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

R. Merrouche[1], A. Yekkour[1,2], Y. Coppel[3], N. Bouras[1,4], A. Zitouni[1], A. Lebrihi[5], F. Mathieu[5] and N. Sabaou[1]

1 Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Alger, Algeria
2 Institut National de Recherche Agronomique d'Algérie, Alger, Algeria
3 Laboratoire de Chimie de Coordination, CNRS, Université de Toulouse, UPS, INPT, Toulouse, France
4 Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, Ghardaïa, Algeria
5 Laboratoire de Génie Chimique, Université de Toulouse, CNRS, Toulouse, France

Significance and Impact of the Study: Given the strong activities of dithiolopyrrolones 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 dithiolopyrrolone coumpounds. In this study, sorbic acid addition combined to long fermentation duration was shown to induce the biosynthesis of a novel dithiolopyrrolone derivative. After purification and full spectroscopic and spectrometric study, the compound was characterized as iso-hexanoyl-pyrrothine. In the future investigation for novel dithiolopyrrolone discovery, fermentation duration should be regarded as a key parameter as well.

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

Correspondence
Nasserdine Sabaou, Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Alger, Algeria.
E mail: sabaou@yahoo.fr

doi:10.1111/lam.13207

Abstract

Saccharothrix algeriensis NRRL B 24137 is an actinobacterium isolated from Algerian Saharan soil. It produces bioactive compounds belonging to the dithiolopyrrolone class of antibiotics, which are characterized by the possession of a unique pyrrolinonodithiole nucleus. Dithiolopyrrolones 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 dithiolopyrrolone derivative. After HPLC analysis and purification, the chemical structure of this antibiotic was determined by ^{1}H and ^{13}C nuclear magnetic resonance, mass and UV visible data. PR11 was thus characterized as an iso hexanoyl pyrrothine, a novel dithiolopyrrolone 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 dithiolopyrrolones 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).

Dithiolopyrrolones 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 dithiolopyrrolone groups are mainly distinguished: pyrrothins ($R1 = CH_3$) 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 dithiolopyrrolone derivatives characterized by their different N acyl groups, such as acetyl pyrrothine (thiolutin), senecioyl pyrrothine, tigloyl pyrrothine, isobutyrylpyrrothine and butanoyl pyrrothine (Fig. 1) (Lamari *et al.* 2002b). However, the production levels of these five dithiolopyrrolones 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* dithiolopyrrolone derivatives in the SS medium (Bouras *et al.* 2008). Another study using the same bacterial strain, but supplemented with valeric acid (5×10^{-3} mol) allowed formation of three new dithiolopyrrolone derivatives: formylpyrrothine, valerylpyrrothine and isovalerylpyrrothine (Merrouche *et al.* 2010). Likewise, the addition of sorbic acid (5×10^{-3} mol) allowed formation of four new dithiolopyrrolone derivatives: crotonyl pyrrothine, sorbyl pyrrothine, 2 hexonyl pyrrothine and 2 methyl 3 pentenylpyrrothine (Fig. 1) (Merrouche *et al.* 2011); while cinnamic acid addition (5×10^{-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 dithiolopyrrolones derivatives.

In general, all these dithiolopyrrolone 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 dithiolopyrrolone 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 dithiolopyrrolone derivative was investigated.

Results and discussion

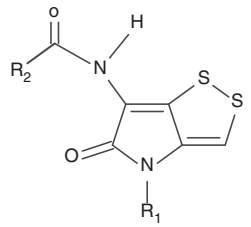
Effect of sorbic acid addition on antibiotic production in *S. algeriensis*

The HPLC profiles shown in Fig. 2 indicated that *S. algeriensis* NRRL B 24137 still produced several dithiolopyrrolones 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 senecioyl 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 dithiolopyrrolones 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 dithiolopyrrolone (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 dithiolopyrrolone 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 dithiolopyrrolone (Bouras *et al.* 2006a,2006b, 2008; Chorin *et al.* 2009).

Likewise all characterized *S. algeriensis* induced dithiolopyrrolones, 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 dithiolopyrrolone 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 $0{\cdot}12 \pm 0{\cdot}03$ mg l^{-1}.



