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PRELIMINARY NOTE

THE ROLE OF PHOSPHATE ON THE PRODUCTION OF NUCLEIC ACID-RELATED SUBSTANCES BY *ASPERGILLUS NIDULANS*

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ABSTRACT: A chemically defined medium was developed for the nucleic acid-related substances accumulation by Aspergillus nidulans. *The production level is dependent on the medium composition where phosphate concentration is the most important factor involved in increasing the production of substances. The accumulation of substances in the growth medium is a process closely related to the microbial activity in all growth phases.*

KEYWORDS: Aspergillus nidulans; UV-absorbing substances; phosphate.

INTRODUCTION

An intensive study was made on the fermentation production of nucleotides, nucleosides, bases and related substances by microorganisms[1,13]. The object of the present study was to discover the nutritional requeriments and condition of cultivation for microbial growth, production, and accumulation of nucleic acid-related substances through *A. nidulans*.

MATERIAL AND METHODS

Basal Medium

The composition of the basic chemically defined medium is as follows: sucrose – 50 g; $(NH_4)_2SO_4$ – 3.96 g (30 mM); K_2HPO_4 – 1.306 g (7.5 mM) and $MgSO_4.7H_2O$ – 0.2 g. Distilled water was added to give 1 liter of medium. After autoclave sterilization (20 minutes at 121°C), the pH was adjusted to 6.0-7.0.

Fermentation

The microorganism was cultivated on a rotary shaker at 250 rpm in revolving cycles of 3 cm diameter and kept in a constant temperature room at 30° C, in 125 ml Erlenmeyer

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flasks containing 30 ml of fermentation medium inoculated with 1.0 ml of inoculum culture. The flasks were closed with 1 cm thick polyurethane foam covers. All experiments were made using duplicate pairs of flasks for each medium or condition studied.

Analytical Methods

The production of the nucleic acid-related substances (S_{260}) was measured by the average corrected 260 nm optical density of the culture filtrate flask pairs (A_{260}). The correction included the sterilized media blank absorption and the fermented volume corrected to the initial volume (30 ml).

One unit of material (S_{260}) was defined as the quantity per milliliter of material needed to give absorption of 1.0 at 260 nm in 1 cm optical path cells, similar to that proposed by BENDICH[14].

The microorganism growth was measured by the average dry weight of the distilled water washed mycelium, dried at 105°C for 15 hours and expressed as miligrams per milliliters referred to the initial medium volume. When the media contained calcium carbonate the mycelia were washed with perchloric acid 0.1 M before washing with water, to remove the possible unreacted portion of that solid.

RESULTS AND DISCUSSION

Using sucrose as a carbon source, several assimilable nitrogen compounds were suitable for both growth and substances accumulation (Table 1), among which were urea, potassium nitrate, potassium L-aspartate and ammonium sulfate plus potassium L-aspartate.

The S_{260} production level is dependent on the medium composition where phosphate concentration seems to be the most important factor involved in increasing the production of substances (Table 2). Low phosphate levels increases the material concentration, suggesting that the microorganism is secreting, in the culture medium, some of the biosynthesized nucleic acid precursors, due to the lack of enough phosphate to produce the nucleosides triphosphates necessary for nucleic acid synthesis. A 4.5 mM concentration of phosphate is the most efficient for maximum accumulation.

Fermentation conditions were studied, such as the influence of the nutrient medium volume or the inoculum volume and fermentation time. Higher S_{260} productions are obtained using 125 ml Erlenmeyer flasks, containing 40 ml of fermentation medium inoculated with 1 ml of inoculum culture, in experiments involving 264 hours fermentation (250 rpm; 30°C).

The accumulation of substances in the growth medium is a process closely related to the microbial activity in all growth phases (Figure 1), and proves to be an active metabolic deviation, not a process linked to the degradation of pre-formed nucleic acid and the decline phase. In the present work this deviation was nutritionally manipulated with the object of attaining an adequate culture medium for maximum accumulation of the secreted material.

Preliminary chromatographic analysis of the culture filtrate, made by classical methods[15,18], indicated, similar to a previous paper[1], the presence of nucleic acid-related substances in the complex composition, as will be shown in a forthcoming paper.

