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# A new dithiopyrrolone antibiotic triggered by a long fermentation of *Saccharothrix algeriensis* NRRL B-24137 in sorbic acid-amended medium

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**Significance and Impact of the Study:** Given the strong activities of dithiopyrrolones against diverse prokaryotic and eukaryotic micro-organisms including potent selective-anticancer activity, the discovery of new-related derivatives draw continuous attention for therapeutic research. Depending on nature and concentration of added precursor, *Saccharothrix algeriensis* NRRL B-24137 produce several dithiopyrrolone compounds. In this study, sorbic acid addition combined to long fermentation duration was shown to induce the biosynthesis of a novel dithiopyrrolone derivative. After purification and full spectroscopic and spectrometric study, the compound was characterized as iso-hexanoyl-pyrrothine. In the future investigation for novel dithiopyrrolone discovery, fermentation duration should be regarded as a key parameter as well.

## Keywords

antimicrobial activity, dithiopyrrolone antibiotics, long fermentation, *Saccharothrix algeriensis*, sorbic acid.

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## Abstract

*Saccharothrix algeriensis* NRRL B 24137 is an actinobacterium isolated from Algerian Saharan soil. It produces bioactive compounds belonging to the dithiopyrrolone class of antibiotics, which are characterized by the possession of a unique pyrrolinodithiole nucleus. Dithiopyrrolones are known for their strong antibacterial and antifungal activities. This class of antibiotics generated great interest after the discovery of their anticancer properties. In this study, an antibiotic named PR11, produced after a long bacterial fermentation (11 days) in sorbic acid containing culture broth, was characterized as a new dithiopyrrolone derivative. After HPLC analysis and purification, the chemical structure of this antibiotic was determined by <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance, mass and UV visible data. PR11 was thus characterized as an iso hexanoyl pyrrothine, a novel dithiopyrrolone derivative. The minimum inhibitory concentrations of the new induced antibiotic were determined against several pathogenic micro organisms. A moderate to strong activity was noted against all Gram positive bacteria, filamentous fungi and yeasts tested.

## Introduction

Although resistance to existing antibiotic that increase at an alarming rate, there is an urgent need for new antibiotics with novel cellular targets. However, only four new structural classes of antibiotics have been introduced to

the clinic in the last 50 years (Qin *et al.* 2013). With their strong activities against variety of prokaryotic and eukaryotic micro organisms as well as potent selective anticancer activity, one of promising investigate group of antibiotics are dithiopyrrolones derivatives (Minamiguchi *et al.* 2001; Webster *et al.* 2002; Lamari *et al.* 2002a; Jia *et al.*

2010; Li *et al.* 2014; Merrouche *et al.* 2017; Guo *et al.* 2019).

Dithiopyrrolones are a class of antibiotics that possess the unique pyrrolinonodithiole (4H [1,2] dithiolo [4,3 b] pyrrol 5 one) skeleton linked to two variable acyl groups, R1 and R2 (Fig. 1) (Jiang *et al.* 2012). Depending on the nature of R1, two dithiopyrrolone groups are mainly distinguished: pyrrothins (R1 = CH<sub>3</sub>) and holothins (R1 = H) that differ from each other by the side chain R2 (Stachel *et al.* 2002). These compounds have been found to be produced by few species of actinobacteria (*Streptomyces* and *Saccharothrix*), *Xenorhabdus* and some marine bacteria such as *Alteromonas rava*, *Photobacterium halotolerans*, *Yersinia ruckeri* and *Pseudoalteromonas* sp. SANK 73390 (Qin *et al.* 2013; Ding *et al.* 2017; Dreyer *et al.* 2018). Among these producing strains, *Saccharothrix algeriensis* NRRL B 24137, a rare actinobacteria isolated from a palm grove soil in Southern Algeria (Zitouni *et al.* 2004), was shown to commonly produce in complex ISP2 broth medium (glucose yeast extract malt extract, Shirling and Gottlieb 1966) at least five dithiopyrrolone derivatives characterized by their different N acyl groups, such as acetyl pyrrothine (thiolutin), senecieryl pyrrothine, tigloyl pyrrothine, isobutyrylpyrrothine and butanoyl pyrrothine (Fig. 1) (Lamari *et al.* 2002b). However, the production levels of these five dithiopyrrolones were shown to be modified by the addition of amino acids as precursors to a basal semi synthetic (SS) medium containing glucose, yeast extract and several mineral sources (Bouras *et al.* 2006a,2006b).

