Nutritional requirements for the production of dithiolopyrrolone antibiotics by *Saccharothrix algeriensis* NRRL B-24137

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Abstract

The amino acid and humic acid requirements of *Saccharothrix algeriensis* NRRL B-24137 for growth and production of the dithiolopyrrolone antibiotics were studied in a semi-synthetic medium (SSM). Nature and concentration of amino acids and humic acid strongly influenced the growth and dithiolopyrrolone specific production.

The highest value of thiolutin (acetyl-pyrrothine) specific production was obtained in the presence of 1 g/l humic acid (336 mg/g DCW), and in the presence of 5 mM L-cystine (309 mg/g DCW) as compared to 19 mg/g DCW obtained with the control. Furthermore, thiolutin production was increased about six-fold, four-fold and three-fold in the presence of L-proline, L-glutamic acid and DL-histidine, respectively. In contrast, the production of thiolutin was reduced by addition of other amino acids such as L-glutamine, DL-ethionine, L-methionine and L-arginine. The highest value of isobutyryl-pyrrothine production was obtained in the presence of 2,6-diaminopimelic acid and L-lysine (7.8 and 1.0 mg/g DCW, respectively). However, the highest value of butanoyl-pyrrothine production was obtained in the presence of humic acid (6.6 mg/g DCW), followed by L-cysteine and L-proline (3.6 and 3.2 mg/g DCW, respectively). In addition, the maximum specific production of senecioyl-pyrrothine (29 mg/g DCW) and tigloyl-pyrrothine (21 mg/g DCW) was obtained in the presence of humic acid. We found that, except for isobutyryl-pyrrothine, production of all dithiolopyrrolones was favoured by addition of L-proline. The maximum specific production was obtained with L-proline at concentrations of 2.50 mM for thiolutin (133 mg/g DCW), 1.25 mM for senecioyl-pyrrothine, tigloyl-pyrrothine and butanoyl-pyrrothine production (29, 23 and 3.9 mg/g DCW, respectively). Production of all dithiolopyrrolones strongly decreased as the L-methionine or DL-ethionine concentration was increased in the culture medium.

Keywords: Antibiotic production; Dithiolopyrrolone; *Saccharothrix algeriensis*; Amino acid; Humic acid; Regulation

1. Introduction

Dithiolopyrrolones are members of the pyrrothine class of naturally occurring antibiotics. This class of compounds includes thiolutin (acetyl-pyrrothine), aureothricin (propionyl-pyrrothine), isobutyryl-pyrrothine (2-methylpropanoyl-pyrrothine), butanoyl-pyrrothine (xenorhabdin VII), senecioyl-pyrrothine (3-methyl-2-butenoyl-pyrro-thine), tigloyl-pyrrothine, propionyl-holothin, holomycin, xenorhabdins, xenorxides and thiomarinols [1–6]. Dithiolopyrrolones were initially isolated from species of *Streptomyces* in the 1940s and from other microorganisms such as *Alteromonas rava*, *Xenorhabdus bovienii* and *Xenorhabdus nematophilus* [3,4,7,8]. In addition, *Saccharothrix algeriensis* NRRL B-24137 produces at least five dithiolopyrrolone antibiotics: thiolutin, senecioyl-pyrrothine (SEP), tigloyl-pyrrothine (TIP), isobutyryl-pyrrothine (ISP) and butanoyl-pyrrothine (BUP) [9]; structures are shown in Fig. 1.

Dithiolopyrrolone derivatives have very strong activities against a variety of Gram-positive and Gram-negative bacteria and eukaryotic microorganisms (yeasts, fungi and parasites) [1,6,9–11]. Furthermore, they have strong anticancer activity against several human cancer cell lines and are especially useful in the treatment of malignant mammary cells [6,12,13]. Conse-

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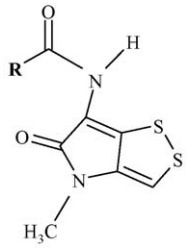

R =	CH_3	Thiolutin.
R =	$CH(CH_3)_2$	Isobutyryl-pyrrothine (ISP).
R =	$(CH_2)_2$-CH_3	Butanoyl-pyrrothine (BUP).
R =	$CH{=}C(CH_3)_2$	Senecioyl-pyrrothine (SEP).
R =	$C(CH_3){=}CH(CH_3)$	Tigloyl-pyrrothine (TIP).

