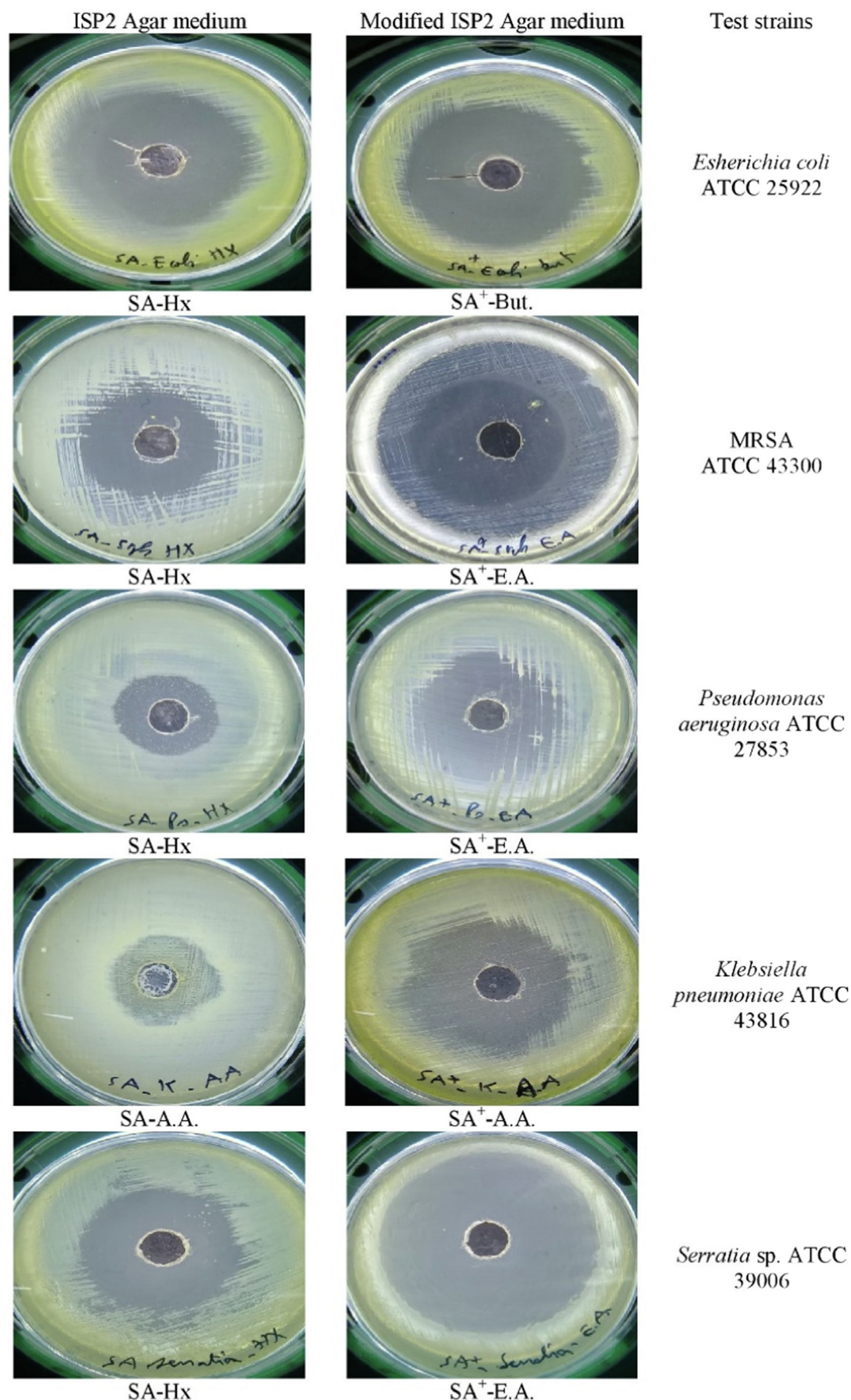


Fig. 2. Best solvents in antibacterial activities of *Streptomyces ayarius* strain S115 in ISP2 and modified ISP2 medium: Hx.: Hexane; But: Butanol; A.A.: Acetic Acid; E.A.: Ethyl acetate; SA: *Streptomyces ayarius* strain S115 in ISP2 Agar medium; SA+: *Streptomyces ayarius* strain S115 in modified ISP2 Agar medium.