The so called precursor directed biosynthesis (PDB) method also led to the production of new *S. algeriensis* dithiopyrrolone derivatives in the SS medium (Bouras *et al.* 2008). Another study using the same bacterial strain, but supplemented with valeric acid ( $5 \times 10^{-3}$  mol) allowed formation of three new dithiopyrrolone derivatives: formylpyrrothine, valerylpyrrothine and iso valerylpyrrothine (Merrouche *et al.* 2010). Likewise, the addition of sorbic acid ( $5 \times 10^{-3}$  mol) allowed formation of four new dithiopyrrolone derivatives: crotonyl pyrrothine, sorbyl pyrrothine, 2 hexonyl pyrrothine and 2 methyl 3 pentenylpyrrothine (Fig. 1) (Merrouche *et al.* 2011); while cinnamic acid addition ( $5 \times 10^{-3}$  mol) permitted the production of benzoyl pyrrothine (Merrouche *et al.* 2019) (Fig. 1). Thus, depending on precursors added, which determine the activated organic acid (acyl CoA) type incorporated into the pyrrothine nucleus, *S. algeriensis* has the ability to produce different dithiopyrrolones derivatives.

In general, all these dithiopyrrolone derivatives were shown to reach a maximum production between 3 and 8 days of incubation. However, in this work, during a longer fermentation of *S. algeriensis*, a new dithiopyrrolone

antibiotic was induced in the 11th day by adding sorbic acid in the culture broth. After purification process, the chemical structure and minimum inhibitory concentrations of this new dithiopyrrolone derivative was investigated.

## Results and discussion

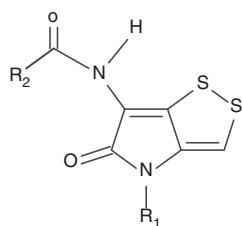
### Effect of sorbic acid addition on antibiotic production in *S. algeriensis*

The HPLC profiles shown in Fig. 2 indicated that *S. algeriensis* NRRL B 24137 still produced several dithiopyrrolones compounds after 11 days of fermentation. Five compounds were produced in the basal SS medium (control without addition of precursors) (Fig. 2a) and correspond (with respect to their retention times and absorbance at 390 nm) to thiolutin, iso butyryl pyrrothine, butanoyl pyrrothine, tigloyl pyrrothine and senecieryl pyrrothine (Fig. 1), as reported by Lamari *et al.* (2002b). As previously described by Merrouche *et al.* (2011), the addition of sorbic acid to the SS medium triggered both modification in production levels of this five known dithiopyrrolones and induction of four other derivatives characterized as crotonyl pyrrothine, sorbyl pyrrothine, 2 hexonyl pyrrothine and 2 methyl 3 pentenyl pyrrothine (Figs 1 and 2b). However, another dithiopyrrolone (noted PR11) that has never been reported before, with regard to its retention time of 20.92 min, was detected (Fig. 2b).

*Saccharothrix algeriensis* has a remarkable ability to produce a wide range of dithiopyrrolone derivatives depending on the composition of the culture medium, and both the nature and concentration of precursors added (Lamari *et al.* 2002a; Bouras *et al.* 2008; Merrouche *et al.* 2010, 2011). These culture conditions determine the enzymatic process involved in attaching a variety of radicals (R) into the pyrrothine ring (Fig 1) which thus determine the nature of the produced dithiopyrrolone (Bouras *et al.* 2006a,2006b, 2008; Chorin *et al.* 2009).

Likewise all characterized *S. algeriensis* induced dithiopyrrolones, the compound PR11 appeared yellow to yellow orange. Furthermore, the compound PR11, which subsequently expressed antimicrobial activity, exhibited intense absorption at 390 nm (Fig. 2c) as it was generally mentioned for dithiopyrrolone derivatives (Lamari *et al.* 2002b; Bouras *et al.* 2008; Merrouche *et al.* 2010).

During the time course of fermentation in SS medium supplemented with sorbic acid, antibiotic production and dry cell weight were monitored (Fig. 3). The maximal production of PR11 was obtained on the 11th day of fermentation with an amount of  $0.12 \pm 0.03$  mg l<sup>-1</sup>.



(I) Dithiopyrrolones produced in both complex ISP2 and semi-synthetic media

R1 = CH<sub>3</sub> (pyrrothin group)

R2 = CH <sub>3</sub>	Acetyl-pyrrothine (thiolutin)
CH(CH <sub>3</sub> ) <sub>2</sub>	Iso-butyryl-pyrrothine
(CH <sub>2</sub> ) <sub>2</sub> -CH <sub>2</sub>	Butanoyl-pyrrothine
CH=C(CH <sub>3</sub> ) <sub>2</sub>	Senecioid-pyrrothine
C(CH <sub>3</sub> )=CH(CH <sub>3</sub> )	Tigloyl-pyrrothine