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

R1 = CH_3 (pyrrothin group)		
R2 = CH_3	Acetyl-pyrrothine (thiolutin)	
$CH(CH_3)_2$	Iso-butyryl-pyrrothine	
$(CH_2)_2$–CH_2	Butanoyl-pyrrothine	
$CH=C(CH_3)_2$	Senecioyl-pyrrothine	
$C(CH_3)=CH(CH_3)$	Tigloyl-pyrrothine	
(II) PDB-induced dithiolopyrrolones insemi-synthetic medium		
R1 = CH3		
R2 = $CH_2CH_2CH_2CH_3$	Valeryl-pyrrothine	*Val*
$CH(CH_3)(CH_2CH_3)$	Isovaleryl-pyrrothine	*Val*
H	Formyl-pyrrothine	*Benz, Cin, Val*
$CH=CH(CH_3)$	Crotonyl-pyrrothine	*Sorb*
$CH=CH–CH=CH(CH_3)$	Sorbyl-pyrrothine	*Sorb*
$CH=CH–CH_2CH_2CH_3$	2-Hexonyl-pyrrothine	*Sorb*
$CH=C(CH_3)(CH_2CH_{3)}$	2-methyl-3-pentenyl-pyrrothine	*Sorb*
C_6H_6	Benzoyl-pyrrothine	*Benz, Cin*
R1 = (holothin group)		
R2 = CH_2CH_5	Demethyl-benzoyl-pyrrothine (Benzoyl-holothine)*	*Benz*

*Mass spectrum-based putatively determined structure.
Precursor used: *Benz*, benxoic acid; *Cin*, cinnamic acid; Sorb, sorbic acid; *Val*, valeric acid.

Figure 1 Dithiolopyrrolones 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. More over, the PR11 production was observed at the end of the idiophase, in agreement with previous studies that have shown for all *S. algeriensis* synthesized dithiolopyrrolones (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 con centrated 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 dithiolopyrrolones with respect to retention time, was effectively identified as a new dithi olopyrrolone derivative according to its spectral character istics.

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 corre spond to a pyrrothin group of dithiolopyrrolone class as previously reported for other dithiolopyrrolones (McIner ney *et al.* 1991; Bouras *et al.* 2008).