and negative target species: *Escherichia coli* ATCC 25922, Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 43816 and *Serratia* sp. ATCC 39006 compared with that of the fermented broth ISP2 (Table 1 and Fig. 2). The diameter of the inhibition zone of antibacterial activity against Gram-negative bacteria was significantly higher than that of Gram-positive bacteria showing values varying from 13.5 mm to 60.5 mm. As reported, the actinomycetes are bacteria rich in secondary metabolites, extracellular enzymes, and enzyme inhibitors. Hence, this study has proved the ability of the tested actinomycetes to assimilate plant polyphenol compounds from the modified ISP2 medium to synthesize bioactive molecules of high antibacterial activity. Regarding the extraction solvent optimization, ethyl acetate was found to be the best solvent to extract all antibacterial agents from the modified ISP2 fermentation broth against MRSA ATCC 43300, *P. aeruginosa* ATCC 27853, and *Serratia* sp. ATCC 39006. Meanwhile, butanol and acetic acid are respectively, the best extraction solvent for *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 4381. In the ISP2 broth devoid of the plant powder, the hexane solvent was the adequate solvent for efficient extraction of bioactive molecules against *E. coli* ATCC 25922, MRSA ATCC 43300, *P. aeruginosa* ATCC 27853, and *Serratia* sp, however, acetic acid has proven to be an effective solvent for extracting antibacterial agents against *K. pneumoniae* ATCC 43816.

A previous study using different solvents, including ethyl acetate, methanol, and chloroform to extract the bioactive compounds and to compare the inhibition zones of the solvent extracts provedethyl acetateas the best among the tested solvents [24,25], and this coincides with these findings. It was reported that the antibacterial potential of the culture filtrate might be related to the high production of secondary antibacterial compounds as antibiotics in many *Streptomyces* species [19,21,26]. These results suggest that the inhibition of bacterial growth by the culture filtrates is likely referred to the production of extracellular hydrolytic enzymes and secondary antibacterial compounds that were activated by the plant polyphenols supplemented ISP2 medium. Most of the *Streptomyces* bioactive secondary metabolites are antimicrobial, anticancer, and immunosuppressive agents [27]. In addition, these bacteria contribute to producing nearly of the two-third commercial antibiotics [19]. Of note, some of the used antibiotics in therapy, such as streptomycin, kanamycin, tetracycline, chloramphenicol, and neomycin are derived from *S. griseus*, *S. kanamyceticus*, *S. venezuelae*, and *S. fradiae* [28,29].

3.4. Biomass determination in ISP2 and modified ISP2 medium

The amount of biomass was twice as higher as that found in International *Streptomyces* Project-2 (ISP2) (Fig. 3). The maximum biomass value was noticed on the 5th day in the two media, where it reached 0.0271 in the ISP2 medium, and 0.06001 in the modified ISP2 medium. As the medium composition can influence biomass production, the production of secondary metabolites is believed to occur when growth is limited by one of the medium substrates [30]. The three main factors causing this limitation are namely the sources of carbon, nitrogen, and phosphate. Overall, when one of these elements becomes limiting, growth slows down and consequently, the production of secondary metabolites such as antibiotics takes place during the idiophase. Under certain experimental conditions, high biomass production can occur during the growth phase without nutritional limitations but with slowly metabolizable substrates, and this is alike to the case of plant compounds. Accordingly, Voelker and Altaba [31], have reported production of pristinamycins during the growth phase in the culture medium of *Streptomyces pristinaespiralis* containing sodium nitrate as the sole source of nitrogen.

