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Investigations of *Saccharothrix algeriensis* Growth on Synthetic Media

Caroline Strub,¹ Cédric Brandam,¹ Xuan Meyer,¹ and Ahmed Lebrihi^{2*}

Université de Toulouse, Laboratoire de Génie Chimique, UMR CNRS-INPT 5503, Institut National Polytechnique de Toulouse, BP 34038, 6 allée Emile Monso, 31029 Toulouse cedex 4, France¹ and Université de Toulouse, Laboratoire de Génie Chimique, Ecole Nationale Supérieure Agronomique de Toulouse, 1 avenue de l'agrobiopôle, BP 32607 Auzeville-Tolosane, F 31326 Castanet-Tolosan Cedex, France²

Development of bacterial resistance to antibiotics has lead to investigations of rare bacteria, which produce new bioactive molecules. *Saccharothrix algeriensis* 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 $2\text{ g}\cdot\text{L}^{-1}$ 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 $1.35\text{ g}\cdot\text{L}^{-1}$ compared to $0.32\text{ g}\cdot\text{L}^{-1}$ 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 *Staphylococcus 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, seneciroyl-pyrrothine (SEP), tigloyl-pyrrothine (TIP), isobutyryl-pyrrothine (ISP), butanoyl-pyrrothine (BUP) and benzoyl-pyrrothine (5, 4) as shown in Fig. 1 [Remark 5]. 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 nine-fold 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.

* Corresponding author. e-mail: lebrihi@ensat.fr
phone: +33-5-62-19-39-44 fax: +33-5-62-19-39-44

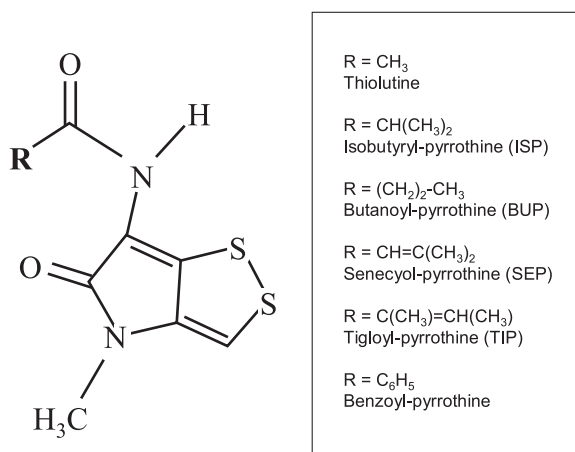


FIG. 1. Dithiopyrrolone antibiotic structure.

MATERIALS AND METHODS

Microorganism 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 CaCO₃ (MM_{CaCO3}) was used in previous studies (8). However, from a practical point of view, CaCO₃ 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; (NH₄)₂SO₄, 2 g; NaCl, 2 g; KH₂PO₄, 0.5 g; K₂HPO₄, 1 g; MgSO₄·7H₂O, 0.2 g; and CaCO₃, 5 g; per liter of distilled water. MM_{MOPS} contains: glucose, 15 g; (NH₄)₂SO₄, 2 g; NaCl, 2 g; KH₂PO₄, 0.5 g; K₂HPO₄, 1 g; MgSO₄·7H₂O, 0.2 g; MOPS, 20 g; and CaCl₂·2H₂O, 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

TABLE 1. Oligoelements mix (1X) composition

Oligoelements Composition	mix 1X g·l ⁻¹
CuSO ₄ ·5H ₂ O	0.02968
NiCl ₂ ·6H ₂ O	0.003353
H ₂ BO ₃	0.0014
MnSO ₄ ·H ₂ O	0.0525
ZnSO ₄ ·7H ₂ O	0.0308
CoCl ₂ ·6H ₂ O	0.007
NaMoO ₄ ·2H ₂ O	0.0035
KI	0.00231
AlCl ₃ ·6H ₂ O	0.0028
FeSO ₄ ·7H ₂ O	0.09

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.

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 (μ) is calculated from the DCW.

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

(r_{DCW} is the DCW evolution rate,

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

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 amperometric quantification was linear for glucose concentration ranging from

0–25 g·L⁻¹. The relative error is 3%.