(II) PDB-induced dithiopyrrolones in semi-synthetic medium

R1 = CH<sub>3</sub>

R2 = CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	Valeryl-pyrrothine	Val
CH(CH <sub>3</sub> )(CH <sub>2</sub> CH <sub>3</sub> )	Isovaleryl-pyrrothine	Val
H	Formyl-pyrrothine	Benz, Cin, Val
CH=CH(CH <sub>3</sub> )	Crotonyl-pyrrothine	Sorb
CH=CH-CH=CH(CH <sub>3</sub> )	Sorbyl-pyrrothine	Sorb
CH=CH-CH <sub>2</sub> CH <sub>2</sub> CH <sub>3</sub>	2-Hexonyl-pyrrothine	Sorb
CH=C(CH <sub>3</sub> )(CH <sub>2</sub> CH <sub>3</sub> )	2-methyl-3-pentenyl-pyrrothine	Sorb
C <sub>6</sub> H <sub>6</sub>	Benzoyl-pyrrothine	Benz, Cin

R1 = (holothin group)

R2 = CH <sub>2</sub> CH <sub>5</sub>	Demethyl-benzoyl-pyrrothine (Benzoyl-holothine)*	Benz
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\*Mass spectrum-based putatively determined structure.

Precursor used: Benz, benzoic acid; Cin, cinnamic acid; Sorb, sorbic acid; Val, valeric acid.

**Figure 1** Dithiopyrrolones produced by *Sacharothrix algeriensis* NRRL B 24137.

As expected, the addition of sorbic acid allowed to maintain *S. algeriensis* biomass in the end of fermentation at an appreciable level in comparison to control. Moreover, the PR11 production was observed at the end of the idiophase, in agreement with previous studies that have shown for all *S. algeriensis* synthesized dithiopyrrolones (common or PDB induced) an optimal production in the biomass decline phase (Lamari *et al.* 2002a; Bouras *et al.* 2008; Merrouche *et al.* 2011).

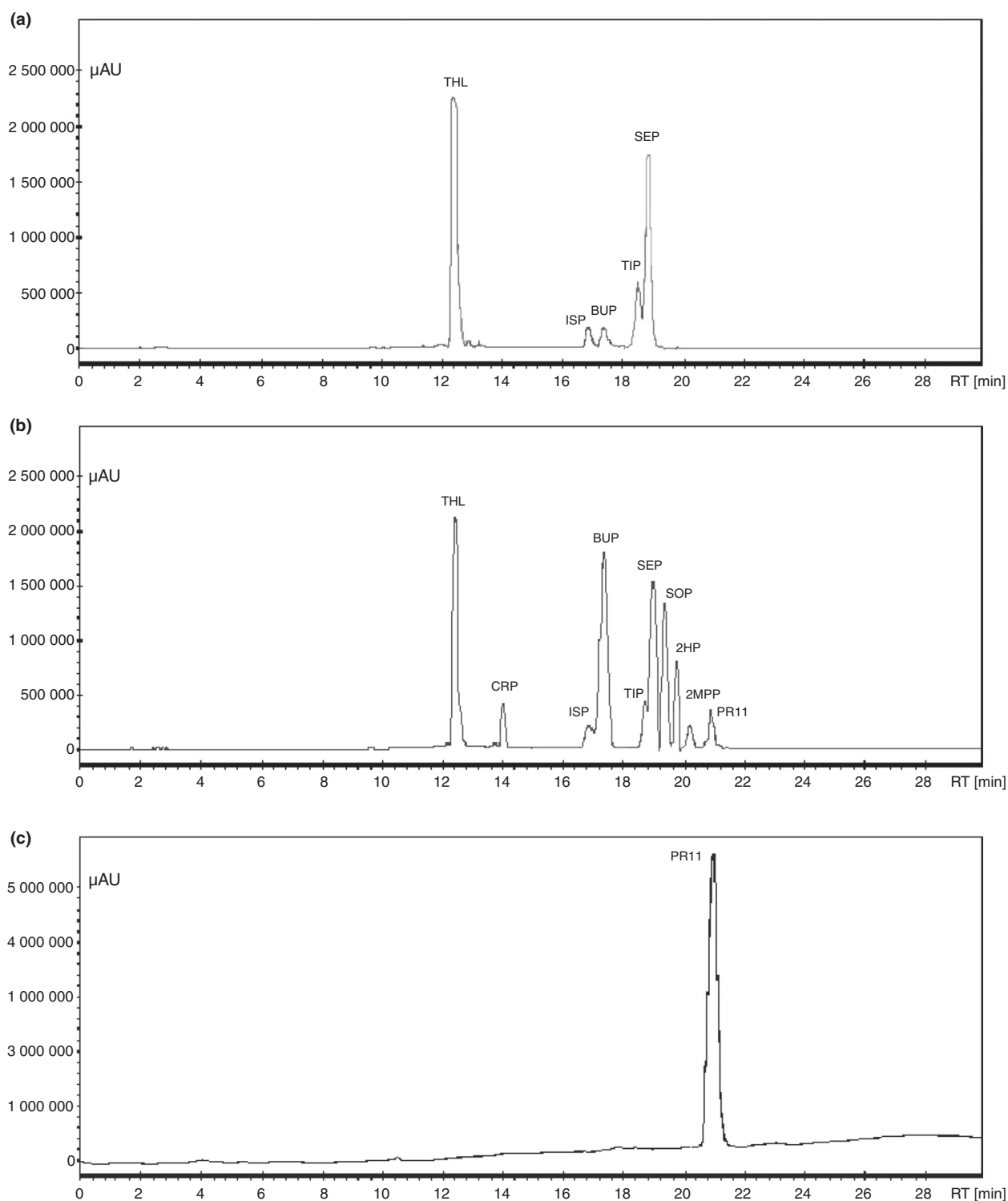
#### Isolation and purification of the new antibiotic