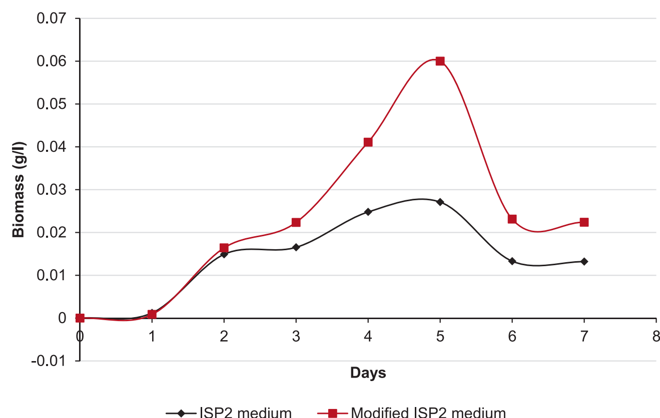


Fig. 3. Kinetics of biomass of *Streptomyces ayarius* strain S115 in ISP2 and modified ISP2 medium.

3.5. Kinetics and optimization of growth and secondary metabolites production in ISP2 and modified ISP2 medium

ISP2 medium was found as the best culture medium among those tested in several previous studies, showing optimal production of antimicrobial agents. ISP2 supplemented with plant powder showed higher biomass than that found in ISP2 alone. In ISP2 medium containing different carbon sources, including glycerol, maltose, and sucrose instead of glucose as a carbon source showed that the glycerol was the optimal carbon source in ISP2 (Fig. 4) since glucose was the optimal carbon source in ISP2 modified medium in 3rd d of culture.

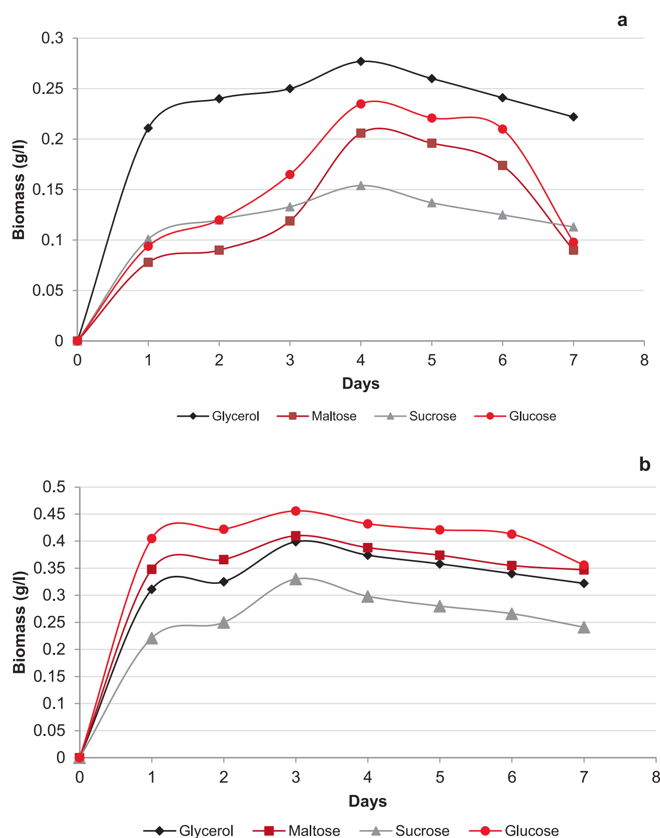


Fig. 4. Kinetics and optimization of growth (effect of carbon sources) of *Streptomyces ayarius* strain S115 in ISP2 (a) and modified ISP2 medium (b).

On top of that, the glycerol was identified as the optimal carbon source among the tested carbon sources used to replace glucose in ISP2 medium, exhibiting the largest inhibition zone against *Escherichia coli* ATCC 25922, Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853 and *Serratia* sp. ATCC 39006 on the 4th day of culture (Table 2). A previous study has reported that the maximum production of some antibiotics was achieved using glucose and lactose as carbon sources [25,32].