Fig. 1. Structure of dithiolopyrrolone antibiotics produced by *Saccharothrix algeriensis*.

quently, these compounds may be of pharmaceutical interest as new anticancer agents [14].

The selection of appropriate carbon and nitrogen sources can have a great effect on the production levels of secondary metabolites [15]. Nutrients such as amino acids can often act as both carbon and nitrogen sources. Amino acids, as precursor, stimulated the production of secondary metabolites either by increasing the amount of a limiting precursor, or by inducing a biosynthetic enzyme (synthase) or both [16]. *Sa. algeriensis* is Gram-positive mycelial bacterium with a complex life cycle. However, little is known about this microorganism. In *Streptomyces* spp., the catabolic products of amino acids can be incorporated into the carbon skeleton of some antibiotics [17]. Precursors (from amino acid catabolism) are incorporated into the pyrrothine nucleus in the form of activated substrates (acetyl-, propionyl-, and butyryl-CoA) that lead to the different dithiolopyrrolones.

This study was initiated to identify key nutritional parameters influencing dithiolopyrrolone production. Also, the potential use of amino acids and humic acid was tested in order to get a suitable fermentation medium for dithiolopyrrolone production by *Sa. algeriensis*. The results presented here should be useful for future studies on dithiolopyrrolone biosyntheses and manufacture.

2. Materials and methods

2.1. Producing strain

Sa. algeriensis NRRL B-24137 (=DSM 44581) was used. Stock cultures were maintained at 4 °C on ISP 2 (International *Streptomyces* Project 2) medium slants composed of (per litre of distilled water): 4 g D(+) glucose (Fisher Labosi), 10 g malt extract (Difco), 4 g yeast extract (Difco) and 18 g agar (Difco). The pH of the medium was adjusted to 7 using 2N NaOH before autoclaving for 20 min at 121 °C.

2.2. Culture medium

The basal semi-synthetic medium (SSM), developed in our laboratory, was used for both pre-culture and production of antibiotics. This medium contained the following components (per litre of distilled water): 10 g D(+) glucose (Fisher Labosi), 2 g $(NH_4)_2SO_4$ (Prolabo), 2 g NaCl (Fisher Labosi), 0.5 g KH_2PO_4 (Acros), 1 g K_2HPO_4 (Acros), 0.2 g $MgSO_4{\cdot}7H_2O$ (Acros), 5 g $CaCO_3$ (Prolabo) and 2 g yeast extract (Difco). The pH of the medium was adjusted to 7 using 2N NaOH prior to autoclaving. The glucose was autoclaved separately, and added aseptically to the culture medium before inoculation. Humic acid and all amino acids (Table 1) were autoclaved separately and added at the required concentrations to the medium before inoculation.

2.3. Culture conditions

The effect of amino acids and their related compounds (at a concentration of 5 mM) and humic acid (at a concentration of 1 g/l) were investigated in SSM for dithiolopyrrolone antibiotic production during 72 h of fermentation, which is the optimal time for antibiotic production [18]. The selected amino acids (including the best one for antibiotic production and two amino acids which have a negative effect on antibiotic productions at 5 mM) and humic acid were investigated for antibiotic productions at different concentrations: 0.25, 0.5, 1 and 1.5 g/l for humic acid, and 1.25, 2.5, 5 and 7.5 mM for the selected amino acids. The pre-culture (500-ml Erlenmeyer flask containing 100 ml of the medium) was incubated for 48 h at 30 °C and 260 rpm on a rotary shaker (New Brunswick Scientific Company, New Jersey, USA). The resultant seed was then homogenized and 5 ml were used to inoculate 100 ml of the same medium in 500-ml Erlenmeyer flasks, under the same conditions. Control flasks (basal SSM medium without amino acids) were also included for comparison. All data shown represent the average of triplicate flasks.

2.4. Measurement of DCW

Culture broth samples were taken every 24 h of fermentation. For the dry cell weights (DCWs), we used the method of Pfefferle et al. [19] with slight modifications. The DCWs were determined by centrifuging (Microlitre Centrifuges, Heraeus Instruments, Biofuge) 4 ml of homogenized culture broth in pre-weighed Eppendorf tubes for 10 min at 16 000 × *g*. The pellet was washed three times with HCl (0.35N) in order to eliminate $CaCO_3$, followed by distilled water. The Eppendorf tubes containing pellet were dried at 105 °C for 24 h, cooled in a desiccator, and weighed. The results were expressed as g/l.

