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Influence of culture media on the production of antibacterial compounds by *Streptomyces* sp. PAL114 isolated from palm grove soil in Ghardaïa

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Abstract

A Saharan actinobacterium, PAL114, isolated from Ghardaïa (Algeria), showed an important antibacterial activity against various pathogenic bacteria tested on different culture media. The results showed that the highest antibacterial activity was obtained after 7 days of growth. The Bennett medium was found to be the best medium for optimal growth and antibiotic production. The secondary metabolites were detected by reverse-phase HPLC using a C18 column and the fractions corresponding to peaks were collected, concentrated then tested against *Listeria monocytogenes* ATCC 13932 to detect the active fractions.

Key words: *Streptomyces*, Antagonistic potential, Antibiotic production, Palm grove, Ghardaïa

Introduction

Currently, bacterial infection caused by resistant strains to commonly used antibiotics is a major global healthcare problem. The constant development of these resistances as well as the appearance of new infectious diseases justify the great attention given to searching for new antibiotics [1].

The actinobacteria, a Gram positive bacteria, are known as the most attractive source of several types of bioactive metabolites, especially members of the genus *Streptomyces*, which biosynthesize over 70 % of the clinically useful antibiotics of natural origin [2]. The Algerian Saharan soils are rich and diversified in actinobacteria with interesting antimicrobial properties [3].

Improvement in the bacterial growth and bioactive secondary metabolite production can be carried out by manipulating the chemical (nutritional) and physical parameters of culture conditions [4].

Streptomyces sp. PAL114, a strain isolated from an Algerian Saharan soil collected in Ghardaïa (Mzab), has shown to produce, in liquid culture, interesting antibiotics, named saquayamycins A and B [5,6]. Nevertheless, many studied showed that the high cost of large scale fermentation technology often limits industrial applications [7].

The selection of a suitable culture medium plays an important role to make any fermentation process cost effective. Therefore, the current study was conducted to find out the most appropriate solid complex medium for the production of antibiotics by *Streptomyces* sp. PAL114.

Materials and methods

Bacterial strain

The actinobacterium PAL114 was isolated from a Saharan soil collected in Béni Isguen, Ghardaïa province (Mzab region), in southern Algeria [5]. The strain was maintained on solid slants of ISP 2 (International *Streptomyces* Project 2 medium [8] containing (in 1L of distilled water): 4 g D(+)-Glucose, 4 g yeast extract ,10 g malt extract and 18 g agar. The pH of the medium was adjusted to 7.2 ± 0.3 with a 2 M NaOH solution prior autoclaving (at 121°C for 20 min).

Culture media used for antibiotic production

The production of antibiotics by the strains PAL114 was studied on four different solid growth media. Each growth medium was incubated at 30°C in Petri plates for 7, 10, 12 and 14 days. The medium Bennett contained (per 1 L of distilled water): 10 g D(+)-Glucose, 1 g yeast extract, 2 g peptones, 1 g beef extract and 18 g agar. GYEA (Glucose Yeast Extract Agar) composed of (per 1 L of distilled water): 10 g D(+)-Glucose, 10 g yeast extract and 18 g agar. The nutrient agar (NA) contained (per 1 L of distilled water): 2 g yeast

extract, 5 g peptone, 1 g beef extract, 5 g NaCl and 18 g agar. The pH of each medium was adjusted to 7.2 ± 0.3 with a 2 M NaOH solution prior autoclaving (at 121°C for 20 min).

Antagonistic properties of the strain

In order to determine its antagonistic activity, strain PAL114 was tested by streak method against several bacterial species; 5 Gram positive (*Bacillus subtilis* ATCC 6633, methicillin resistant *Staphylococcus aureus* 639c, *Listeria monocytogenes* ATCC 13932, *Enterococcus faecalis* F1 and *Clavibacter michiganensis*), one Gram negative bacteria (*Pseudomonas aeruginosa* CIP A22). The experiment was done by streaking a straight line of the actinobacterial inoculum across the surface of medium on 90-mm diameter Petri plates and incubated at 30°C for 7, 10, 12 and 14 days. Then, target-bacteria were seeded in streaks perpendicular to the actinobacterial strain (a single streak for each bacteria at 90° to actinobacterial strain). The bacterial activity was evaluated by measuring the distance of inhibition between target microorganisms and actinobacterial colony margins, after incubation at 30°C for 24 h. All results presented are mean values of three independent experiments.

