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Evaluation of Culture Conditions that Minimize 3-nitropropionic Acid Production by *Arthrinium* Strains

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Abstract: The present study determined the best conditions (parameters such as initial pH of the culture medium, culture system, time and temperature of incubation) for strains of moulds of the genus *Arthrinium* to produce secondary metabolites with antibiotic capacity, avoiding 3-nitropropionic acid (3-NPA) production, due to its neurotoxicity. It was observed that the desirable conditions for these strains to get our aim were: initial pH of the culture medium: 5, colonies developed under static conditions, incubation at 28°C and during 14 and 21 days.

Key words: 3-nitropropionic acid, *Arthrinium*, culture conditions

INTRODUCTION

Arthrinium strains are characterized by their ability of producing and storing secondary metabolites with antibiotic capacity[1-7]. However, some other studies have shown 3-nitropropionic acid (3-NPA) production by some strains of this genus, known to be able of producing serious neurological damage on humans[6].

The main objective of this study was to evaluate the possible influence of incubation conditions as well as culture media, on *Arthrinium* strains, just to determine which are the suitable conditions that allow 3-NPA elaboration and storing by these strains, so that it is possible to establish the optimum culture conditions to produce secondary metabolites with antibiotic capacity, without 3-NPA co-production.

MATERIALS AND METHODS

The *Arthrinium* strains chosen for this study were *Arthrinium aureum* FVB 345 and *Arthrinium phaeospermum* FVB 768 (FVB = Facultat de Veterinària Bellaterra - Barcelona). These strains were cultivated on Sabouraud Dextrose Agar during eight weeks at 28°C. Then, 8 mm diameter culture disks were taken under sterility conditions and were placed on flasks containing Yeast Extract Broth 2%. After inoculation, flasks were distributed into different groups with the aim of studying the influence of the following factors:

- pH: 5 or 7
- Temperature: 24 or 28°C
- Culture system: Static or Agitation (150 rpm)
- Samples from the different cultures were taken at 7, 14, 21 and 28 days.

All the assays were performed in triplicate. Detection of 3-NPA production was performed using Thin Layer Chromatography (TLC) according to the detection procedures described by Wei *et al.*[8]

RESULTS AND DISCUSSION

The results due to pH modifications registered throughout the study under the different conditions, as well as production of pigments into the medium are shown in Table 1 and 2.

Table 3 shows variations on 3-NPA production. When no production was observed, it was marked with the sign "-" whereas 3-NPA production was marked with the sign "+" when low levels were detected or "++" in case that levels were higher.

With the results obtained we may assess that when cultures are obtained under agitation conditions, a higher and a more homogeneous development of the fungal colonies are reached, when comparing with those cultures obtained in a static system.

In addition all the growth conditions evaluated in this study, after fungal development, it was observed that the culture medium pH had decreased, acquiring values from 2 up to 4 (Table 1).

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Table 1: Variation of final pH of the culture medium according to the different culture conditions

	pH 5												pH 7											
	Agitation						Static						Agitation						Static					
	24°C			28°C			24°C			28°C			24°C			28°C			24°C			28°C		
Days	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7	2	5	2	2	5	2	3	5	3	3	5	3	2	7	2	3	7	3	3	7	3	2	7	3
14	2	5	3	3	5	3	3	5	4	3	5	3	2	7	2	4	7	5	3	7	4	3	7	4
21	3	5	2	3	5	4	3	5	4	4	5	4	4	7	2	4	7	4	4	7	5	4	7	5
28	4	5	3	4	5	4	4	5	4	5	5	5	4	7	3	4	7	5	4	7	5	5	7	6

A: *Arthrinium aureum*, B: Control, C: *Arthrinium phaeospermum*

Table 2: Variation of pigment production into the medium

	pH 5												pH 7											
	Agitation						Static						Agitation						Static					
	24°C			28°C			24°C			28°C			24°C			28°C			24°C			28°C		
Days	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7	+	-	++	-	-	+	+	-	++	+	-	++	++	-	++	+	-	+	++	-	+	+	-	++
14	++	-	+++	+	-	+	++	-	+++	++	-	++	+++	-	++	+	-	+	+	-	+	+	-	++
21	+	-	+	+	-	+	+	-	+++	+	-	++	++	-	++	+	-	+	+	-	+	+	-	++
28	+	-	++	+	-	+	+	-	+++	+	-	++	++	-	+++	+	-	++	+	-	++	++	-	+++

A: *Arthrinium aureum*, B: Control, C: *Arthrinium phaeospermum*
-: No pigment production, +: Low pigment production, ++: Moderate pigment production, +++: High pigment production

Table 3: Variation of 3-nitropropionic acid (3-NPA) production

	pH 5												pH 7											
	Agitation						Static						Agitation						Static					
	24°C			28°C			24°C			28°C			24°C			28°C			24°C			28°C		
Days	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
7	+	-	+	+	-	+	+	-	-	+	-	+	++	-	++	++	-	+	+	-	+	-	-	+
14	+	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	-	+
21	++	-	++	++	-	+	++	-	+	-	-	-	++	-	+	++	-	+	+	-	++	+	-	+
28	+	-	+	+	-	-	+	-	+	+	-	-	++	-	+	+	-	-	+	-	+	+	-	+

A: *Arthrinium aureum*, B: Control, C: *Arthrinium phaeospermum*, -: No 3-NPA production, +: Moderate 3-NPA production, ++: High 3-NPA production

It was also observed that initial culture conditions have influence on 3-NPA production, so that it can be indicated that the maximum production takes place in cultures under agitation conditions after 21 days of development (Table 3) and at the same time, the strain *Arthrinium aureum* FVB 345 produces more 3-NPA than the strain *Arthrinium phaeospermum* FVB 768.

Having into account all these data, the culture conditions that seem to be optimum in order to avoid 3-NPA production, according to the studied strains are: Initial pH of the culture medium: 5, static cultures, incubation at 28°C and incubation period of 14 and 21 days.

The results obtained in this study do not coincide with another study[8] that pointed that if the cultures grow under agitation conditions (120 rpm), lower 3-NPA concentrations are produced when comparing to static cultures. Another difference is that in their study no coincidence was found with initial pH conditions of the culture medium.

In previous studies[4,5] it has been observed that the maximum production of inhibitory substances by the strains *Artrinium aureum* FVB 345 and *Arthrinium phaeospermum* FVB 768 against the microorganisms assayed, was reached in static cultures at 28°C during 7 days, which are the conditions that prevent cultures from producing and storing 3-NPA (Table 3).

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