Alpha amino nitrogen quantification Alpha amino nitrogen is quantified using a specific enzymatic method (Microdon kit using glutamate oxidase) and an automatic multiparametric analyzer (Mascott, Lisabio, Pouilly en Aixoie, France). The signal quantification was linear for alpha amino nitrogen concentration ranging from 0–500 mg·L⁻¹. The relative error is 5%.

RESULTS AND DISCUSSION

Calcium carbonate substitution in the composition of the culture medium CaCO₃ was initially used to buffer the culture medium, but DCW determination was complicated, and was replaced by MOPS in the culture medium composition. Calcium was then provided by CaCl₂. Evaluation of the effect of the substitution of MOPS and CaCl₂ on bacterial growth was performed. Figure 2 shows the evolution of biomass as a function of time in the two synthetic media and that after 12 h of culture, the MOPS buffered medium produced 1.48 g·L⁻¹ of biomass compared to 0.17 g·L⁻¹ by CaCO₃ buffered medium. In addition, the medium buffered with MOPS allowed a maximal production of biomass, 2.5 times higher than the biomass obtained on calcium carbonate buffered medium.

Thus, replacement of CaCO₃ by MOPS allowed growth of *S. algeriensis* in a semi-synthetic medium. Moreover, the MOPS buffered medium had a positive impact on biomass production.

Growth on semi-synthetic medium buffered with MOPS

Figure 3 illustrates the growth of *S. algeriensis* on a medium supplemented with a small quantity of YE (2 g·L⁻¹), referred to as semi-synthetic medium (79). Biomass production and glucose consumption were monitored during the 45 h. Biomass yield, biomass formed per gram of glucose consumed, is represented in the same figure.

The results indicate that the growth of the bacterium on this medium could be split into two parts: from starting point to approximately 12 h of culture and from approximately 12 h to the end. In this first period, there was a production of 1.48 g·L⁻¹ of biomass with a biomass yield of 1.1 g_{DCW}·g_{glucose}⁻¹. This yield was much higher than the maximal actinomycete biomass yield of 0.46 g_{DCW}·g_{glucose}⁻¹ referenced in literature (12). This indicates that during this period, biomass was formed mainly from a substrate other than glucose. During

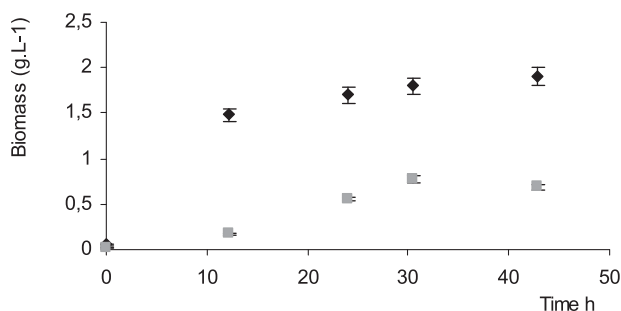


FIG. 2. Evolution of *S. algeriensis* biomass as function of time on two semi-synthetic media. One media is buffered with CaCO₃ (grey square) and the other with MOPS (closed diamond). Batch culture: 30°C, 250 rpm.

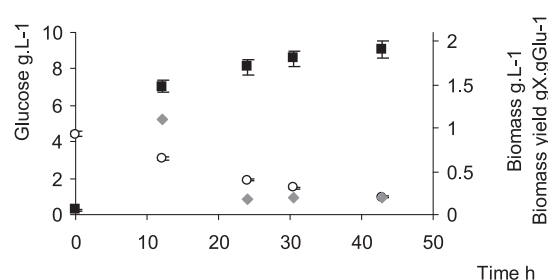


FIG. 3. Evolution of biomass production, glucose consumption, and biomass yield. Closed square, Biomass g·L⁻¹; open circle, glucose g·L⁻¹; and grey diamond, biomass yield (biomass formed per gram of glucose consumed g_X·g_{Glu}⁻¹) as a function of time on MOPS buffered semi-synthetic medium. Batch culture of *S. algeriensis*: 30°C, 250 rpm

this first step of growth, the average specific growth rate was 0.27 h⁻¹.