2.5. Dithiolopyrrolone extraction and high performance liquid chromatography (HPLC) analysis

After centrifuging the samples, the supernatant was kept for further analysis. Four ml of the supernatant were extracted with an equal volume of dichloromethane. The organic phase (containing antibiotics) was collected and dried with anhydrous sodium sulfate. The extracts were concentrated to dryness under vaccum on Rotavapor (Laborata 4000, Heidolph), dissolved in 1 ml of MeOH as crude extract. The analysis of dithiolopyrrolone antibiotics was carried out by HPLC (Bio-Tek Instruments). The equipment consisted of variable wavelength detector (UV-Vis 545V diode detector array), pump system 525, column thermostat 582 and auto-injector 465. Kroma (3000, Bio-Tek, Milan, Italy) was the data acquisition system. The analytical column used was a Zorbax SB, 150 mm × 4.6 mm Uptisphere 5 μm C-18 ODB fitted with a guard column of 10 mm × 4 mm. The samples were analyzed by linear gradient elution using acetonitrile as solvent A and double-distilled water as solvent B. The separation gradient starting with 0% solvent A and 100% solvent B, reaching 30% solvent A and 70% solvent B in 5 min, continuing from 30% to 100% solvent A in 25 min, using a flow rate of 0.8 ml/min. The column temperature was 30 °C. The injection volume was 60 μl. The detection of these antibiotics was carried out at 390 nm. In these conditions, the retention times were recorded at 11.03, 13.42, 14.02, 15.39 and 16.32 min for thiolutin, isobutyryl-pyrrothine, butanoyl-pyrrothine, tigloyl-pyrrothine and senecioyl-pyrrothine, respectively [18]. Quantification of antibiotics was performed using a thiolutin standard calibration curve. The molar extinction coefficient (ε) of thiolutin is nearly the same for all pyrrothines (ε_{390} = 8317–9333 M^{-1} cm^{-1}) as described by Lamari et al. [5]. All results presented are the mean values of three independent experiments. Data were analyzed using analysis of variance (ANOVA) and checked for significant probability ($p \leq 0.05$) level using Sigmastat® 2.03 Statistical Software.

Table 1
Effect of 5 mM concentration of different amino acid sources on growth (DCW) and dithiolopyrrolone specific production by *Saccharothrix algeriensis* in batch culture