The *S. ayarius* strain S115 strain cultured in International *Streptomyces* Project-2 (ISP2) medium showed good production of secondary metabolites, and efficient bacterial growth starting with an exponential growth phase lasting 1 d (0–1 d), followed by a second exponential phase between the 2nd and 4th d for ISP2 and 2nd and 3rd d for modified ISP2 medium. This suggests the presence of a diauxic phenomenon. Additionally, a decline phase begins on the 4th d for ISP2 and the 3rd d for the modified ISP2 medium. The production of antibiotics begins on the 3rd d in ISP2 and on the 2nd d in ISP2 modified medium during the exponential phase, meanwhile, the maximum production was observed on the 4th d for all the tested pathogenic bacteria in the two media, i.e. during the decline phase. The antibiotics produced by the bacterial strain cultured in the modified ISP2 medium were unlike and greater than that of the ISP2 medium until the last day of the culture, showing regular and continuous production of antibiotics. Furthermore, the kinetics of growth and production of secondary metabolites were studied under liquid conditions and agitation using the two media (ISP2 and modified ISP2). This choice was made based on the results from the previously conducted studies, reporting that the ISP2 medium composed of malt extract (10 g/l), yeast extract (4 g/l), and glucose (4 g/l) is one of the most favorable media for the production of antibiotics in actinomycetes [23]. The difference between the two used media is related to the addition of a quantity of the plant powder. For the growth kinetics, certain points in common between the two media were noted, including the absence of a latency phase where the pre-cultures can be performed under the

Table 2
Optimization of secondary metabolites production (effect of carbon sources) of *Streptomyces ayarius* strain S115 in ISP2 and modified ISP2 medium.

Strain days	*Escherichia coli* ATCC 25922							
	GY		M		S		GL	
1	0	0$^+$	0	0$^+$	0	0$^+$	0	0$^+$
2	0	17$^+$	0	15.5$^+$	0	13.5$^+$	0	16.8$^+$
3	19	24.8$^+$	16	17$^+$	12	15.8$^+$	17	23$^+$
4	28	30$^+$	22	24$^+$	22	21$^+$	23	28$^+$
5	24	27$^+$	20.5	22$^+$	20	20$^+$	21	26.2$^+$
6	23	25.5$^+$	19	20.2$^+$	18	18.2$^+$	20.5	24$^+$
7	21	23$^+$	19	19$^+$	16	17$^+$	19	20$^+$
Strain days	Methicillin-Resistant *Staphylococcus aureus* ATCC 43300							
	GY		M		S		GL	
1	0	0$^+$	0	0$^+$	0	0$^+$	0	0$^+$
2	21	22$^+$	0	17$^+$	0	14$^+$	18	20$^+$
3	24	26$^+$	18	20$^+$	14	18$^+$	19	22.5$^+$
4	27.5	30$^+$	20	24$^+$	15.5	22$^+$	23.5	26$^+$
5	24.5	28$^+$	19	20.5$^+$	13.5	16.5$^+$	22	25$^+$
6	22	25$^+$	18	18.5$^+$	12	14.5$^+$	20	21.5$^+$
7	20	21$^+$	17	17$^+$	11	13.5$^+$	19	19$^+$
Strain days	*Pseudomonas aeruginosa* ATCC 27853							
	GY		M		S		GL	
1	0	0$^+$	0	0$^+$	0	0$^+$	0	0$^+$
2	18	19.5$^+$	15.5	16$^+$	13	14.5$^+$	17.2	18.5$^+$
3	21.5	22$^+$	17	18.5$^+$	14	16$^+$	19	21$^+$
4	24	25.5$^+$	19	20.5$^+$	15.5	18$^+$	22	24$^+$
5	23	23$^+$	18.5	19.5$^+$	13.5	17$^+$	21	22.5$^+$
6	20	21.5$^+$	17	18$^+$	13	14.5$^+$	18	19.5$^+$
7	19	19$^+$	16	17$^+$	12	13$^+$	17	18$^+$
Strain days	*Klebsiella pneumoniae* ATCC 43816							
	GY		M		S		GL	
1	0	0$^+$	0	0$^+$	0	0$^+$	0	0$^+$
2	19	20.5$^+$	15.5	17$^+$	14	15.5$^+$	17.5	19.8$^+$
3	19.8	22$^+$	17.8	18$^+$	16.5	17.8$^+$	19	21$^+$
4	23	25$^+$	20	21$^+$	18.5	19.4$^+$	22	24$^+$
5	22.5	21$^+$	18	19$^+$	17	18$^+$	21.2	20$^+$
6	20.9	19$^+$	16	17.5$^+$	15.2	16.2$^+$	19	19$^+$
7	18.5	18$^+$	15.5	16$^+$	14.5	15.5$^+$	18	17$^+$
Strain days	*Serratia* sp. ATCC 39006							
	GY		M		S		GL	
1	0	0$^+$	0	0$^+$	0	0$^+$	0	0$^+$
2	18	19.2$^+$	13	15$^+$	13	17$^+$	17	18$^+$
3	19.5	20.5$^+$	16	17.5$^+$	17	18.5$^+$	19.5	20$^+$
4	23	24.8$^+$	18.5	20.5$^+$	20	21.2$^+$	22	22.5$^+$
5	22.5	23$^+$	16.8	17$^+$	18	19$^+$	19	21$^+$
6	22	22.2$^+$	15	14$^+$	16	17$^+$	17.5	19.8$^+$
7	20	19$^+$	13	13$^+$	13.8	15.8$^+$	15.5	17$^+$