In the second period, the bacteria multiplied slowly. Less than 0.5 g·L⁻¹ of biomass was formed in 30.5 h. At the same time, 2.25 g·L⁻¹ glucose was consumed. The average specific growth rate was 0.007 h⁻¹, which is forty times lower than the previous period. This phase was characterized by a lower biomass yield of 0.19 g_{DCW}·g_{glucose}⁻¹. Such a result indicated that during the second period, glucose could be the only source of carbon used for the bacterial growth. In addition, this low value could indicate that the bacterium growth was limited by an unidentified element.

YE are predominantly composed of field proteins. It is likely that the initial phase of growth uses the amino acid available from the YE as carbon and nitrogen substrates. The *Saccharothrix* genus was relatively and genetically close to the *Streptomyces* genus (13). Moreover, numerous studies have discussed their capacity to catabolize amino acids (14).

Growth on minimum medium No significant growth occurred in the minimum medium. Glucose alone was not sufficient to induce *S. algeriensis* development; thus, the bacterium was missing an essential element for its growth. Yeast extract would be a source of growth factors filling an auxotrophy. Moreover, preliminary experiments have shown that glucose was indispensable for the bacterium development. Based on our results, the hypothesis that glucose was used as an energy source for the metabolism could be debated upon (15, 16).

Influence of carbohydrates on growth in the semi-synthetic and minimum media In order to determine if glucose was the best carbon source for *S. algeriensis* growth, other carbohydrates were tested both in minimal and favorable conditions at 0.83 mM. Results are presented in Fig. 4a and 4b. The specific maximal biomass [X_{max}]_{specific} was the maximum biomass produced, reported as concentration of glucose in g·L⁻¹, because the carbohydrates tested did not contain the same number of carbon atoms. Glucose, galactose, fructose, and mannitol contain 6 carbon atoms. Lactose, maltose, and sucrose contained 12 carbon atoms. Glycerol consisted of 3 atoms of carbon. Dextrin and starch were glucose polymers.

In the presence of YE (Fig. 4b), there was an average of 3.6 times more specific biomass production than in the absence of YE (Fig. 4a). Moreover, the highest level of biomass produced without YE did not reach the minimal value

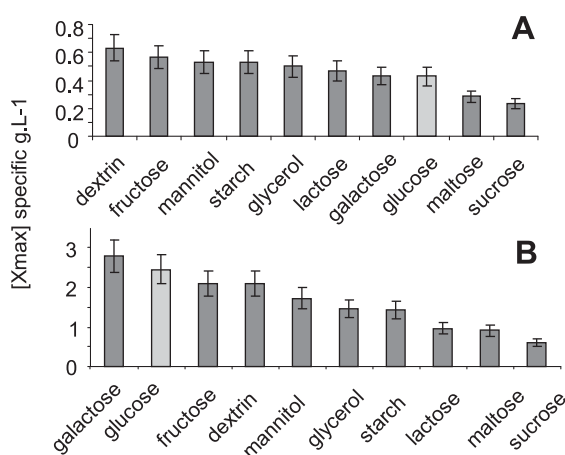


FIG. 4. Maximal specific biomass production in presence of different carbohydrates on (a) MOPS buffered minimum medium and (b) MOPS buffered semi-synthetic medium. Glucose, galactose, fructose, lactose, maltose, sucrose, and glycerol (83 mM). Dextrin and starch (15 g·L⁻¹). Batch culture of *S. algeriensis*, 30°C, 250 rpm.

of biomass produced with YE. The performance of various carbohydrates on the specific production of biomass was different depending upon the presence or absence of YE (Fig. 4a, b). We noted that in presence of YE, carbohydrates that contain 6 carbons produced the best specific production while those containing 12 carbons induced the lowest specific production. In the absence of YE, maltose and sucrose, which are dioses, gave the lowest specific productions.

When the bacterium was in presence of YE that was in adequate nutritional conditions for development, glucose produced the second best specific production of biomass. The carbohydrate responsible for higher biomass, galactose, was not a classic sugar; therefore, glucose was used as the carbon substrate residue for the result of this work.