Antibiotic extraction

At the end of the incubation periods (as indicated above), the best culture medium was cut into small pieces and extracted with methanol (MeOH) under constant agitation of 250 rpm for 2 h (at room temperature). The extracts were concentrated to dryness by a rotary evaporator under vacuum at 40°C. The residues were dissolved in 1 ml of MeOH and subjected to biological assay (paper disk of 6 mm in diameter) against *Listeria monocytogenes* ATCC 13932. The disks received 50 µl of each extract and were placed on the ISP2 medium inoculated with the target-bacterium. Inhibition zones were expressed as diameter (mm) and measured after incubation at 30°C for 24. A paper disk containing the same volume of MeOH acted as control.

High performance liquid chromatography (HPLC) analysis

The concentrations of antibiotic components were determined quantitatively by HPLC. A 100 µl volume of each sample was injected into the HPLC system (Agilent 1260) using a reverse phase C18 column (20 × 1 cm, 5 µm) with a continuous linear gradient solvent system from 20 to 100% MeOH in water during 55 min, then remained at a steady flow of 100% of MeOH for 15 min. A total run time was maintained at a flow rate of 1 ml/min at room temperature. The detection of secondary metabolites was carried out at 220 nm. The fractions corresponding to peaks were collected, concentrated and then tested (6 mm paper disk diffusion method) against *Listeria monocytogenes* ATCC 13932 to detect the active fractions (showing antibacterial activity) and distinguish them from the non-active fractions.

Results and discussion

Antimicrobial activity

As shown in Figure 1, among the tested solid growth media, Bennett was found to be the best medium as it exhibited the highest antagonistic activity. We noticed that, an appreciable antibacterial activity was obtained after 7 (maximum) and 12 days of incubation on Bennett medium. In contrast, ISP2 and GYEA displayed the lowest antibacterial activity (Figure 1B, 1C). The obtained results indicated that the different available nutritional components in culture media directly induced the production level of antibiotics.

All the four complex culture media used are able to supply carbon and nitrogen sources for the formation of primary metabolite (and consequentially secondary metabolites). The nature and concentration of nitrogen plays a very important role in controlling the process of antibiotic biosynthesis in *Streptomyces* [9,10].