GY: Glycerol; M: Maltose; S: Sucrose; GL: Glucose.
+ : *Peganum harmala* powder supplemented ISP2 medium (modified medium).

same conditions as the cultures and be taken in the growth exponential phase. The other common point is that of the production of antibiotics during the exponential phase. In contrast, the production of secondary metabolites by microorganisms generally can be occurred during the slowing and stationary phase (idiophase), while the production period is variable, and can take place in the exponential phase in actinomycetes [23].

4. Conclusions

Up to the authors' knowledge, this study is the first to investigate the effect of a specific culture medium of actinomycetes International *Streptomyces* Project-2 (ISP2) supplemented with *Peganum harmala* L. extract on the growth and the production of secondary metabolites of *Streptomyces*. As a result, the phytochemical compounds of the medicinal plant provided effective conditions promoting the actinomycetes growth and the production of secondary metabolites. The diversity and the associations between various actinobacterial activities and the relationship with the modified ISP2 medium (culture medium supplemented with substrates (*e.g.* plant powder extract)) can provide a new understanding of the synthesis of active biomolecules and offer opportunities to isolate novel therapeutic agents. Beyond the medium composition, fermentation conditions such as temperature and pH effectively promote antibacterial production. The influence of cellular growth, in particular, the pellet morphology of streptomycetes must be taken into consideration for optimal metabolite production.

Author contributions

- Study conception and design: A Nait Marzoug; F Khaldi.
- Data collection: A Nait Marzoug; A Ayari; I Guehria.
- Analysis and interpretation of results: A Nait Marzoug; A Ayari; I Guehria; A Gheid.
- Draft manuscript preparation: A Nait Marzoug; A Ayari; F Khaldi.
- Revision of the results and approval of the final version of the manuscript: A Nait Marzoug Amel; A Ayari; F Khaldi.

Conflict of interest

None.

References

[1] Al-Dhabi NA, Esmail GA, Duraipandiyan V, et al. Chemical profiling of *Streptomyces* sp. Al-Dhabi-2 recovered from an extreme environment in Saudi Arabia as a novel drug source for medical and industrial applications. Saudi. J Biol Sci 2019;26(4):758–66. https://doi.org/10.1016/j.sjbs.2019.03.009. PMid: 31049001.

[2] Al-Dhabi NA, Esmail GA, Ghilan AKM, et al. Isolation and screening of *Streptomyces* sp. Al-Dhabi-49 from the environment of Saudi Arabia with concomitant production of lipase and protease in submerged fermentation. Saudi. J Biol Sci 2020;27(1):474–9. https://doi.org/10.1016/j.sjbs.2019.11.011. PMid: 31889873.