Growth on synthetic medium In order to detect this potential auxotrophy, the possible candidates exhibiting it and that may be potentially present in yeast extract have been listed. Thus, amino acids, nucleobases, and trace elements have been screened on minimum medium. The results of these investigations are presented below.

Effect of amino acids on growth As shown in Fig. 5a, the four groups of amino acids allowed for a better growth than the negative control, but no group equaled the values of biomass produced by the positive control. From a quantitative point of view, group 1 led to the best biomass production, 1.2 g·L⁻¹ in 150 h, groups 3 and 2 resulted in 1.18 g·L⁻¹ in 100 h and 1.05 g·L⁻¹ in 150 h, respectively.

The amino acid group 3 led to an earlier development of the microorganism than the other amino acids, but biomass production in group 3 remained constant after 100 h of culture, unlike the other groups. The amino acids used together (group 4) produced earlier growth than the cluster groups of amino acids (groups 1, 2, and 3). The cluster groups had a positive synergistic effect on initiation of growth; however, this growth stopped rapidly. This may have occurred because one of the amino acids was rapidly depleted. Observation of alpha-amino nitrogen evolution (Fig. 5b) in culture broth demonstrated the consumption of amino acids by bacteria in synthetic medium. Several authors have already em-

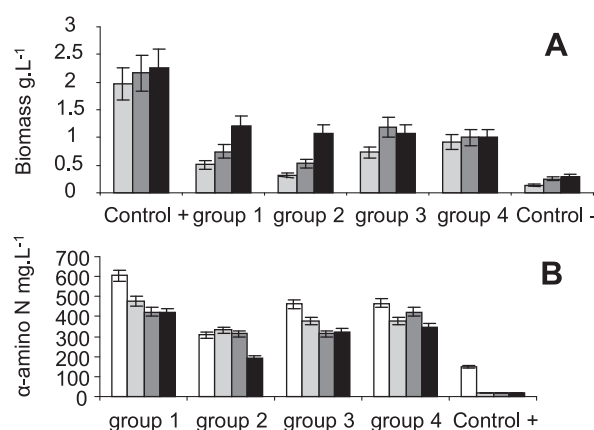


FIG. 5. (a) Biomass production and (b) ammonium ions consumption at different time points: 0 (open bars, only for panel b), 50 (grey bars), 100 (dark grey bars), and 150 (closed bars) h of culture, as a function of amino acid groups. Positive control contains MM_{MOPS} medium + 2 g·L⁻¹ of YE. Negative control contains only MM_{MOPS} medium. Groups 1, 2, 3, and 4 are prepared as explained in Materials and Methods. Batch culture of *S. algeriensis*: 30°C, 250 rpm.

phasized the ability of *Streptomyces* to metabolize amino acids (14). They are either incorporated directly into protein synthesis or de-aminated or used as a source of carbon in the Krebs cycle (17). Because the concentration of alpha-amino nitrogen was not null, it was not a limiting factor as a whole. However, it is still possible that specific amino acids were growth limiting. Specific amino acids may have been consumed at different rates, resulting in depletion of one amino acid while metabolism of others continued.

The observation of alpha-amino nitrogen evolution in the positive control showed that the amino acids from YE were consumed in the first 50 h of culture. In the semi-synthetic medium, the first growth phase occurred on the amino acids from the YE, which was confirmed as stated above. Amino acids belonging to the groups 1 and 3 were rapidly consumed. By contrast, amino acids from group 2 were consumed later, after 100 h of culture. The sulfured amino acid and those with a hydroxyl group were assimilated with more difficulty than nonpolar and charged amino acids by the bacteria. Group 3 could contain one or several important amino acids for the bacterial growth because, in this group, when there was no amino acids consumption left, biomass production stopped. Moreover, the amino acid consumption of the group 1 stagnated while growth continued. In this case, the hydrophobic amino acids would facilitate the formation of biomass from glucose after 100 h.

Evolution of these different cultures made it difficult to determine a specific auxotrophy, or a multi-auxotrophy because amino acids tested as a single group did not induce growth similar to the positive control (see amino acids together group 4). However, the amino acids did produce a positive effect on biomass production by *S. algeriensis*.