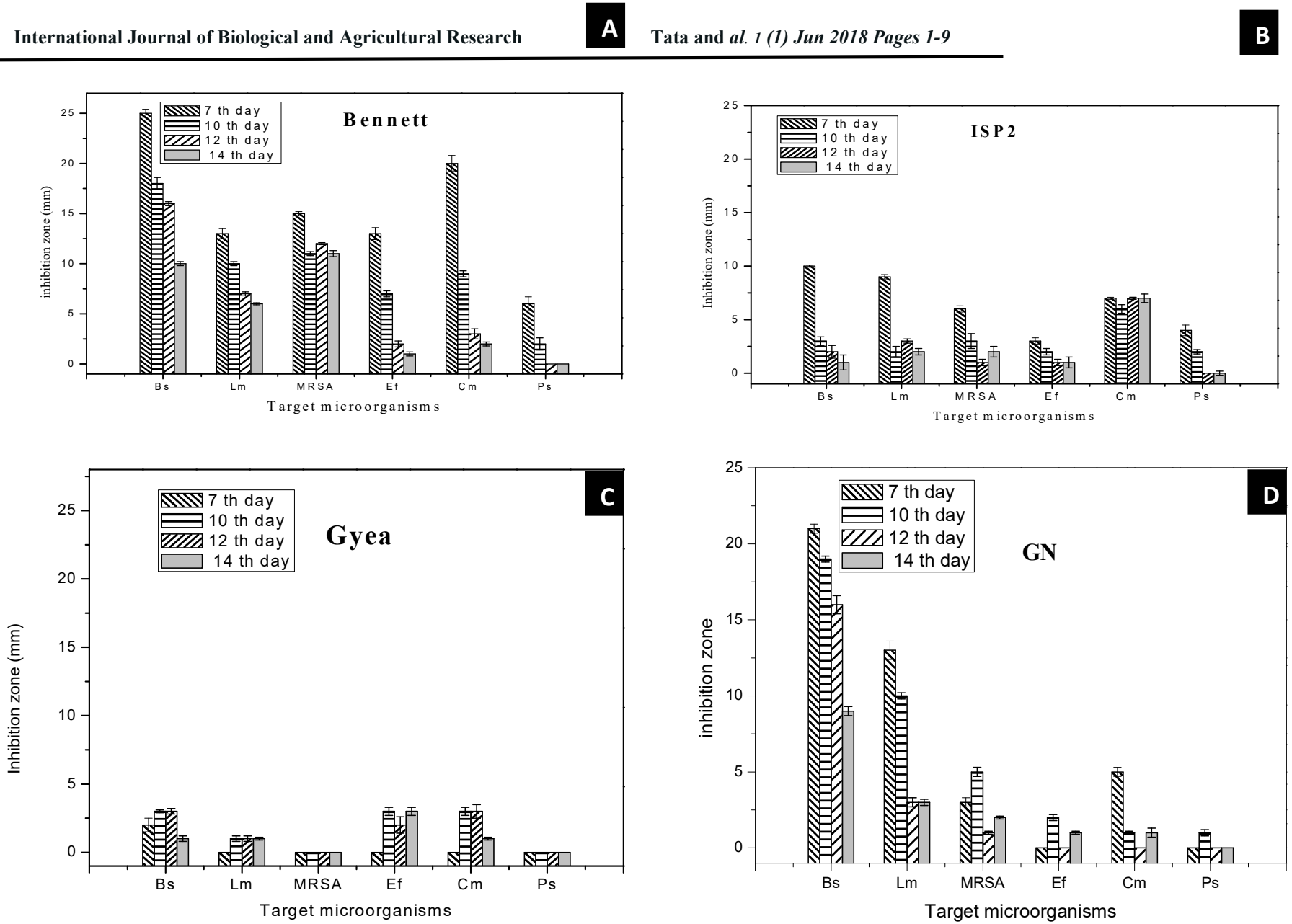


Figure 1. Effect of solid-state Bennett (A), ISP2 (B), GYEA (C) and NA (D) media on the productivity of antibacterial agent by *Streptomyces* sp., PAL114.
Bs: *Bacillus subtilis* ATCC 6633; Lm: *Listeria monocytogenes* ATCC 13932; MRSA: *Staphylococcus aureus* 639c; Ef: *Enterococcus faecalis* F1; Cm: *Clavibacter michiganensis*; Ps: *Pseudomonas aeruginosa* CIP A22.

The production of desired secondary metabolites could be achieved by the utilization of an appropriate nitrogen source in appropriate concentration relative to the carbon source [11]. It was also illustrated that complex nitrogen sources could enhance the production of antibiotics [12]. These sources could maintain high antibiotic titer due to the slow release of nitrogenous components during the fermentation time course in contrast to medium containing directly metabolized nitrogen [13].

Furthermore, the reduction of antibacterial activity in ISP2 and GYEA media (Figure 1B, 1C, 2F, 2G) was probably due to the lack of complex nitrogen sources in the media (peptone and beef extract). Glucose is one of the simple carbon sources that is metabolized easily and very rapidly and it suppresses secondary metabolite formation. It inhibits the formation of most key enzymes in biosynthetic pathways [14].

In contrast, Bennett and NA media (Figure 1A, 1D, 2E, 2H) contains peptone and beef extracts as complex nitrogen sources that possibly enhanced the production of antibiotics by the strain *Streptomyces* sp. PAL114. On the other hand, the NA medium does not contain glucose, which thus likely allowed important antibacterial compound biosynthesis.

Antimicrobial compounds production assay

Since solid Bennett medium was found to be the best for production of antibacterial agents, it was thus chosen as the production medium for further steps. The antibacterial activity exhibited by the strain *Streptomyces* sp. PAL114 on solid Bennett medium during the incubation time course was evaluated against several Gram positive and Gram negative bacteria (Figure 2). These obtained results revealed that the maximum antibacterial activities were obtained after 7 of incubation. Among the targeted strains, the pathogenic bacteria *Clavibacter michiganensis*, *Staphylococcus aureus* 639c and *Listeria monocytogenes* ATCC 13932 were the most sensitive.