[3] Bhakyashree K, Kannabiran K. Actinomycetes mediated targeting of drug resistant MRSA pathogens. J King Saud Univ - Sci 2020;32(1):260–4. https://doi.org/10.1016/j.jksus.2018.04.034.

[4] Suresh RSS, Younis EM, Fredimoses M. Isolation and molecular characterization of novel *Streptomyces* sp. ACT2 from marine mangrove sediments with antidermatophytic potentials. J King Saud Univ - Sci 2020;32(3):1902–9. https://doi.org/10.1016/j.jksus.2020.01.020.

[5] Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 2008;46(supp5):S344–9. https://doi.org/10.1086/533590. PMid: 18462089.

[6] Al-Ansari M, Kalaiyarasi M, Almalki MA, et al. Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from *Streptomyces* sp. AS11 isolated from the marine environment. J King Saud Univ - Sci 2020;32(3):1993–8. https://doi.org/10.1016/j.jksus.2020.02.005.

[7] Bérdy J. Bioactive microbial metabolites. J Antibiot 2005;58(1):1–26. https://doi.org/10.1038/ja.2005.1. PMid: 15813176.

[8] Amin DH, Tolba S, Abolmaaty A, et al. Phylogenetic and antimicrobial characteristics of a novel *Streptomyces* sp. Ru87 isolated from Egyptian soil. Int J Curr Microbiol App Sci 2017;6(8):2524–41. https://doi.org/10.20546/ijcmas.2017.608.300.

[9] Ibrahim SY. Fermentation, extraction and characterization of antimicrobial agent from marine *Saccharomonospora viridis* AHK 190. Egypt J Microbiol 2018;53(9):9–21. https://doi.org/10.21608/ejm.2018.2823.1046.

[10] Zaitlin B, Watson SB. Actinomycetes in relation to taste and odour in drinking water: myths, tenets and truths. Water Res 2006;40(9):1741–53. https://doi.org/10.1016/j.watres.2006.02.024. PMid: 16600325.

[11] Newman DJ, Cragg GM. Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007;70(3):461–77. https://doi.org/10.1021/np068054v. PMid: 17309302.

[12] Kämpfer P. Genus Streptomyces: the Actinobacteria. In: Goodfellow M, Kämpfer P, Busse HJ, et al. editors. Bergey's Manual of Systematic Bacteriology. United Kingdom: Springer Nature; 2012. p. 1777–81.

[13] Vijayakumar R, Selvam KP, Muthukumar C, et al. Antimicrobial potentiality of a halophilic strain of *Streptomyces* sp. VPTSA18 isolated from the saltpan environment of Vedaranyam. India. Ann Microbiol 2021;62:1039–47. https://doi.org/10.1007/s13213-011-0345-z.

[14] Al-shaibani MM, Radin Mohamed RMS, Sidik NM, et al. Biodiversity of secondary metabolites compounds isolated from Phylum Actinobacteria and its therapeutic applications. Molecules 2021;26(15):4504. https://doi.org/10.3390/molecules26154504. PMid: 34361657.

[15] Kannaiyan M, Manuel VN, Raja V, et al. Antimicrobial activity of the ethanolic and aqueous extracts of *Salacia chinensis* Linn. against human pathogens. Asian Pac J Trop Dis 2012;2(1):S416–20. https://doi.org/10.1016/S2222-1808(12)60194-7.

[16] Achour S, Rhalem N, Khattabi A, et al. L'intoxication au *Peganum harmala* L. au Maroc : à propos de 200 cas. Therapies 2012;67(1):53–8. https://doi.org/10.2515/therapie/2012003. PMid: 22487503.

[17] Habbachi W, Benhissen S, Ouakid ML, et al. Effets biologiques d'extraits aqueux de *Peganum harmala* (L.) (Zygophyllaceae) sur la mortalité et le développement larvaire de *Drosophila melanogaster* (Diptera - Drosophilidae). Algerian J Arid Environ 2013;3(1):82–8. https://doi.org/10.12816/0008892.

