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# Investigations of *Saccharothrix algeriensis* Growth on Synthetic Media

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**Development of bacterial resistance to antibiotics has lead to investigations of rare bacteria, which produce new bioactive molecules. *Saccharothrix algriensis* has been isolated from the desert Maghreb. It produces dithiolopyrrolones, some of which were newly identified. In order to optimize and control production of dithiolopyrrolones, investigation regarding microorganism metabolism was required. Growth on semisynthetic medium containing 2 g·$l^{-1}$ of yeast extract was complicated because it was performed on several substrates. Moreover, because development of this bacterium on minimum medium was difficult, its composition was optimized by screening of different compounds led by yeast extract. Uracil added to the minimum medium allowed a maximum biomass production of 1.35 g·$l^{-1}$ compared to 0.32 g·$l^{-1}$ without uracil. Moreover, nonpolar amino acids and trace metal elements stimulated *Saccharothrix algeriensis* growth.**

[**Key words:** actinomycete, dithiolopyrrolone, nucleobase consumption, amino acid consumption, synthetic medium optimization, traces metal elements]

Antibiotic resistance has been increasing rapidly during the last decade (1, 2). This phenomenon is problematic, especially in a hospital environment where multiple antibiotic resistant bacteria such as *Staphyloccocus aureus* appear (3). Two main solutions to overcome this problem are: better use of antibiotics and reduction of their consumption, and identification of new bioactive molecules. In this study, *Saccharothrix algeriensis*, a bacterium belonging to the actinomycetes family and producing dithiolopyrrolone, a new class of antibiotics, was isolated from Saharan soils of Algeria (4).

These antibiotics are also known to have anticancer and anti-allergic activities (Stahl, P. *et al.*, U.S. Patent 1988-4760077, 1988 and Webster, J. M. *et al.*, U.S. patent 2000-6020360, 2000). Recently, it was found that *S. algeriensis* is able to produce at least five dithiolopyrrolone antibiotics containing *N*-acyl derivatives of 6-amino-4,5-dihydro-4-methyl-5-oxo-1,2-dithiolo [4,3-b] pyrrole: thiolutine, senecioyl-pyrrothine (SEP), tigloyl-pyrrothine (TIP), isobutyryl-pyrrothine (ISP), butanoyl-pyrrothine (BUP) and benzoyl-pyrrothine (5, 4) as shown in Fig. 1. TIP and SEP are produced only by *S. algeriensis*. The dithiolopyrrolone core consists of two 5-membered heterocycles: a dithiol cycle and a pyrrole cycle linked to a radical by nitrogen 4 and 7 (Fig. 1). The cyclical core results from the cyclization of cystine (6). Each association with radical-nucleus grants the dithiolopyrrolone different properties.

Previous studies showed the influence of the composition of culture medium on the production of derivatives. Thus, the addition of tiglic and benzoic acids multiplied the production of specific TIP and thiolutine by ninefold and at least 10-fold, respectively (7). It was also shown that the addition of different amino and organic acids can induce the synthesis of 18 new dithiolopyrrolones.

Two separate approaches exist to increase the biomolecule concentration of microbial productions. The first approach is to improve the specific production of the bioactive molecule of interest and the next is to increase the total biomass production. The first approach has already been investigated; previous studies carried out on this bacterium (8, 9) have focused on the specific production of these secondary metabolites. As antibiotic production is easily induced by the addition of various organic acids, we focused on increasing the growth of *S. algeriensis* in the present manuscript.

The present work proposes to define a synthetic-medium devoid of yeast extract (YE), to explore the development of *S. algeriensis* from a qualitative and quantitative point of view. Thus, carbohydrates, amino acids, and nucleobases were screened on minimum medium to develop a synthetic media. Moreover, the influence of trace elements was assessed.

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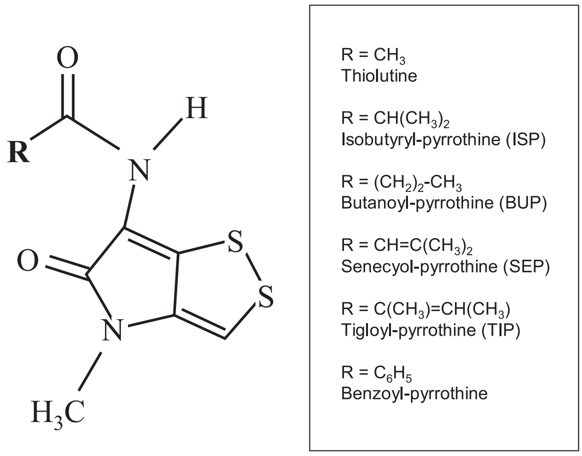


FIG. 1. Dithiolopyrrolone antibiotic structure.

## MATERIALS AND METHODS

**Microoganism and flasks culture conditions** *S. algeriensis* NRRL B-24137 was used as the study bacterium. Microbial spores were obtained from solid culture on petri dishes filled with conservation medium. They were maintained in 25% glycerol at –20°C. A 100 ml volume of semi-synthetic medium was inoculated by 3.5 ml of this suspension and incubated on a rotary shaker (New Brunswick Scientific, Edison, NJ, USA) at 250 rotation per minute (rpm) at 30°C for 52 h. Five ml of the resulting preculture was used to inoculate each culture. Before inoculation, the pH was adjusted to 7 by addition of 1 mol·$l^{-1}$ NaOH. Cultures were performed in 500 ml Erlenmeyer flasks containing 100 ml of medium under the same conditions. They were carried out in, at a minimum, duplicate for at least 200 h.