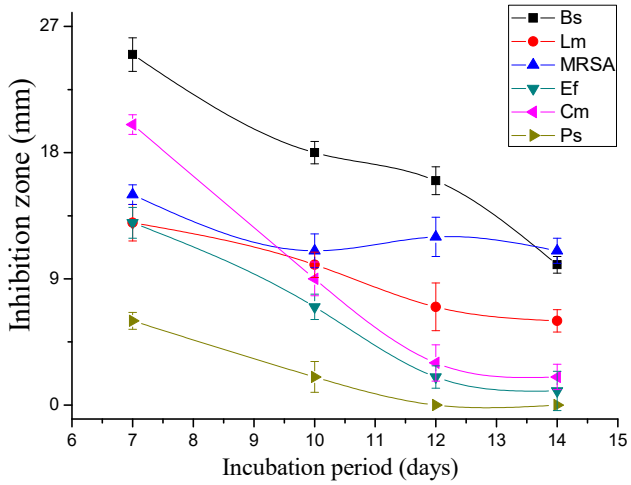


Figure 2. Antibacterial activity of *Streptomyces* sp. PAL114 expressed on solid Bennett medium.

Note: Bs: *Bacillus subtilis* ATCC 6633, Lm: *Listeria monocytogenes* ATCC 13932, MRSA: *Staphylococcus aureus* 639c, Ef: *Enterococcus faecalis* F1, Cm: *Clavibacter michiganensis*, Ps: *Pseudomonas aeruginosa* CIP A22.