Fifteen litres of the culture broth was extracted by dichloromethane and the yellow organic phase was concentrated to dryness. Through semi preparative HPLC, the compound PR11 was repetitively recovered than finally purified after two successive reinjections in the

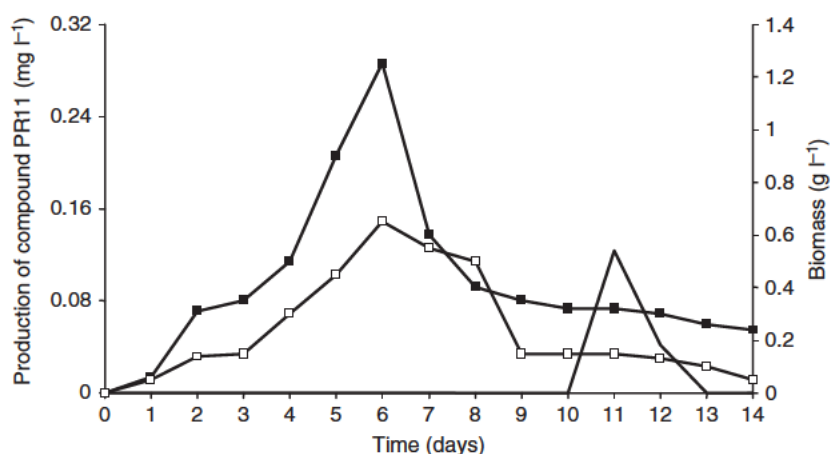
HPLC system. This new induced compound, which did not correspond to known dithiopyrrolones with respect to retention time, was effectively identified as a new dithiopyrrolone derivative according to its spectral characteristics.

#### Characterization of induced antibiotic PR11

The UV visible spectrum of the induced compound PR11 showed three absorption maxima at 207, 307 and 392 nm. The EIMS spectrum analysis showed a molecular weight of  $M = 284$  (Fig. S1) and a prominent fragment ion of  $m/z$  186. This fragment indicated the presence of an extra methyl group on the heterocyclic ring that correspond to a pyrrothin group of dithiopyrrolone class as previously reported for other dithiopyrrolones (McInerney *et al.* 1991; Bouras *et al.* 2008).



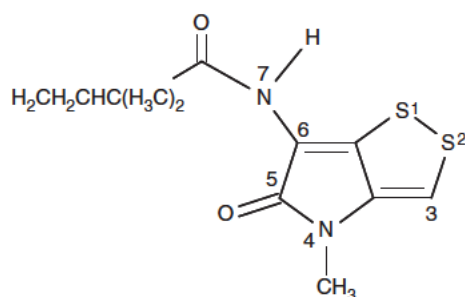
**Figure 2** Effect of sorbic acid addition on dithiopyrrolones produced by *Saccharothrix Algeriensis* NRRL B 24137 under a long fermentation condition. (a) Compounds produced in standard conditions (basal SS medium). (b) Compounds produced after addition of sorbic acid to the SS medium. (c) Final purification of compound PR11. The HPLC analysis was done at an UV detection of 390 nm of 11 days old crude culture from *S. Algeriensis*. The produced dithiopyrrolones with corresponding retention times (min) were as follows: THL, Thiolutine (12.50); CRP, Crotonyl pyrrothine (14.02); ISP, Iso butyryl pyrrothine (16.80); BUP, Butanoyl pyrrothine (17.60); TIP, Tigloyl pyrrothine (18.60); SEP, Senecieryl pyrrothine (18.90); SOP, Sorbyl pyrrothine (19.40); 2HP, 2 hexonyl pyrrothine (19.72); 2MPP, 2 methyl 3 pentenyl pyrrothine (20.10) and PR11 (20.92).



**Figure 3** Effect of sorbic acid addition to the SS medium on evolution of biomass and production of new dithiopyrrolone PR11 by *Saccharothrix algeriensis* NRRL B 24137. —■—, evolution of biomass in sorbic acid supplemented medium; —□—, evolution of biomass in control condition (SS medium without sorbic acid supplementation); —●—, production of PR11 dithiopyrrolone.