Effect of nucleobases on growth Figure 6 shows the performance of the 5 nucleobases (adenine, thymine, cytosine, guanine, and uracil) on the maximum production of biomass at different times of culture (100, 150, and 200 h). Nucleobases were tested separately at 20 mM each and together at 5 mM each.

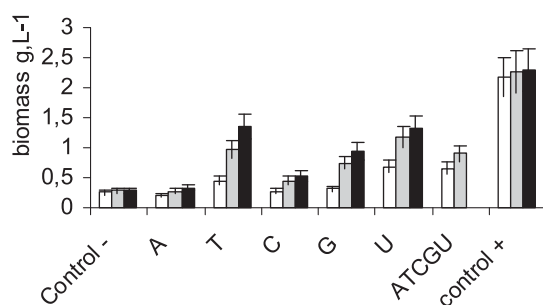


FIG. 6. Biomass production at different times: 100 (white), 150 (grey), and 200 (black) h of culture, as a function of nucleobases. Nucleobases were tested alone at 20 mM, adenine (A), thymine (T), cytosine (C), guanine (G), and uracil (U) and together (ATCGU) at 5 mM each. Positive control contains MM_{MOPS} medium + 2 g·l⁻¹ of YE. Negative control contains only MM_{MOPS} medium. Batch culture of *S. algeriensis*: 30°C, 250 rpm.

Figure 6 shows that uracil and thymine at 20 mM allowed production of biomass, which was higher than the negative control and other nucleobases. Thus, 1.35 and 1.33 g·l⁻¹ of biomass were produced in 200 h in the presence of uracil and thymine, respectively. These values remained encouraging but were 40% lower than the amounts of biomass produced by positive culture control: 2.17, 2.27, and 2.3 g·l⁻¹ in 100, 150, and 200 h, respectively. The growth on uracil took place with less latency than growth on thymine. Adenine, guanine, and cystine induced similar or better production of biomass compared to our negative control, with biomass concentrations of 0.215, 0.315, 0.275, and 0.260 g·l⁻¹ in 100 h, 0.28, 0.75, 0.46, and 0.29 g·l⁻¹ in 150 h and 0.33, 0.95, 0.56, and 0.28 g·l⁻¹ in 200 h, respectively.

Although these nucleobases had a positive effect on the amount of biomass produced, they induced later growth than the positive culture control. In effect, between 100–200 h, the amount of biomass increased by only 10% of the growth increase seen in the positive control culture; biomass production was increased three- with addition of thymine and two-fold with uracil. It should be noted that nucleobases used together at 5 mM each did not show a positive effect on maximal produced biomass but did induce earlier growth: 0.65 and 0.9 g·l⁻¹ of biomass were produced from this last culture in 100 and 150 h, respectively.

The positive effect of nucleobases such as uracil on bacterial growth has been described in the literature (18). Moreover, nucleobases could be a carbon source for *S. algeriensis* because *Streptomyces*, which are genetically close to *Saccharothrix*, are able to catabolize nucleobases (14). It is not surprising that these two nucleobases (uracil and thymine) provide similar results. Uracil is the nucleobase that replaces thymine in RNA. They have a similar structure, with the difference of an additional methyl group at position 5 of thymine.

Effect of mineral solution on growth Preliminary experiments identified molecules that have a positive effect on growth but did not allow identification of a specific auxotroph. Effect of trace metal elements on growth has been estimated. An element mixture borrowed from the literature (19, 20) was added in the minimum culture medium as described in Materials and Methods.