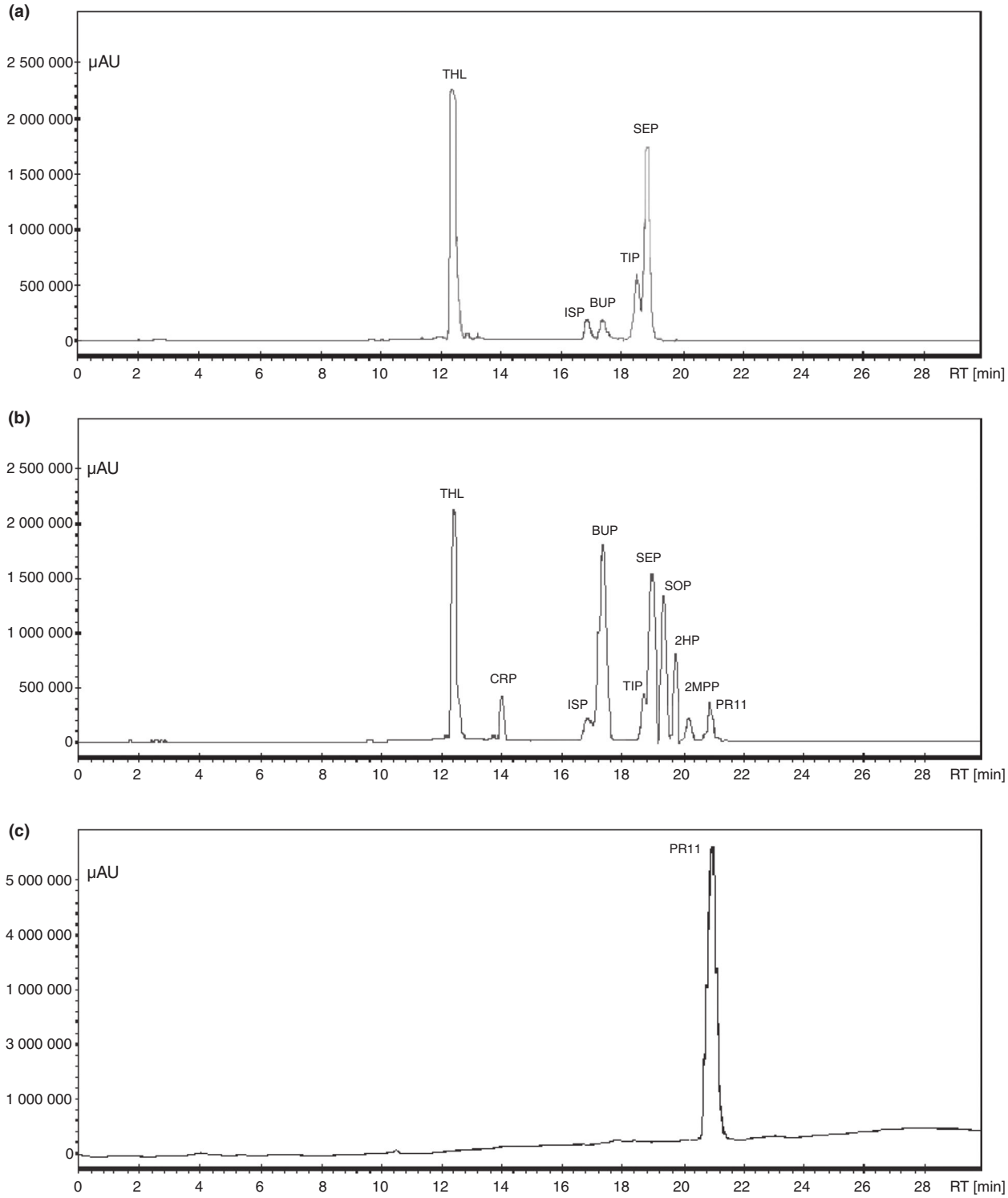


Figure 2 Effect of sorbic acid addition on dithiolopyrrolones produced by *Saccharothrix algeriensis* NRRL B 24137 under a long fermentation con dition. (a) Compounds produced in standard conditions (basal SS medium). (b) Compounds produced after addition of sorbic acid to the SS med ium. (c) Final purification 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 dithiolopyrrolones 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, Senecioyl 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).

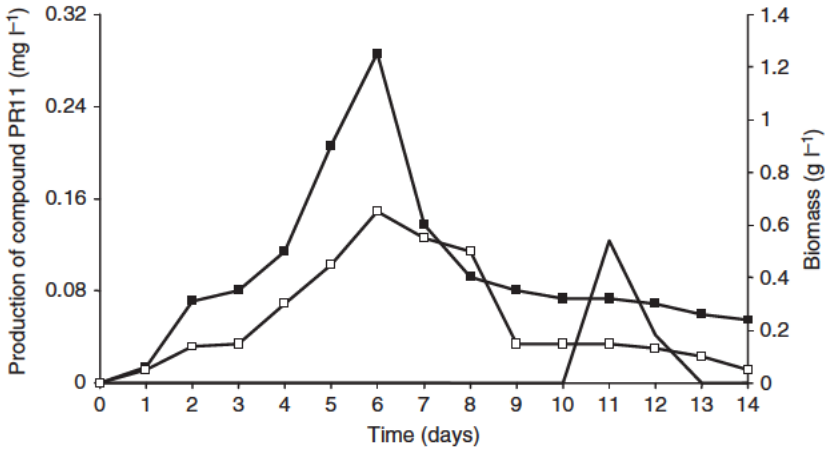


Figure 3 Effect of sorbic acid addition to the SS medium on evolution of biomass and production of new dithiolopyrrolone PR11 by *Saccharothrix algeriensis* NRRL B 24137.–■–, evolution of biomass in sorbic acid supplemented medium;–□–, evolution of biomass in control condition (SS medium without sorbic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

Through ^{1}H and ^{13}C NMR spectral features it is possible to discern; two carbonyl groups (δ_c 166·7/171·5 and δ_c 164·6/166·8), one olefinic group (δ_H 6·75/6·68 and δ_c 108·8/108·0), one N CH_3 group (δ_H 3·40/3·35 and δ_c 27·6/27·7) and one NH group (δ_H 7·92/7·50). In addition, compound PR11 show two additional sp3 methylenes (δ_H 2·37 and 1·60 and δ_c 34·2 and 34·2), one sp3 methine group (δ_H 1·64 and δ_c 27·6) and one methyl group (δ_H 0·96 and δ_c 22·2). The 2D ^{1}H ^{1}H and ^{1}H ^{13}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 $C_{12}H_{16}N_2O_2S_2$. This new dithiolopyrrolone 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).