Through  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral features it is possible to discern; two carbonyl groups ( $\delta_c$  166.7/171.5 and  $\delta_c$  164.6/166.8), one olefinic group ( $\delta_H$  6.75/6.68 and  $\delta_c$  108.8/108.0), one  $\text{N-CH}_3$  group ( $\delta_H$  3.40/3.35 and  $\delta_c$  27.6/27.7) and one  $\text{NH}$  group ( $\delta_H$  7.92/7.50). In addition, compound PR11 show two additional  $\text{sp}^3$  methylenes ( $\delta_H$  2.37 and 1.60 and  $\delta_c$  34.2 and 34.2), one  $\text{sp}^3$  methine group ( $\delta_H$  1.64 and  $\delta_c$  27.6) and one methyl group ( $\delta_H$  0.96 and  $\delta_c$  22.2). The 2D  $^1\text{H}$   $^1\text{H}$  and  $^1\text{H}$   $^{13}\text{C}$  experiments established the presence of an iso pentyl group (Fig. 4).

On the basis of NMR and EIMS data, the molecular formula of PR11 was determined as  $\text{C}_{12}\text{H}_{16}\text{N}_2\text{O}_2\text{S}_2$ . This new dithiopyrrolone derivative was named iso hexanoyl pyrrothine (Fig. 4) and it was never cited in the literature. It was also different from hexanoyl pyrrothine (called xenorhabdine IV) secreted by the proteobacteria *Xenorhabdus bovienii*, *Xenorhabdus nematophilus* and *Xenorhabdus* sp. (McInerney *et al.* 1991; Li *et al.* 1995; Paik *et al.* 2001).



**Figure 4** Structure of a new dithiopyrrolone PR11 induced by adding sorbic acid to the SS medium.

#### Minimum inhibitory concentrations (MIC) of purified dithiopyrrolone antibiotic

The antimicrobial activity of the new dithiopyrrolone antibiotic PR11 is shown in Table 1. This antibiotic compound showed generally a moderate activity against Gram positive bacteria tested, but no activity against Gram negative bacteria. Dithiopyrrolone antibiotic PR11 showed a moderate to strong activity against all filamentous fungi and yeasts tested, the most sensitive fungi were *Aspergillus carbonarius* M333 (CMI =  $3 \mu\text{g ml}^{-1}$ ), *Fusarium moniliforme* FM1 (CMI =  $6 \mu\text{g ml}^{-1}$ ) and *Candida albicans* IPA 200 (CMI =  $3 \mu\text{g ml}^{-1}$ ).

Our previous studies showed that the antibacterial and antifungal activities of the dithiopyrrolones are related to their variable acyl groups (Merrouche *et al.* 2010, 2011, 2019). The new dithiopyrrolone antibiotic PR11 showed moderate to strong activity against all Gram positive bacteria, filamentous fungi and yeasts tested, but no activity against Gram negative bacteria. Similar results were observed with other known dithiopyrrolones produced by our strain (Lamari *et al.* 2002b; Merrouche *et al.* 2010, 2011, 2019).

In conclusion, this work demonstrates that *Saccharothrix algeriensis* NRRL B 24137 produced a new dithiopyrrolone antibiotic, characterized as an iso hexanoyl pyrrothine, after a long bacterial fermentation (11 days) in a sorbic acid containing ( $5 \times 10^{-3}$  mol) semi synthetic culture medium. Thus, along with the nature and the concentration of the added organic acids, which were already shown as determinant in the production of novel potentially interesting dithiopyrrolone compounds, fermentation duration, should be regarded as a key parameter as well in the future investigations for prospecting novel dithiopyrrolone derivatives.

**Table 1** Minimum inhibitory concentrations (MIC) of the new dithiopyrrolone antibiotic PR11 produced by *Saccharothrix algeriensis*

Test organism	MIC ( $\mu\text{g ml}^{-1}$ )*
<i>Bacillus subtilis</i> (ATCC 6633)	25
<i>Bacillus coagulans</i> (CIP 6625)	25
<i>Listeria monocytogenes</i> (CIP 82110)	13
<i>Micrococcus luteus</i> (ATCC 9314)	95
<i>Staphylococcus aureus</i> (CIP 7625)	>100
<i>Agrobacterium tumefaciens</i> (2410 LB)	>100
<i>Escherichia coli</i> (ATCC 10536)	>100
<i>Klebsiella pneumoniae</i> (CIP 82-91)	>100
<i>Salmonella enterica</i> (CIP 81-3)	>100
<i>Pseudomonas aeruginosa</i> (CIPA22)	>100
<i>Aspergillus carbonarius</i> (M333)	3
<i>Fusarium oxysporum</i> f. sp. <i>lini</i> (Foln 3)	70
<i>Fusarium monilliforme</i> (FM1)	6
<i>Fusarium equiseti</i> (FE1)	40
<i>Fusarium culmorum</i> (FC1)	40
<i>Fusarium graminearum</i> (FG1)	40
<i>Umbelopsis ramanniana</i> (NRRL 1829)	13
<i>Penicillium expansum</i> (PE1)	50
<i>Candida albicans</i> (IPA 200)	3
<i>Saccharomyces cerevisiae</i> (ATCC 4226)	10

\*Values confirmed by two successive experiments, each conducted with two replicates.