Amino acids[a]	Dithiolopyrrolone specific production[b] (mg/g DCW)					
	DCW[c] (g/l) at 72 h	Thiolutin	ISP	BUP	SEP	TIP
Control	0.455 ± 0.100	18.52 ± 4.28	0.21 ± 0.09	0.17 ± 0.02	2.54 ± 0.48	2.65 ± 0.42
Humic acid (Fluka)	0.625 ± 0.253	336.04 ± 11.91	0.16 ± 0.09	6.62 ± 1.65	28.80 ± 5.86	21.12 ± 6.20
L-Cystine (Fluka)	0.675 ± 0.156	309.12 ± 18.13	0.04 ± 0.02	0.02 ± 0.01	1.33 ± 0.10	0.25 ± 0.15
L-Proline (Fluka)	1.150 ± 0.786	110.57 ± 23.15	0.08 ± 0.01	3.20 ± 0.08	16.16 ± 4.52	6.51 ± 3.14
L-Glutamic acid (Acros)	1.146 ± 0.333	64.71 ± 8.16	0.06 ± 0.03	1.06 ± 0.22	5.55 ± 0.98	6.50 ± 1.30
DL-Histidine (Acros)	1.173 ± 0.064	57.50 ± 18.43	0.06 ± 0.02	0.47 ± 0.25	4.49 ± 0.49	1.21 ± 0.43
L-Phenylalanine (Acros)	0.872 ± 0.217	38.92 ± 2.10	0.01 ± 0.01	0.05 ± 0.01	5.75 ± 0.65	2.68 ± 1.07
L-Lysine monohydrochlorid (Merck)	1.136 ± 0.057	37.86 ± 4.54	1.03 ± 0.15	0.89 ± 0.16	3.43 ± 0.39	4.10 ± 0.56
3,4-Dihydroxy-L-phenylalanine (Merck)	1.181 ± 0.027	26.93 ± 7.38	ND[d]	0.03 ± 0.02	0.11 ± 0.08	0.05 ± 0.03
DAP (Riedel-de-Haën)[e]	0.914 ± 0.079	26.85 ± 4.26	7.77 ± 0.68	0.99 ± 0.18	20.86 ± 4.01	18.27 ± 3.60
L-Hydroxy-proline (Merck)	0.904 ± 0.056	16.57 ± 4.33	0.02 ± 0.01	0.34 ± 0.08	1.23 ± 0.42	1.49 ± 0.50
L-Alanine (Fluka)	1.212 ± 0.102	16.53 ± 0.61	0.04 ± 0.02	0.18 ± 0.04	2.04 ± 0.26	2.64 ± 0.55
L-(−)-Tryptophan (Acros)	1.987 ± 0.181	15.65 ± 3.48	0.01 ± 0.01	0.02 ± 0.01	0.71 ± 0.18	0.52 ± 0.24
DL-Valine (Roche)	1.973 ± 0.219	11.59 ± 0.60	0.03 ± 0.01	0.19 ± 0.07	0.83 ± 0.29	1.10 ± 0.26
DL-Threonine (Roche)	1.143 ± 0.389	8.47 ± 1.75	0.02 ± 0.01	0.10 ± 0.01	3.33 ± 0.28	4.50 ± 0.62
L-Cysteine (Sigma–Aldrich)	0.578 ± 0.204	11.70 ± 2.14	0.65 ± 0.18	3.55 ± 0.51	2.40 ± 0.79	0.55 ± 0.39
L-Asparagine monohydrate (Fluka)	1.783 ± 0.125	7.70 ± 2.14	ND[d]	0.23 ± 0.01	1.03 ± 0.15	1.56 ± 0.38
L-Serine (Acros organics)	2.345 ± 0.641	4.13 ± 0.23	0.02 ± 0.01	0.05 ± 0.01	0.97 ± 0.16	0.70 ± 0.36
L-Methionine (Sigma–Aldrich)	0.550 ± 0.182	2.93 ± 0.55	0.02 ± 0.02	0.20 ± 0.02	0.51 ± 0.13	0.34 ± 0.30
DL-Ethionine (Sigma–Aldrich)	1.100 ± 0.444	2.77 ± 1.09	ND[d]	0.03 ± 0.01	0.16 ± 0.02	0.12 ± 0.05
L-Glutamine (Fluka)	2.345 ± 0.485	0.26 ± 0.06	ND[d]	0.01 ± 0.00	0.09 ± 0.04	0.08 ± 0.01
L-(+)-Arginine (Prolabo)	1.583 ± 0.520	ND[e]	ND[d]	0.02 ± 0.01	0.28 ± 0.01	0.32 ± 0.10
LSD	0.559	14.330	0.260	0.708	3.040	2.859

Specific dithiolopyrrolone production is given as mg per g of biomass at 72 h of fermentation. The amino acids are ranked in order of thiolutin production. Values are averages ± S.D. of triplicate experiments. LSD: Fisher's least significantly difference at probability level ($p < 0.05$).

[a] Each amino acid was added to the medium at a final concentration of 5 mM.

[b] Dithiolopyrrolone specific production was determined at 72 h of fermentation.

[c] Mycelial biomass is given as g of dry weight per liter of culture broth at 72 h of fermentation.

[d] Not detected.

[e] 2,6-Diaminopimelic acid.

The difference between the means was determined using LSD (Fisher's last significant difference) at a probability ($p \leq 0.05$).

3. Results

3.1. Effect of different amino acids and humic acid on growth and dithiolopyrrolone specific production

The effect of amino acids and humic acid on the dithiolopyrrolone biosyntheses was studied during batch cultivation. Fifteen common amino acids and five of their related compounds were used. All amino acids were tested at 5 mM, but humic acid was tested at 1 g/l. They were added initially to the semi-defined medium (SSM) and incubated for 72 h.

The effects of various amino acids and some of their analogues on growth of *Sa. algeriensis* and dithiolopyrrolone specific production are summarized in Table 1. Among the various amino acids, the highest value of DCW (2.345 g/l) was obtained in the presence of L-serine and L-glutamine. Furthermore, L-tryptophan, DL-valine, L-asparagine and L-arginine also stimulated cell production.