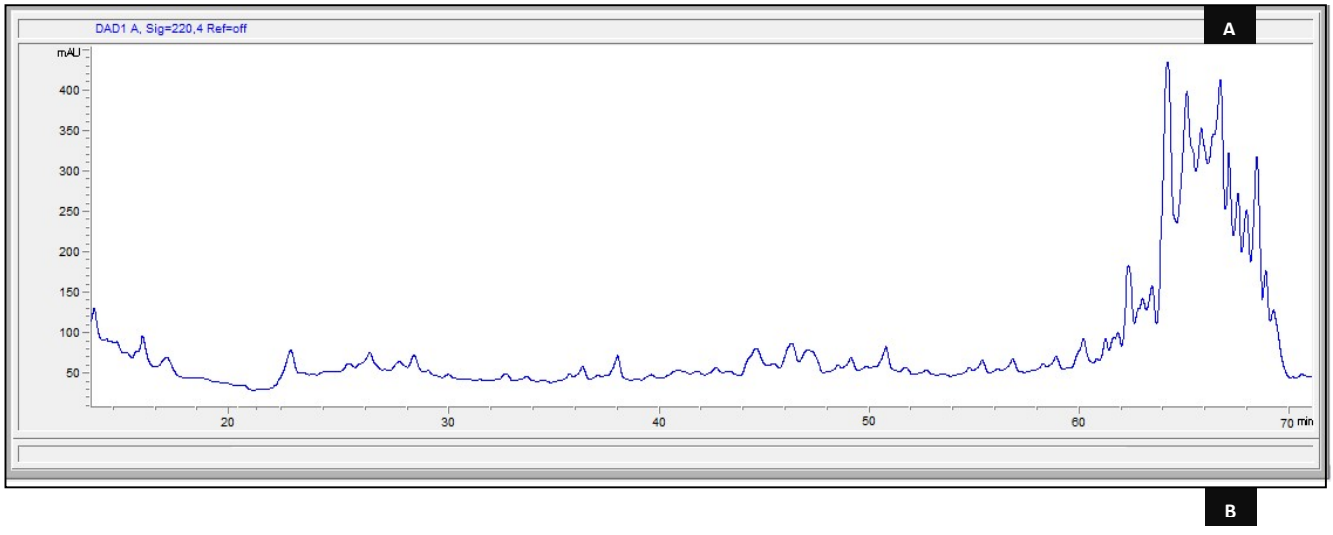
HPLC analysis

The antibiotic compounds present in the Bennett medium on the 7th and 12th day were revealed by the HPLC analysis. As shown in figure 3, the HPLC profiles indicated that the Bennett medium supported the biosynthesis of several antibacterial molecules at 7th and 12th day of fermentation. In order to test their antibacterial activity, the HPLC profile obtained on the 12th day of fermentation (figures 3B) was divided into four groups designated G1 (retention time (RT) ranging from 0 to 30 min), G2 (RT from 30 to 50 min), G3 (RT from 50 to 60 min) and G4 (RT from 60 to 70 min). Each group of molecules was tested against *Listeria monocytogenes* ATCC 13932. The results obtained are summarized in table 1.

Table 1. Antibacterial activities of the purified antibiotics produced by the strain *Streptomyces* sp. PAL114 in the Bennett medium at the 7th and 12th day of incubation.

Fractions	Antibacterial activitiy (mm) against *Listeria monocytogenes*	
	7th day	12th day
G1	-	-
G2	-	11
G3	-	14
G4	28	24

According to table 1, the antibacterial activity observed on the 7th day was totally attributed to the group G4 with a diameter of 28 mm. Moreover, the antibacterial activity observed on the 12th day was attributed to the three groups of metabolites, G1, G2 and G4 showing diameter ranging between 11 and 24 mm.



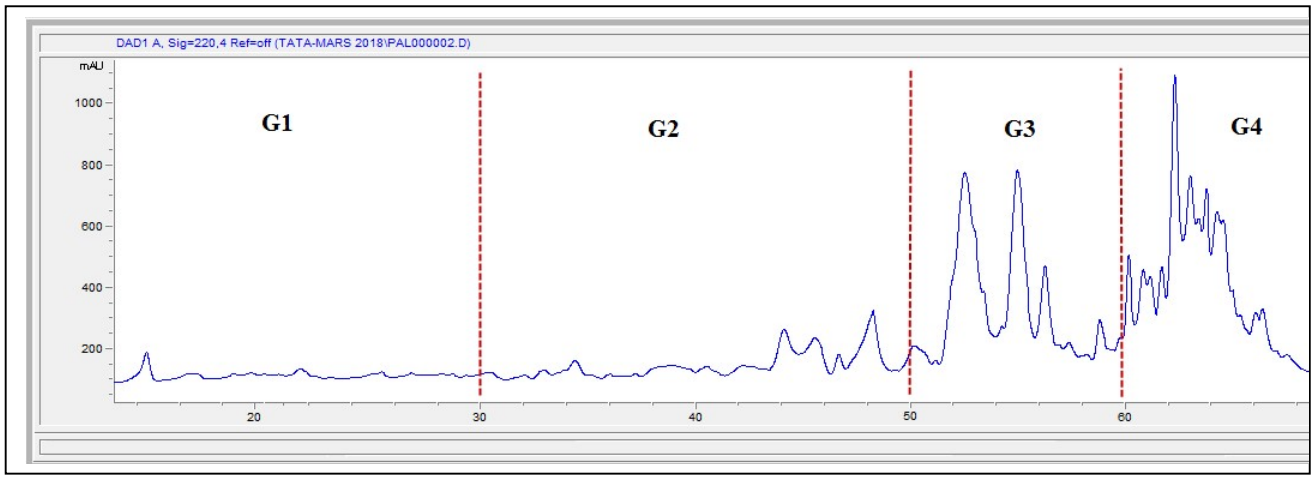


Figure 3. HPLC profile of secondary metabolites secreted by the strain *Streptomyces* sp. PAL114 in the Bennett medium at the 7th (A) and 12th (B) day (detection at 220 nm, column C18 (Agilent 1260); gradient system, 20-100% MeOH in water during 55 min, then 100% MeOH for 15 min.

Conclusion

This current study showed that the antibacterial metabolites obtained from the actinobacterium were enhanced in the Bennett medium. Changes in the type and nature of carbon and nitrogen sources in culture media were shown to affect the formation of cell biomass and the biosynthesis of bioactive secondary metabolites in *Streptomyces* sp. PAL114.

The preliminary data obtained from this work will be useful for the consecutive full characterization of the *Streptomyces* strain PAL114 induced antibiotics.

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