## Materials and methods

### Producing strain

*Saccharothrix algeriensis* NRRL B 24137 (Zitouni *et al.* 2004) was cultivated at 4°C on slants of International *Streptomyces* Project 2 (ISP2) medium containing glucose 4.0 g, malt extract 10.0 g, yeast extract 4.0 g and agar 18.0 g in 1 l distilled water. The pH of the medium was adjusted to 7.0 with 2 mol l<sup>-1</sup> NaOH solution before autoclaving at 120°C for 20 min.

### Fermentation conditions

A mature slant culture of the strain *S. algeriensis* NRRL B 24137 was inoculated into 500 ml Erlenmeyer flasks each containing 100 ml of a basal semi synthetic (SS) medium consisting of glucose 10.0 g, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.0 g, NaCl 2.0 g, KH<sub>2</sub>PO<sub>4</sub> 0.5 g, K<sub>2</sub>HPO<sub>4</sub> 1.0 g, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.2 g, CaCO<sub>3</sub> 5.0 g and yeast extract 2.0 g in 1 l distilled water. The pH of the medium was adjusted to 7.0 using a 2 mol l<sup>-1</sup> NaOH solution prior to autoclaving. The sorbic acid was then aseptically added as precursor at a concentration of 5 × 10<sup>-3</sup> mol to the medium prior to inoculation. Concomitantly, a control (SS medium with out sorbic acid) was also carried out. The cultures were incubated on a rotary shaker (240 rev min<sup>-1</sup>) at 30°C for 14 days.

### Kinetics of biomass and PR11 production

The changes in biomass were evaluated by assessing the dry cell weights (DCWs) of mycelium obtained during the time course (14 days) of strain fermentation on SS medium supplemented with 5 × 10<sup>-3</sup> mol sorbic acid or without supplementation (control). DCWs were determined as previously described by Bouras *et al.* (2006a) and expressed as gram per litre. Concurrently, the quantification of the induced PR11 dithiopyrrolone was performed daily (in triplicate) using a thiolutin standard calibration curve (thiolutin molar extinction coefficient is nearly the same for all fractions) (Lamari *et al.* 2002a; Bouras *et al.* 2006a).

### HPLC analysis of dithiopyrrolones

After centrifuging the samples, the culture supernatant was extracted with an equal volume of dichloromethane.

The organic phase was collected and dried with anhydrous sodium sulphate. The extract was concentrated to dryness under vacuum rotary evaporator, dissolved in 1 ml of methanol and kept as crude extract. The analysis of dithiopyrrolones in the SS medium with or without sorbic acid was carried out by a HPLC system equipped with a C18 reverse phase column (Uptisphere UP5ODB, 150 × 4.6 mm; BioTek Instruments, Milan, Italy). The samples were analysed as described by Lamari *et al.* (2002b) and Bouras *et al.* (2006a). Briefly, the formation of dithiopyrrolones was monitored by comparison of the peak retention times and UV spectra with those of known dithiopyrrolone standards. Appearing dithiopyrrolone products could be easily detected by HPLC analysis due to their intense absorption at 390 nm (Lamari *et al.* 2002b). Concurrently, the antimicrobial activity corresponding to each fraction was monitored using the well method (10 mm well filled with 0.2 ml of each fraction and activity checked towards *Bacillus subtilis* ATCC 6633 and *Umbelopsis ramanniana* NRRL1829. These two target micro organisms were selected as representative of bacteria and filamentous fungi with regard to their sensitivity.

### Purification of new dithiopyrrolone antibiotic

The fermentation procedure was repeated to obtain a total of 15 l of culture broth. This culture was centrifuged and filtered to remove mycelium. The culture filtrate was extracted with an equal volume of dichloromethane, and the organic layer was dried with anhydrous sodium sulphate, and then concentrated under vacuum to generate a crude extract. The latter was subjected to semi preparative HPLC purification on a Waters system using a C18



column (UP5ODB, 250 × 7.8 mm, Waters, Milford, MA). The samples were analysed by linear gradient elution from 10 to 100% methanol in bi distilled water for 40 min, a flow rate of 1.5 ml min<sup>-1</sup> and UV detection at 220 and 390 nm. Final purification of the active fraction was achieved after the second re injection in the HPLC in the same conditions.