The highest values of thiolutin specific production were obtained in the presence of humic acid (336 mg/g DCW) and L-cystine (309 mg/g DCW) as compared to 18.5 mg/g DCW with control. Furthermore, the thiolutin specific production was increased about six-fold, four-fold and three-fold when L-proline, L-glutamic acid and DL-histidine, respectively, were supplemented to the medium. In contrast, the production of thiolutin was reduced by addition of the other amino acids. In the presence of L-methionine, DL-ethionine or L-glutamine, thiolutin production was strongly reduced (6–70-fold). ISP and BUP were not detected in cultures supplemented with DL-ethionine, L-methionine and L-glutamine, and only trace amounts were detected in media containing L-serine, 3,4-dihydroxyphenylalanine, L-phenylalanine, L-arginine and tryptophan. High values of ISP specific production were obtained in the presence of DAP (2,6-diaminopimelic acid) and L-lysine (7.8 and 1.0 mg/g DCW, respectively), but all other amino acids had a negative effect on this production. However, the highest value of BUP specific production was obtained in the presence of humic acid (6.6 mg/g DCW), followed by L-cysteine and L-proline (3.6 and 3.2 mg/g DCW, respectively). The maximum specific production of SEP (29 mg/g DCW) and TIP (21 mg/g DCW) were obtained with humic acid. Furthermore, we observed that the presence of DAP, L-proline and L-glutamic acid in the culture medium, also favoured the production of SEP (21, 16 and 5.6 mg/g DCW) and TIP (18, 6.5 and 6.5 mg/g DCW), respectively.

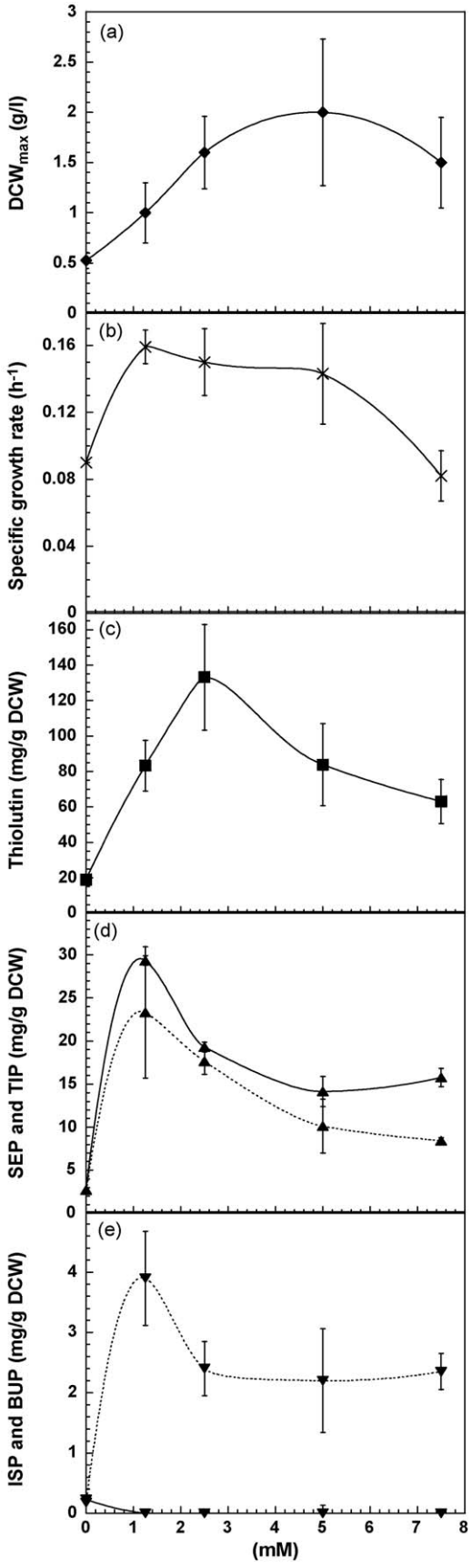

Fig. 2. Effect of initial concentration of L-proline on (a) maximal dry cell weight (♦); (b) specific growth rate (×); (c) specific production of thiolutin (■); (d) SEP (▲, continuous lines) and TIP (▲, dotted lines); (e) ISP (▼, continuous lines) and BUP (▼, dotted lines). Specific dithiolopyrrolone production is given as mg per g of biomass at the time of maximal production during 96 h of fermentation. Vertical bars denote standard deviations.