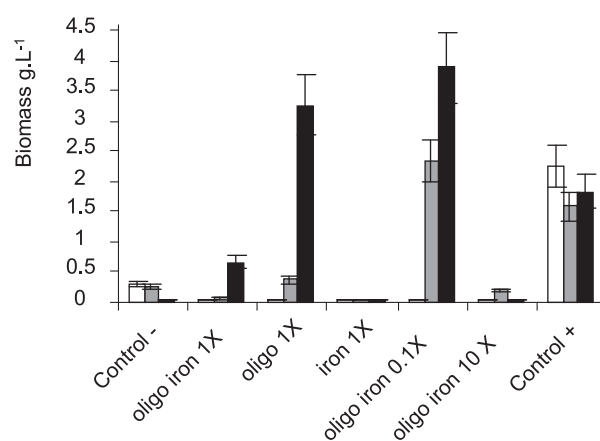


FIG. 7. Biomass production at different times: 150 (open bars), 260 (grey bars), and 330 (black bars) h of culture, as a function of oligo-elements mix. The mixture is tested at 3 concentrations (Table 1): oligo iron 1X, 10 times lower (oligo iron 0.1X), and 10 times higher (oligo iron 10X). The iron is tested in association with the mixture and separately (iron). The mixture is evaluated without iron (oligo 1X). Controls: MM_{MOPS} medium (negative control) and MM_{MOPS} medium + 2 g·l⁻¹ YE (positive control). Batch culture of *S. algeriensis*: 30°C, 250 rpm.

The following figure presented DCW at various time points: 150, 260, and 330 h according to the various trace metal element mixtures added. The oligo iron 1X, oligo 1X, iron 1X, oligo iron 0.1X, and oligo iron 10X mix allowed maximum biomass production of 0.67, 3.27, 0.04, 3.9 and 0.2 g·l⁻¹, respectively. The mixtures of oligo 1X and oligo iron 0.1X induced growth higher than that of the positive control. We noted that in 330 h of cultures, biomass decreased in the negative control, possibly due to bacterial cell lysis.

In all cases, the development of the bacterium occurred after more than 150 h of culture. Such latency could be explained by the composition of the mixtures. Each oligo mix contained more than 10 micronutrients, e.g., some may have had a stimulatory effect, while others may have been inhibitory. Thus, this complex and competing regulation could explain the long latency of more than 150 h.

The addition of iron only induced low growth (0.04 g·l⁻¹ biomass in 330 h) and this element associated with the standard mixture (oligo iron 1X) allowed a biomass production of almost five times lower than that seen in the presence of oligoelements mixed alone without iron (oligo 1X). In our conditions, it seemed that the iron had an inhibiting effect on *S. algeriensis* growth.

The performances, in term of biomass production, were inversely proportional to the trace metal concentrations: oligo iron 10X allowed a production of 0.2 g·l⁻¹, oligo iron 1X produced 0.67 g·l⁻¹, and oligo iron 0.1X produced 3.9 g·l⁻¹. This would emphasize a strong inhibitor trait of one (or more) of the oligoelements mixture component.

Thiolutine specific production on synthetic medium: key-data A synthetic medium containing uracil at 20 mM allowed a specific production of 2.3 mg of thiolutine per g of biomass. The oligoelements mixture (without iron) permits a specific production of 2.1 mg of thiolutine per g of biomass. Galactose and maltose used in synthetic medium

in place of glucose enable a specific production of 8.3 and 7.2 mg of thiolutine per g of biomass, respectively.

Adding benzoic acid (at 5 mM) to the mixture of the 5 nucleobases (at 5 mM each), in a culture medium devoid of yeast extract, allows a specific production of 32.3 mg of thiolutine per g of biomass. This data shows the possibility of thiolutine production by *S. algeriensis* in synthetic medium. Moreover, this specific production is improved by the addition of benzoic acid.

Nucleobases, amino acids, and oligoelements stimulated *S. algeriensis* development on synthetic media, particularly uracil and thymine nucleobases and non polar amino acids. Low concentrations of oligoelements allowed bacterial growth. No auxotrophy was detected. Two assumptions are made from these results: an auxotrophy or multi-auxotrophy target has not been discovered yet, or there is no auxotrophy and optimization of the synthetic medium that is not complete. In the state of current knowledge, we suggest a minimum medium containing 20 mM of uracil for the development of *S. algeriensis* in synthetic conditions.

This research has helped develop a synthetic basis media which facilitates the growth of *S. algeriensis*. Although these media permit a certain bacterial growth, biomass production could still be improved. However, the media determined in the present experiment could be used as tracks for the development of a nutritionally matched synthetic medium allowing for batch cultivation of the strain. Thus, these different medium compositions could be a starting point for accurate and quantitative studies on the metabolism and induction of dithiolopyrrolones production in *S. algeriensis*.

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