#### Chemical characterization of induced new antibiotic

The UV spectrum was determined with a Shimadzu UV1605 spectrophotometer. The molecular weight of new antibiotic compound was obtained by electron impact MS (EIMS) recorded at 70 eV with a Nermag R 10 10C spectrometer. NMR sample was prepared by dissolving 3 mg of the pure new molecule in CD<sub>2</sub>Cl<sub>2</sub> (600 μl). 1D and 2D <sup>1</sup>H and <sup>13</sup>C experiments were recorded on a Bruker Avance 500 spectrometer equipped with a 5 mm triple resonance inverse Z gradient probe (TBI <sup>1</sup>H, <sup>31</sup>P, BB). All chemical shifts for <sup>1</sup>H and <sup>13</sup>C are relative to TMS using <sup>1</sup>H (residual) or <sup>13</sup>C chemical shifts of the solvent as a secondary standard. The temperature was set at 298 K. All the <sup>1</sup>H and <sup>13</sup>C signals were assigned on the basis of chemical shifts, spin spin coupling constants, splitting patterns and signal intensities, and using <sup>1</sup>H <sup>1</sup>H COSY45, <sup>1</sup>H <sup>13</sup>C HMQC and <sup>1</sup>H <sup>13</sup>C HMBC experiments.

#### Minimum inhibitory concentrations (MIC) of purified antibiotic

MIC values of the new antibiotic were determined by the conventional agar dilution method (Oki *et al.* 1990), towards a selection of 20 target micro organisms. These micro organisms were inoculated, in two replicates, onto nutrient agar medium containing different concentrations of PR11 compound (1, 2, 3, 5, 8, 10, 15, 20, 25, 30, 40, 50, 60, 80 and 100 μg ml<sup>-1</sup>). The antimicrobial activity was observed after 24 48 h incubation at 37°C for bacteria and 48 72 h incubation at 28°C for fungi and yeasts, and the lowest concentration that inhibited the growth of each organism was determined. Medium without PR11 compound and inoculated with target micro organisms was used as control. The experiment was repeated twice to confirm the obtained MIC values.

#### Conflict of Interest

The authors declare no conflict of interest.

#### References

Bouras, N., Mathieu, F., Sabaou, N. and Lebrihi, A. (2006a) Effect of amino acids containing sulfur on

- dithiopyrrolone antibiotic productions by *Saccharothrix algeriensis* NRRL B 24137. *J Appl Microbiol* **100**, 390 397.
- Bouras, N., Mathieu, F., Sabaou, N. and Lebrihi, A. (2006b) Nutritional requirements for the production of dithiopyrrolone antibiotics by *Saccharothrix algeriensis* NRRL B 24137. *Enzyme Microb Technol* **39**, 1423 1429.
- Bouras, N., Merrouche, R., Lamari, L., Mathieu, F., Sabaou, N. and Lebrihi, A. (2008) Precursor directed biosynthesis of new dithiopyrrolone analogs by *Saccharothrix algeriensis* NRRL B 24137. *Process Biochem* **43**, 1244 1252.
- Chorin, A.C., Bijeire, L., Monje, M.C., Baziard, G., Lebrihi, A. and Mathieu, F. (2009) Expression of pyrrothines N acyltransferase activities in *Saccharothrix algeriensis* NRRL B 24137: new insights into dithiopyrrolone antibiotic biosynthetic pathway. *J Appl Microbiol* **107**, 1751 1762.
- Ding, H., Wang, J., Zhang, D. and Ma, Z. (2017) Derivatives of holomycin and cyclopropaneacetic acid from *Streptomyces* sp. DT A37. *Chem Biodivers* **14**, e1700140. <https://doi.org/10.1002/cbdv.201700140>.
- Dreyer, J., Malan, A.P. and Dicks, L.M.T. (2018) Bacteria of the genus *Xenorhabdus*, a novel source of bioactive compounds. *Front Microbiol* **9**, 3177.
- Guo, C., Chen, Y., Zhu, J., Wang, J., Xu, Y., Luan, H., Zhu, Z., Hu, M. *et al.* (2019) Preparation, optimization of intravenous ZL 004 nanosuspensions by the precipitation method, effect of particle size on *in vivo* pharmacokinetics and tissue distribution. *J Drug Deliv Sci Technol* **50**, 313 320.
- Jia, Y.F., Wu, S.L., Isenberg, J.S., Dai, S.J., Sipes, J.M., Field, L., Zeng, B., Bandle, R.W. *et al.* (2010) Thiolutin inhibits endothelial cell adhesion by perturbing Hsp27 interactions with components of the actin and intermediate filament cytoskeleton. *Cell Stress Chaperones* **15**, 165 181.
- Jiang, C., Muller, W.E.G., Schroder, H.C. and Guo, Y. (2012) Disulfide and polysulfide containing metabolites from marine organisms. *Chem Rev* **112**, 2179 2207.
- Lamari, L., Zitouni, A., Boudjella, H., Badji, B., Sabaou, N., Lebrihi, A., Lefebvre, G., Seguin, E. *et al.* (2002a) New dithiopyrrolone antibiotics from *Saccharothrix* sp. SA 233 I. Taxonomy, fermentation, isolation and biological activities. *J Antibiot* **55**, 696 701.
- Lamari, L., Zitouni, A., Dob, T., Sabaou, N., Lebrihi, A., Germain, P., Seguin, E. and Tillequin, F. (2002b) New dithiopyrrolone antibiotics from *Saccharothrix* sp. SA 233 II. Physicochemical properties and structure elucidation. *J Antibiot* **55**, 702 706.
- Li, J., Chen, G. and Webster, J.M. (1995) Antimicrobial metabolites from a bacterial symbiont. *J Nat Prod* **58**, 1081 1086.
- Li, B., Wever, W.J., Walsh, C.T. and Bowers, A. (2014) Dithiopyrrolones: biosynthesis, synthesis and activity of unique class of disulfide containing antibiotics. *Nat Prod Rep* **31**, 905 923.
- McInerney, B.V., Gregson, R.P., Lacey, M.J., Akhurst, R.J., Lyons, G.R., Rhodes, S.H., Smith, D.R.J., Engelhardt, L.M.