3.2. Effect of L*-proline,* L*-methionine and* DL*-ethionine concentrations on growth and dithiolopyrrolone specific production*

To study the effect of amino acid concentration, we selected from the previous experiment, L-proline, which was among the amino acids which yielded high dithiolopyrrolone production; as well as L-methionine and DL-ethionine, which exhibited the most negative effect on antibiotic production. The concentrations tested were 1.25, 2.5, 5 and 7.5 mM and the time was 96 h of fermentation.

We observed that when the concentration of L-proline ranged from 0 to 5 mM, a significant increase was observed in DCW$_{max}$ from 0.5 to 2 g/l (Fig. 2a). The maximum specific growth rate for L-proline was obtained in the presence of 1.25 mM (0.159 h^{-1}) (Fig. 2b).

The present study showed that, except for ISP production, formation of all dithiolopyrrolone was favoured by addition of L-proline (Fig. 2c–e). The maximum specific production was observed in the presence of 2.50 mM L-proline for thiolutin (133 mg/g DCW), and in the presence of 1.25 mM for SEP, TIP and BUP productions (29, 23 and 3.9 mg/g DCW, respectively).

In the presence of different concentrations of L-methionine, the DCW$_{max}$ was slightly increased. However, DCW$_{max}$ was stimulated even better in the case of DL-ethionine (1.25 g/l) (Fig. 3a). The maximum specific growth rate was obtained in the presence of 7.50 mM for DL-ethionine (0.137 h^{-1}). However, the addition of L-methionine had a negative effect on specific growth rate value (Fig. 3b). Moreover, there was still an increasing effect on specific growth rate when concentration of DL-ethionine was increased up to 7 mM (Fig. 3b).

The formation of all dithiolopyrrolones was strongly decreased with increasing concentrations of L-methionine or DL-ethionine in the culture medium (Fig. 3c–e).

3.3. Effect of humic acid concentration on growth and dithiolopyrrolone specific production

To study the effect of humic acid concentration on growth and dithiolopyrrolone specific production, we tested the concentrations of 0.25, 0.5, 1 and 1.5 g/l during 96 h fermentations. The DCW$_{max}$ was enhanced by different concentrations of humic acid and the highest value (0.89 g/l) was obtained at 1 g/l (Fig. 4a). On the other hand, the maximum specific growth rate was reduced at all concentrations of humic acid (Fig. 4b). Humic acid concentrations from 0 to 1.25 g/l stimulated dithiolopyrrolone production. Specific productions of thiolutin, SEP and TIP were strongly enhanced in the presence of 0.25 g/l humic acid (447, 39 and 36 mg/g DCW) (Fig. 4c and d). However, the maximum specific production of BUP was obtained with