[18] Bousliman Y, Ait EL Cadi M, EL Jaoudi R, et al. Les plantes toxiques au Maroc. Médecine du Maghreb 2012;196:9–26.

[19] Ayari A, Morakchi H, Kirane-Gacemi D. Evaluation of antifungal activity of novel marine actinomycete, *Streptomyces* sp. AA13 isolated from sediments of Lake Oubeira (Algeria) against *Candida albicans*. Afr. J Microbiol Res 2016;10(6):156–71. https://doi.org/10.5897/AJMR2013.7765.

[20] Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. Int J Syst Evol Microbiol 1966;16(3):313–40. https://doi.org/10.1099/00207713-16-3-313.

[21] Ayari A, Morakchi H, Kirane GD. Identification and antifungal activity of Streptomyces sp. S72 isolated from Lake Oubeira sediments in North-East of Algeria. Afr J Biotechnol 2012;11(2):305–11. https://doi.org/10.5897/AJB10.2345.

[22] Pfefferle C, Theobald U, Gürtler H, et al. Improved secondary metabolite production in the genus *Streptosporangium* by optimization of the fermentation conditions. J Biotechnol 2000;80(2):135–42. https://doi.org/10.1016/S0168-1656(00)00249-2. PMid: 10908794.

[23] Bouali H, Hamza K, Bouras N, et al. Effet de milieux de culture sur la croissance et la production de chloramphenicol chez deux souches de *Saccharothrix* sp. Pal 54 et Pal 42 isolées d'un sol de palmeraie de Ghardaïa. Algerian J Arid Environ 2017;7(1):71–83. https://doi.org/10.12816/0045918.

[24] Parthasarathi S, Sathya S, Bupesh G, et al. Isolation, characterization and extraction of antimicrobial compound from marine actinomycete Streptomyces hygroscopicus BDUS 49. Res J Biotechnol 2013;8(3):40–8.

[25] Sebak M, Saafan AE, Abdelghani S, et al. Isolation and optimized production of putative antimicrobial compounds from Egyptian soil isolate *Streptomyces* sp. MS. 10. Beni-Suef Uni J Basic Appl Sci 2021;10(8):1–12. https://doi.org/10.1186/s43088-021-00099-7.

[26] El-Shanshoury AER, Metwally AM, Sabha ME, et al. Biocontrol of *Aspergillus flavus* producing Aflatoxin B1 by *Streptomyces exfoliatus*. Egypt J Bot 2022;62(2):457–73. https://doi.org/10.21608/ejbo.2022.7763.1287.

[27] Salwan R, Sharma V. Molecular and biotechnological aspects of secondary metabolites in actinobacteria. Microbiol Res 2020;231:126374. https://doi.org/10.1016/j.micres.2019.126374 PMid: 31756597.

[28] Quinn GA, Banat AM, Abdelhameed AM, et al. *Streptomyces* from traditional medicine: sources of new innovations in antibiotic discovery. J Med Microbiol 2020;69(8):1040–8. https://doi.org/10.1099/jmm.0.001232. PMid: 32692643.

[29] Pandey BP, Pradhan SP, Adhikari K, et al. Screening and molecular identification of *Streptomyces* species isolated from high altitude soil of

Nepal. Korean J Microbiol 2021;57(3):174–82. https://doi.org/10.7845/kjm.2021.1035.

[30] Demain AL, Dijkhuizen L. Ecology and industrial microbiology. Curr Opin Microbiol 2006;9(3):237–9. https://doi.org/10.1016/j.mib.2006.04.009.

[31] Voelker F, Altaba S. Nitrogen source governs the patterns of growth and pristinamycin production in *Streptomyces pristinaespiralis*. Microbiol 2001;147(9):2447–59. https://doi.org/10.1099/00221287-147-9-2447. PMid: 11535785.

[32] Ilić SB, Konstantinović SS, Savić DŠS, et al. The impact of Schiff bases on antibiotic production by *Streptomyces hygroscopicus*. Med Chem Res 2010;19(7):690–7. https://doi.org/10.1007/s00044-009-9223-7. PMid: 20700374.