- et al.* (1991) Biologically active metabolites from *Xenorhabdus* spp., Part I. Dithiopyrrolone derivatives with antibiotic activity. *J Nat Prod* **54**, 774–784.
- Merrouche, R., Bouras, N., Coppel, Y., Mathieu, F., Monge, M.C., Sabaou, N. and Lebrihi, A. (2010) Dithiopyrrolone antibiotic formation induced by adding valeric acid to the culture broth of *Saccharothrix algeriensis*. *J Nat Prod* **73**, 1164–1166.
- Merrouche, R., Bouras, N., Coppel, Y., Mathieu, F., Sabaou, N. and Lebrihi, A. (2011) New dithiopyrrolone antibiotics induced by adding sorbic acid to the culture medium of *Saccharothrix algeriensis* NRRL B 24137. *FEMS Microbiol Lett* **318**, 41–46.
- Merrouche, R., Yekkour, A., Lamari, L., Zitouni, A., Mathieu, F. and Sabaou, N. (2017) Efficiency of *Saccharothrix algeriensis* NRRL B 24137 and its produced antifungal dithiopyrrolones compounds to suppress *Fusarium oxysporum* induced wilt disease occurring in some cultivated crops. *Arab J Sci Eng* **42**, 2321–2327.
- Merrouche, R., Yekkour, A., Coppel, Y., Bouras, N., Lamari, L., Zitouni, A., Mathieu, F., Lebrihi, A. *et al.* (2019) Effective biosynthesis of benzoyl pyrrothine dithiopyrrolone antibiotic by cinnamic acid precursor addition in culture of *Saccharothrix algeriensis* NRRL B 24137. *Lett Appl Microbiol* **68**, 165–172.
- Minamiguchi, K., Kumagai, H., Masuda, T., Kawada, M., Ishizuka, M. and Takeuchi, T. (2001) Thiolutin, an inhibitor of huvec adhesion to vitronectin, reduces paxillin in huvecs and suppresses tumor cell induced angiogenesis. *Int J Cancer* **93**, 307–316.
- Oki, T., Tenmyo, O., Tomatsu, K. and Kamei, H. (1990) Pradimicins A, B and C: new antifungal antibiotic. II. *In vitro* and *in vivo* biological activities. *J Antibiot* **43**, 763–770.
- Paik, S., Park, Y.H., Suh, S.I., Kim, H.S., Lee, I.S., Park, M.K., Lee, C.S. and Park, S.H. (2001) Unusual cytotoxic phenethylamides from *Xenorhabdus nematophilus*. *Bull Korean Chem Soc* **22**, 372–374.
- Qin, Z., Huang, S., Yu, Y. and Deng, H. (2013) Dithiopyrrolone natural products: isolation, synthesis and biosynthesis. *Mar Drugs* **11**, 3970–3997.
- Shirling, E.B. and Gottlieb, D. (1966) Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Stachel, H.D., Eckl, E., Immerz Winkler, E., Kreiner, C., Weigand, W.W., Roblb, C., Wünsch, R., Dick, S. *et al.* (2002) Synthesis and reactions of new dithiopyrrolones. *Helv Chim Acta* **85**, 4453–4467.
- Webster, J.M., Chen, G., Hu, K. and Li, J. (2002) Bacterial metabolites. In *Entomopathogenic nematology* ed. Gaugler, R. pp. 99–114. New Jersey, NJ: The Center for Agricultural Bioscience International.
- Zitouni, A., Lamari, L., Boudjella, H., Badji, B., Sabaou, N., Gaouar, A., Mathieu, F., Lebrihi, A. *et al.* (2004) *Saccharothrix algeriensis* sp. nov., isolated from a Saharan soil. *Int J Syst Evol Microbiol* **54**, 1377–1381.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Electron impact mass spectrum (EIMS) of the antibiotic PR11.