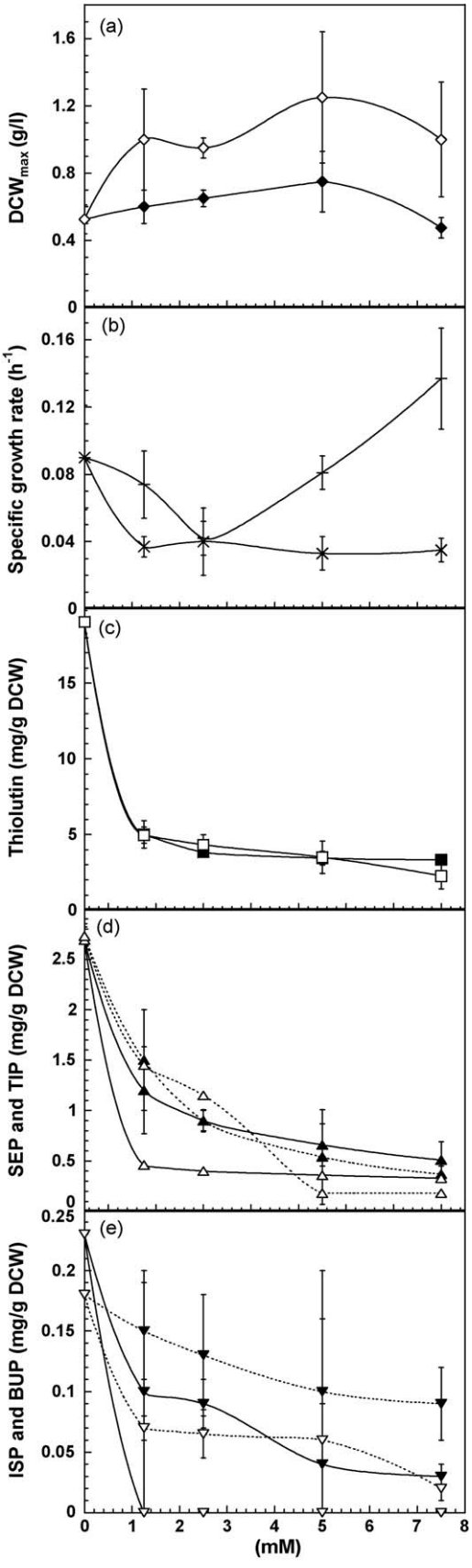

Fig. 3. Effect of initial concentrations of L-methionine (closed symbols) and DL-ethionine (open symbols) on (a) maximal dry cell weight (♦, ◊); (b) specific growth rate (×, +); (c) specific production of thiolutin (■, □); (d) specific production of SEP (▲, △, continuous lines) and TIP (▲, △, dotted lines); (e) specific production of ISP (▼, ▽, continuous lines) and BUP (▼, ▽, dotted lines). Specific dithiolopyrrolone production is given as mg per g of biomass at the time of maximal production during 96 h of fermentation. Vertical bars denote standard deviations.

0.5–1 g/l humic acid (4.7–4.8 mg/g DCW) (Fig. 4e). In contrast, different concentrations of humic acid did not affect ISP specific production (Fig. 4e). The results represented the first report about the use of humic acid for inducing dithioliopyrrolone production in microorganisms.

4. Discussion

Amino acids exhibited different patterns on growth and dithiolopyrrolone antibiotic production in *Sa. algeriensis*. Production depended upon the amino acid added and its concentration. We observed that certain amino acids inhibit, while others stimulate dithiolopyrrolone biosynthesis. Humic acid, L-cystine, L-proline and DAP exerted a major stimulatory effect on earlier steps of pyrrothine biosynthesis. Furumai et al. [20] reported that L-cystine may be a precursor of dithiolopyrrolone biosyntheses. The addition of L-cystine has been shown to increase the phenazine-1-carboxylic acid yield in *Pseudomonas fluorescens* [21].

Several natural products that have a wide range of biological activities contain pyrrole moieties. Precursor labelling studies of some of these natural products have shown that L-proline can serve as the biosynthetic precursor for these moieties, including those found in coumermycin A_1, chlorobiocin and pyoluteorin [22]. Several studies showed that the possible mechanism for the conversion of the pyrrolidine ring of L-proline to a pyrrole is by an enzymatic system [23,24]. Accordingly, L-proline may play an important role in controlling the rate of dithiolopyrrolone biosyntheses.

In the present study, L-glutamic acid, DL-histidine, L-phenylalanine and L-lysine stimulated the production of dithiolopyrrolones. Pen-Chaur et al. [25] have been reported that DL-histidine was a good source of nitrogen and/or carbon for antibiotic production of the different actinomycete strains.

The addition of L-glutamine, L-arginine or L-serine inhibited dithiolopyrrolone specific production, even though growth was not affected. This negative effect was thought to be due to intermediates generated from the catabolism of these amino acids sources interfering with enzymes in the secondary metabolism process [26], or by direct negative effects of these sources. A high rate of oxidative deamination of amino acids causes an accumulation of NH_4^+ [27]. As a consequence, the negative effect of some amino acids may be caused by a strong ammonium release from their catabolism. Formation of secondary metabolites in actinomycetes is usually delayed or reduced by an excess of a readily available nitrogen [28]. The ammonium effect might be explained by inhibition of antibiotic synthetases or, alterna-