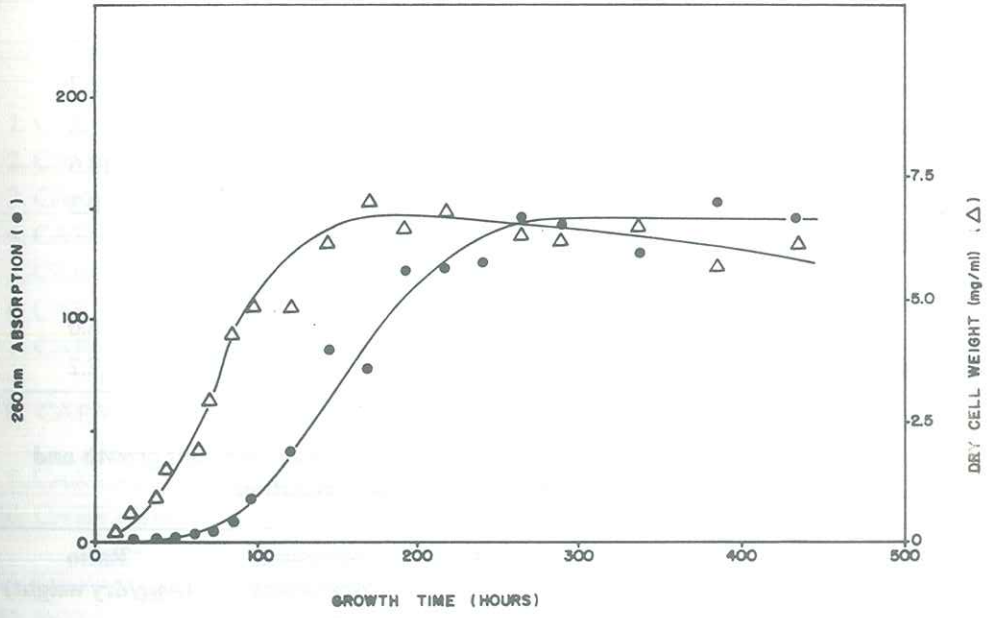

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TABLE 1 – The influence of the nitrogen source on *Aspergillus nidulans* growth and nucleic acid-related substances accumulation in the basal medium

Nitrogen source and concentration	Final pH	Filtrate A_{260}	Mycelium dry weight (mg/ml)
Urea (30 mM)	3.0	58.0	8.2
Potassium nitrate (60 mM)	3.0	42.2	8.5
Potassium L-aspartate (60 mM)	4.5	27.1	13.1
Ammonium sulfate (15 mM) plus Potassium L-aspartate (30 mM)	3.5	11.2	16.4
Glycine (60 mM)	4.7	8.2	12.7
Ammonium sulfate (30 mM)(basal medium)	2.1	7.1	15.8
Gelatin (5 g/l)	3.5	4.2	10.6
None	5.8	3.5	0.3
Ammonium sulfate (30 mM) [medium "buffered" with calcium carbonate (4.0 g/l)]	3.6	3.0	8.0
Ethanolamine (60 mM)	4.7	0.8	2.2

TABLE 2 – Effect of phosphate concentration on *Aspergillus nidulans* growth and nucleic acid-related substances accumulation

K_2HPO_4 (mM)	K_2SO_4 (mM)	Final pH	Filtrate A_{260}	Mycelium dry weight (mg/ml)	Ratio (A_{260}/dry weight)
0.0	10.0	4.0	1.0	7.6	0.1
0.5	9.5	3.2	33.7	7.0	4.8
1.5	8.5	3.3	49.9	7.0	7.1
3.0	7.0	3.3	60.9	6.4	9.5
4.5	5.5	3.5	70.7	7.2	9.8
6.0	4.0	3.5	63.3	7.0	9.1
7.5	2.5	3.4	55.1	6.6	8.3
9.0	1.0	3.5	41.5	7.0	5.9
10.0	0.0	3.7	22.1	7.6	2.9

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RESUMO: Desenvolveu-se meio de cultivo, quimicamente definido, para a produção de substâncias relacionadas a ácidos nucléicos por Aspergillus nidulans. A produção do material depende da composição do meio e a concentração de fosfato é o fator mais importante envolvido no aumento da produção das substâncias. O acúmulo do material no meio de cultura é um processo diretamente relacionado à atividade microbiana em todas as fases do crescimento.

UNITERMOS: Aspergillus nidulans; substâncias que absorvem no ultravioleta; fosfato.

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