An alternative to CREA composition of the medium used in the Aspergillus taxonomy

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Abstract

Background: Aspergillus spp. is an important cause of food degradation and biodeterioration, in addition to being pathogenic to animals. Moreover, some species are used in biotechnology and can produce mycotoxins. This paper reports the use of CREA compounds combined with three alternative pH indicators for the Aspergillus systematics. CREA was added to the basal medium with different pH indicator combinations (bromocresol green and bromothymol blue).

Results: Among the 16 Aspergillus isolates, no difference was observed in the three conditions tested in CREA medium, using different pH indicators. A. ostianus, A. sydowii and Aspergillus sp showed good growth and sporulation. Aspergillus niger 1 and A. niger 2 reacted within three days of growth. As for A. carbonarius and A. nidulans, they reacted in 5 days. Additionally, Aspergillus sp showed a delayed reaction on the tenth day. When considering the intensity relation of the reaction among the five positive isolates, A. niger 1 and A. niger 2 presented a larger and distinct zone around the colony.

Conclusion: Bromocresol green and bromothymol blue are easy to prepare, and they are recommended for diagnostic purposes in Aspergillus, specially the Nigri section.

Introduction

The genus Aspergillus contains approximately 250 species. Some of these species have been used for many years in the fermentation industry for the production of organic acids and, more recently, for the production of a variety of enzymes and other secondary metabolites. On the other hand, some Aspergillus species produce mycotoxins that cause adverse effects in animal and human health. Further descriptions of new species have been published, raising their total number to approximately 250. Fungal identification has been performed mostly according to the morphological criteria. Even though several physiological tests have been proposed for filamentous fungi, they are still used mostly in the classification and identification of bacteria and yeast. However, creatine sucrose agar (CREA), containing a pH indicator, was developed as a differential medium for Penicillium subgenus Penicillium, and modified for use in the Aspergillus and Penicillium taxonomy.

The reactions observed in the medium are consistent and presented great results as another tool for the black Aspergillus taxonomy. pH indicators are frequently weak acids or bases. When added to a solution, pH indicators bind to H+ or OH-. This association causes a change in the electronic configuration of indicators, thus changing their color. This paper reports the use of CREA compounds with three alternative pH indicators for Aspergillus systematics.

Materials and Methods

Strains

All fungal strains were obtained based on the literature and research available at the Laboratório de Micologia da Núcleo de Pesquisa Micológica e Micotoxicológica do Departamento de Microbiologia e Imunologia Veterinária, Projeto Sanidade Animal/Embrapa/UFRRJ. The Aspergillus spp. identification was based on the morphological taxonomy, performed according to the colony diameter, conidial color, exudates, mycelium texture, soluble pigment, excretions, shape and size of conidiophores, vesicles, metulae, phialides, conidia, in addition to their texture and color, observing the identification system for the Aspergillus genus.

Media

The creatine sucrose agar consisted of bromocresol purple, 0.05 g L⁻¹; agar, 15 g; mineral solution, 10 mL L⁻¹; trace metal solution, 1 mL L⁻¹. The mineral solution consisted of (g/100 mL): KCl, 5; MgSO₄.7H₂O, 5; FeSO₄.7H₂O, 0.1. The trace metal solution consisted of (g/100 mL): ZnSO₄.7H₂O, 1; CuSO₄.5H₂O, 0.5; the final pH was 8.0, adjusted after the medium was autoclaved.

A different combination of pH indicator (bromocresol green and bromothymol blue) was added to the basal medium. Twelve
isolates of Aspergillus were tested in the CREA compound containing three different pH indicators, and then they were compared to all media mentioned above. A hemocytometer was used to count mould spores. Spore suspensions were adjusted by serial dilutions of NaCl (0.85 g/100 mL) plus Tween 80 (0.1 %), containing approximately 10^6 spores/mL. The culture duplicates consisted of three inoculated points, incubated at 25°C in the dark, being examined after 3, 5 and 10 days.

Results and Discussion

In this study, the growth abilities of Aspergillus strains were tested in CREA medium (Table 1). CREA is useful in the classification of various fungal cultures. Colonial growth characteristics, acid production (medium color change from purple to yellow) and base production can be used as diagnostic features in CREA.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Growth</th>
<th>Acid Production</th>
<th>Base Production</th>
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<tbody>
<tr>
<td>A. flavus</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. niger</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>A. carbonarius</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. terreus</td>
<td>+</td>
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</tr>
</tbody>
</table>

Table 1: Growth, acid and base production by Aspergillus on CREA with three different pH indicators.

Growth: ++, good sporulation; + little sporulation; +/- limited growth; Acid production: ++, a distinct yellow zone around the colony; +, a small yellow zone around the colony; -, no yellow colours in the medium.

Among 16 Aspergillus isolates, no difference was observed in the three conditions tested in CREA medium, containing different pH indicators. The reaction time, in days, was identical for those with positive Aspergillus, as well as the intention of reaction. Among the 16 isolates, A. flavipes developed a very limited profile, displaying other fungi such as Penicillium and Aspergillus.

According to Frisvad, this characteristic is due to the presence of N-sources in the medium, since A. ostianus, A. sydowii and Aspergillus sp showed good growth and sporulation. Aspergillus niger 1 and A. niger 2 presented reactions within three days of growth. A. carbonarius and A. nidulans presented reactions within 5 days. Aspergillus sp showed an overall delayed reaction on the tenth day. When considering the intensity relation of the reaction among the five positive isolates, A. niger 1 and A. niger 2 presented a larger and distinct zone around the colony (Table 1), and this feature was cited by Frisvad.

For diagnostic purposes, both pH indicators (bromocresol green and bromothymol blue) can be used to replace bromocresol purple. Bromocresol green and bromothymol blue are easy to prepare, and they are recommended for diagnostic purposes in Aspergillus, specially the Nigri section.

References