**Media composition** Sporulation and conservation medium: ISP2 had the following composition (per liter of distilled water): malt extract, 10 g; yeast extract, 4 g; glucose, 4 g; and agarose, 18 g. Minimum media buffered with $CaCO_3$ ($MM_{CaCO3}$) was used in previous studies (8). However, from a practical point of view, $CaCO_3$ complicated the determination of dry cell weight. Thus, a second minimum medium buffered with morpholinepropanesulfonic acid MOPS ($MM_{MOPS}$) was tested in place of the first one and used as a negative control in this study. $MM_{CaCO3}$ contains: glucose, 15 g; $(NH_4)_2SO_4$, 2 g; NaCl, 2 g; $KH_2PO_4$, 0.5 g; $K_2HPO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; and $CaCO_3$, 5 g; per liter of distilled water. $MM_{MOPS}$ contains: glucose, 15 g; $(NH_4)_2SO_4$, 2 g; NaCl, 2 g; $KH_2PO_4$, 0.5 g; $K_2HPO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; MOPS, 20 g; and $CaCl_2 \cdot 2H_2O$, 1 g.

A semi-synthetic medium was used as positive control. It contained (per liter of distilled water): 2 g·$l^{-1}$ of YE added to the minimum medium ($MM_{CaCO3}$ or $MM_{MOPS}$).

The culture media used to evaluate the influence of different compounds on the bacterium growth was based on the minimum medium to which the various components to be screened were added. Carbohydrates tested were glucose, sucrose, fructose, galactose, maltose, mannitol, glycerol, lactose at 0.83 mM each, and starch and dextrin at 15 g·$l^{-1}$ each. Amino acids were screened in groups. Groups 1, 2, and 3 contained amino acids at 5 mM each. Group 1 contained glycine, alanine, leucine, isoleucine, valine, tryptophan, phenylalanine, and proline. Group 2 contained tyrosine, serine, threonine cysteine, and methionine. Group 3 contained lysine, arginine, histidine, glutamate, aspartate, glutamine, and asparagine. Group 4 contained 20 amino acids at 1.7 mM each. Nucleobases (adenine, guanine, cytosine, thymine, and uracil) were evaluated individually at 20 mM each and in combination at 5 mM each. Oligoelements mix (X) was tested at three concentrations: 0.1X, 1X, and 10X. Composition of X is given in Table 1.

TABLE 1. Oligoelements mix (1X) composition

| Oligoelements composition | mix 1X g·$l^{-1}$ |
|---|---|
| $CuSO_4 \cdot 5H_2O$ | 0.02968 |
| $NiCl_2 \cdot 6H_2O$ | 0.003353 |
| $H_3BO_3$ | 0.0014 |
| $MnSO_4 \cdot H_2O$ | 0.0525 |
| $ZnSO_4 \cdot 7H_2O$ | 0.0308 |
| $CoCl_2 \cdot 6H_2O$ | 0.007 |
| $NaMoO_4 \cdot 2H_2O$ | 0.0035 |
| KI | 0.00231 |
| $AlCl_3 \cdot 6H_2O$ | 0.0028 |
| $FeSO_4 \cdot 7H_2O$ | 0.09 |

The nature and the concentration of the compounds to be tested were selected on basis of the composition of YE used in this study and on previous investigations (8, 9) conducted on *S. algeriensis*. The amino acid concentrations tested in this study are in the same range as those amino acids produced in the culture medium by 2 g·$l^{-1}$ of YE (reference no. 212720 from Difco). These choices allowed comparison of the experiments results with those of the positive control.

The effect of glucose on biomass production was evaluated in minimal and semi-synthetic conditions. Sucrose, starch, dextrin, fructose, galactose, glycerol, maltose, lactose, and mannitol were evaluated at the same molarity as glucose. Performances of amino acids, nucleobases, and trace metal element (oligoelements) were evaluated on minimal medium. Amino acids are classified into four groups based on physical and chemical properties. Group 1 contains nonpolar amino acids; group 2, amino acids with sulfur or alcohol groups; group 3, polar amino acids remaining and charged amino acids; and group 4, all amino acids.

Table 1 represents the oligoelements composition used in this study. The chemical elements can potentially produce activating or inhibitory effects on the microbial metabolism; they were added to span a wide range of concentration. For example, in addition to the trace metal solution met in literature at the concentration 1X (oligo iron 1X), the mixture diluted ten times (oligo iron 0.1X), and the mixture concentrated 10 times (oligo iron 10X) were used. Moreover, iron usually plays an enzymological role in microbial metabolism (10, 11) and was separately added (iron 1X) to the basic mixture (oligo 1X). Media were sterilized at 120°C during 20 min.

**Growth estimation** To estimate the dry cell weight (DCW), 3 ml samples of homogenized culture broth were centrifuged at 16,000×*g* for 10 min in pre-weighed Eppendorf tubes. The pellet was washed twice with distilled water; the supernatant was reserved for other analysis. Eppendorf tubes containing pellets were dried at 105°C for 48 h and cooled for 30 min in a dessicator. The relative error is 5%.

The average specific growth rate ($\mu$) is calculated from the DCW.

$$\mu = \frac{r_{DCW}}{DCW}$$

($r_{DCW}$ is the DCW evolution rate,

$r_{DCW} = \frac{\Delta DCW}{\Delta t}$. DCW is in g·$l^{-1}$ and t is in h)

**Determination of glucose concentration** A biochemical analyzer with enzymes fixed on a membrane was used (YSI 2700 select) with glucose oxidase. Glucose was determined with amperometric quantification after its enzymatic oxidation. The amperometer quantification was linear for glucose concentration ranging from