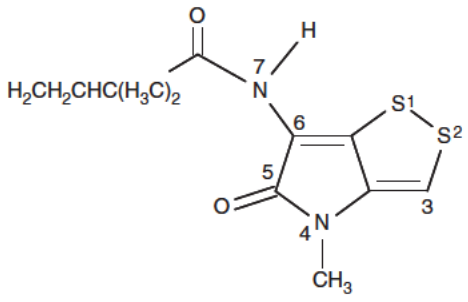


Figure 4 Structure of a new dithiolopyrrolone PR11 induced by adding sorbic acid to the SS medium.

Minimum inhibitory concentrations (MIC) of purified dithiolopyrrolone antibiotic

The antimicrobial activity of the new dithiolopyrrolone 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. Dithiolopyrrolone 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 μg ml^{-1}), *Fusarium moniliforme* FM1 (CMI = 6 μg ml^{-1}) and *Candida albicans* IPA 200 (CMI = 3 μg ml^{-1}).

Our previous studies showed that the antibacterial and antifungal activities of the dithiolopyrrolones are related to their variable acyl groups (Merrouche *et al.* 2010, 2011, 2019). The new dithiolopyrrolone 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 dithiolopyrrolones 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 dithiolopyrrolone antibiotic, characterized as an iso hexanoyl pyrrothine, after a long bacterial fermentation (11 days) in a sorbic acid containing (5×10^{-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 dithiolopyrrolone compounds, fermentation duration, should be regarded as a key parameter as well in the future investigations for prospecting novel dithiolopyrrolone derivatives.

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

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

*Values confirmed by two successive experiments, each conduced 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^{-1} 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, $(NH_4)_2SO_4$ 2·0 g, NaCl 2·0 g, KH_2PO_4 0·5 g, K_2HPO_4 1·0 g, $MgSO_4{\cdot}7H_2O$ 0·2 g, $CaCO_3$ 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^{-1} NaOH solution prior to autoclaving. The sorbic acid was then aseptically added as precursor at a concentration of 5×10^{-3} mol to the medium prior to inoculation. Concomitantly, a control (SS medium without sorbic acid) was also carried out. The cultures were incubated on a rotary shaker (240 rev min^{-1}) 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^{-3} 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 dithiolopyrrolone 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 dithiolopyrrolones

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 dithiolopyrrolones 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 dithiolopyrrolones was monitored by comparison of the peak retention times and UV spectra with those of known dithiolopyrrolone standards. Appearing dithiolopyrrolone 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 dithiolopyrrolone 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 elu tion from 10 to 100% methanol in bi distilled water for 40 min, a flow rate of 1·5 ml min^{-1} 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 spec trometer. NMR sample was prepared by dissolving 3 mg of the pure new molecule in CD_2Cl_2 (600 μl). 1D and 2D ^{1}H and ^{13}C experiments were recorded on a Bruker Avance 500 spectrometer equipped with a 5 mm triple resonance inverse Z gradient probe (TBI ^{1}H, ^{31}P, BB). All chemical shifts for ^{1}H and ^{13}C are relative to TMS using ^{1}H (residual) or ^{13}C chemical shifts of the solvent as a secondary standard. The temperature was set at 298 K. All the ^{1}H and ^{13}C signals were assigned on the basis of chemical shifts, spin spin coupling constants, splitting patterns and signal intensities, and using ^{1}H ^{1}H COSY45, ^{1}H ^{13}C HMQC and ^{1}H ^{13}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^{-1}). The antimicrobial activity was observed after 24 48 h incubation at 37°C for bacte ria 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 dithiolopyrrolone 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 dithiolopyrrolone 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 dithiolopyrrolone 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 dithiolopyrrolone 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 multisulfide 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 dithiolopyrrolone 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 dithiolopyrrolone 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) Dithiolopyrrones: 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. Dithiolopyrrolone 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) Dithiolopyrrolone 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 dithiolopyrrolone 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 dithiolopyrrolones 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 dithiolopyrrolone 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 angiogensis. *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) Dithiolopyrrolone 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 Winklera, E., Kreiner, C., Weigand, W.W., Roblb, C., Wünsch, R., Dick, S. *et al*. (2002) Synthesis and reactions of new dithiolopyrroles. *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.