Boron in aqueous extracts of non-nutrient agars

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Summary Boron was observed to be an easily extractable mineral constituent of five different proprietary sources of standard non-nutrient agars. The quantity of boron extracted from each agar exceeded that considered to be inhibitory to normal plant growth.

Introduction

A requirement for boron for the in vitro germination of numerous species of angiosperm pollen has been well established. Five different proprietary sources of standard non-nutrient agars have been observed to induce the in vitro germination of Impatiens holstii pollen in the complete absence of any exogenously supplied boron which led to the assumption that such germination occurred under boron-free conditions. This assumption has subsequently been found to be incorrect as evidence is presented in this communication which demonstrates the presence of easily extractable boron from five different proprietary sources of standard non-nutrient agars.

Materials and methods

Two grams each of the following proprietary sources of standard non-nutrient agars were placed in 100 cm³ of deionized distilled water in non-borosilicate glass bottles: (i) Oxoid Agar No. 3 (Oxoid Ltd., Hampshire, U.K.); (ii) BDH Agar Powder, fine, Product of Japan (BDH Chemicals Ltd., Poole, U.K.); (iii) Difco Bacto-Agar, “Difco Certified” (Difco Laboratories, Detroit, Michigan, U.S.A.); (iv) Lab m Agar No. 2 (London Analytical and Bacteriological Media Ltd., London, U.K.); and (v) Davis Bacteriological Agar, Product of New Zealand (Davis Gelatine Ltd., Leamington Spa, U.K.). The agars were melted by briefly heating them in an autoclave and each medium was distributed to five 85 mm diameter sterile plastic Petri dishes (20 cm³ of medium per dish) and cooled to and solidified at room temperature. The surface of the solidified agar in each Petri dish was overlayed with 10 cm³ of deionized distilled water, covered with the lid, and placed in the dark at 25°C for 2 h. At the end of the 2 h period, the water in each Petri dish was carefully decanted into non-borosilicate glass containers and quantitatively tested for the presence of boron in the form of borate using the carminic acid procedure recommended for borate determinations in water analysis. Deionized distilled water heated in non-borosilicate glass bottles for the same length of time and at the same temperature used for melting the agars and placed in five sterile plastic Petri dishes (10 cm³ of water per dish) in the dark for 2 h served as an analytical control. Unheated deionized distilled water obtained directly from the deionizer also served as an analytical control. Borate standards were prepared with analytical grade boric acid in deionized distilled water.
Table 1. Boron analyses of aqueous agar extracts

<table>
<thead>
<tr>
<th>Agar</th>
<th>Boron concentration (ppm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxoid No. 3</td>
<td>4.4</td>
</tr>
<tr>
<td>BDH</td>
<td>5.8</td>
</tr>
<tr>
<td>Difco Bacto</td>
<td>9.9</td>
</tr>
<tr>
<td>Lab m No. 2</td>
<td>11.8</td>
</tr>
<tr>
<td>Davis Bacteriological</td>
<td>1.3</td>
</tr>
<tr>
<td>Control No. 1**</td>
<td>Not detectable</td>
</tr>
<tr>
<td>Control No. 2**</td>
<td>Not detectable</td>
</tr>
</tbody>
</table>

* Analyses based on five replicates per agar sample.

** Control No. 1 = heated deionized distilled water from non-borosilicate glass bottles and transferred to sterile plastic Petri dishes. Control No. 2 = unheated deionized distilled water taken directly from the deionizer.

Results and discussion

Boron concentrations in the form of borate anions ranging from a low of 1.3 to a high of 11.8 ppm were evident in the water overlays from the five proprietary standard non-nutrient agars (Table 1). No borate anions were detected in the heated or unheated deionized water controls, thus demonstrating that neither the non-borosilicate glass bottles nor the sterile plastic Petri dishes in themselves were significant sources of extractable borate anions. A 2 h extraction period was employed here so as to be in keeping with the 2 h pollen germination period employed previously on such solid agar-water media.

Although the standards of purity of commercially produced standard non-nutrient agars have been shown to vary with the different manufacturers, the data in Table 1 show that boron was a common easily extracted mineral constituent of all five proprietary non-nutrient agars used here. In addition, the amounts of boron extracted from each of these agars exceeded the 1.0 ppm concentration considered to be harmful to higher plant growth. Although seawater contains over 4 mg l⁻¹ of boron, seaweeds accumulate relatively large amounts of this mineral, and seaweeds accumulate relatively large amounts of this mineral, the producers of the non-nutrient agars used here do not provide any technical data concerning the presence of absence of this mineral from their products. A wide variety of growth responses have been observed in cultured excised shoot apices of *Picea abies* elicited by varying the agar component of an otherwise standard nutrient medium. In the case of boron sensitive plants, variations of extractable boron with different batches of standard non-nutrient agars may be responsible for inconsistent growth of developmental behaviour of the particular organism being investigated.

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References