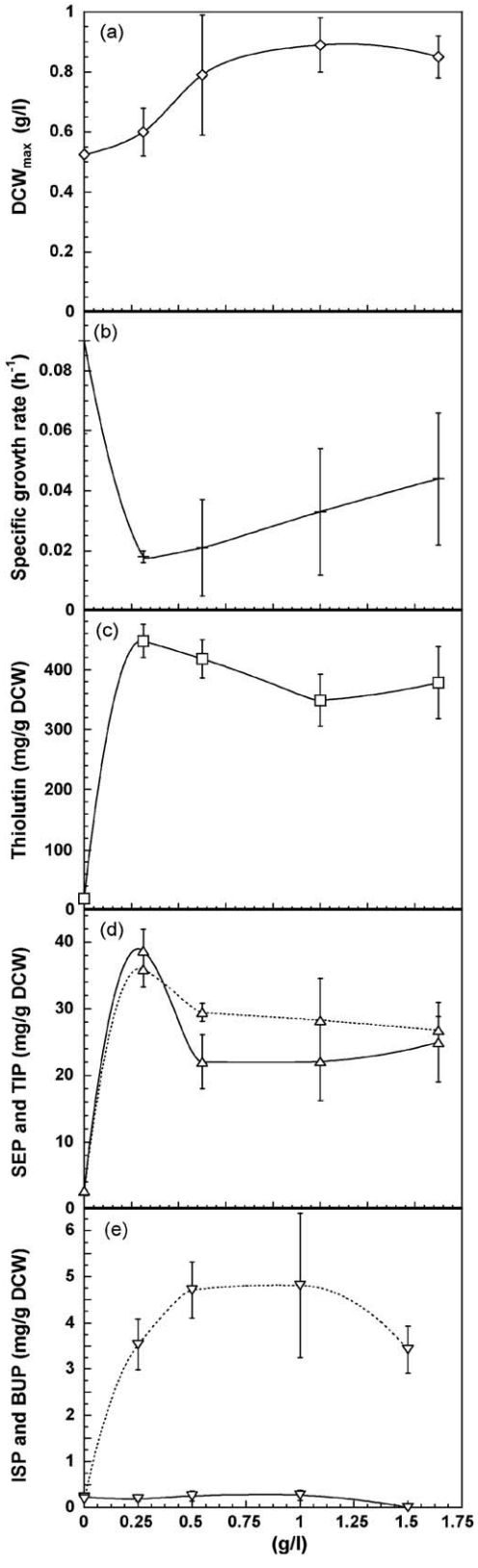

Fig. 4. Effect of initial concentration of humic acid on (a) maximal dry cell weight (◊); (b) specific growth rate (+); (c) specific production of thiolutin (□); (d) SEP (△, continuous lines) and TIP (△, dotted lines); (e) ISP (▽, continuous lines) and BUP (▽, dotted lines). Specific dithiolopyrrolone production is given as mg per g of biomass at the time of maximal production during 96 h of fermentation. Vertical bars denote standard deviations.

tively, by an effect at some other step(s) in the biosynthesis of antibiotics [29].

L-Methionine and DL-ethionine appeared to exert catabolite repression or inhibition; they supported growth but not dithiolopyrrolone production. Many studies showed the negative effect of methionine and ethionine on secondary metabolites biosynthesis such as thiolutin and aureothricin by *Streptomyces kasugaensis* [10], and ochratoxin A by *Aspergillus ochraceus* [30].

Our results showed that humic acid supported maximum production of all dithiolopyrrolones. Humic acid is a complex macromolecule consisting of an array of aromatic and aliphatic structures with sulfur-containing amino acids, amino sugars, other amino acids, peptides, fatty acids and other organic compounds [31]. Mc Carthy et al. [32] reported that during the stages of microbial decomposition, many organic compounds in humic acid were transformed by β-oxidation. Previous studies of the effect of humic acid on plant growth consistently showed positive effects on plant biomass as stimulation of root growth, germination, seeding growth and activation of enzymes [32].

In conclusion, it is suggested that humic acid, L-cystine, L-proline and DAP could serve to enhance the production of dithiolopyrrolones by *Sa. algeriensis*.

Dithiolopyrrolones have many important applications for employing them as medicaments, particularly in the treatment of human and animal cancers. The obtained results can be served for the optimization of the culture medium necessary for an industrial scale of dithiolopyrrolone productions.

Additionally, we have observed that *Sa. algeriensis* produces new dithiolopyrrolones when supplied with some amino acids (data not shown). Further studies on the regulation of dithiolopyrrolone production by *Sa. algeriensis* under different culture conditions and the characterization of the new dithiolopyrrolones are currently underway in our laboratory.

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