A MASS CULTURE BIOASSAY METHOD FOR CAENORHABDITIS BRIGGSAE USING POPULATION GROWTH RATE AS A RESPONSE PARAMETER

BY

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A bioassay method for C. briggsae using population growth rate as a response parameter is described in which the nematodes are rotated at 1.0 rpm in 5.0 ml medium in 18 mm tubes on a tissue culture rotator. This method permits higher peak populations of nematodes than a previous mass culture method and also allows nephelometric monitoring of population growth. Population growth rate during the initial phase of growth is an effective index of growth and avoids the variations due to inoculum size and accumulation of metabolites associated with use of direct population counts at fixed time intervals.

The use of mass culture for nutrient bioassay using Caenorhabditis briggsae provides a less variable index of reproduction and is often more convenient than the commonly used larval bioassay method (Lower, Hansen & Yarwood, 1966). Tomlinson & Rothstein (1962) described a method in which nematodes were grown in a shallow layer of culture medium in 50 ml flasks. We have found that the use of 18 mm culture tubes and aeration by gentle rotation permits higher peak populations of C. briggsae. Use of tubes also permits nephelometric monitoring of population growth (Watson, Pinnock, Stokstad & Hieb, 1974). Either growth rate or the log of the number of nematodes after a standard time interval during logarithmic growth of the population provides a reliable response parameter.

MATERIALS AND METHODS

A basal medium consisting of C. briggsae Maintenance Medium (CbMM — Grand Island Biological Co.), plus 50 μg/ml β-sitosterol (Sigma Chemical Co.) dissolved in Tween 80, and 50 μg/ml cytochrome c (Sigma Chemical Co.) was supplemented with purified casein (Difco Laboratories) dissolved in dilute KOH and adjusted to pH 6.4. Inocula were drawn from mixed age cultures in, or just after the logarithmic phase of growth (8-15 days after inoculation). Nematodes were washed twice in 7.0 ml sterile distilled water before inoculation at a final population density of 400 nematodes per ml. The nematodes were incubated at 20° C on a tissue culture rotator (Model TC8, New Brunswick Scientific Co.) in either 5 ml of medium in 18 × 50 mm rimless tubes with Morton closures or in 1.0 ml of medium in 13 × 100 mm screw cap tubes. Rotation speed was 1 rpm at an angle of 11° to the horizontal. Transfers were made using wide bore prothrombin pipets and aliquots counted using a binocular microscope after serial
dilution. Measurement of population size using a nephelometer is described elsewhere (Watson, Pinnock, Stokstad & Hieb, 1974).

RESULTS AND DISCUSSION

Growth of *C. briggsae* in mass culture is typically exponential during the first 8-11 days, followed by a decline in growth rate. Death of larvae and reproductive adults was observed only after the peak population was reached. Aeration by rotation greatly increased peak population compared to stationary flask cultures used in the Rothstein method (Table 1). Growth rate during the exponential phase was not altered, however. This, together with pH change from 6.6 at 600 nematodes per ml to pH 8.5 at 120,000, suggests that decline and death of the population is due to the accumulation of toxic metabolites rather than depletion of nutrients. Measurement of population size after the log phase would therefore be complicated by this factor. Changes in rotation speed (0.55 to 2 rpm) and angle from the horizontal (6° and 11°) did not significantly affect the growth rate but did affect peak population. Rotation of 1 rpm at 11° to the horizontal was chosen as standard procedure. Increases in population size due to aeration by bubbling air through cultures have been reported by other workers (Buecher & Hansen, 1971). However, we feel that the method described in this paper maintains the experimental flexibility of small volumes and the convenience of handling required for a bioassay system.

**Table I**

*Effect of rotation on population growth of C. briggsae in casein supplemented medium (10 mg/ml)*

<table>
<thead>
<tr>
<th>Population Size</th>
<th>k (growth rate)</th>
<th>Maximum Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotated Tube Culture (1 rpm)</td>
<td>3.88 ± .029</td>
<td>0.206 ± .028</td>
</tr>
<tr>
<td>Stationary Flask Culture 1</td>
<td>3.85 ± .029</td>
<td>0.200 ± .029</td>
</tr>
</tbody>
</table>

1 5 ml in 50 ml flask.
2 5 ml in 18 mm × 150 mm rotated tubes.
3 95% confidence interval.

Variation of inoculum size gave a series of parallel curves, showing growth rate to be independent of inoculum size but total population to be strongly dependent on it. Evidence of a lag effect was noticed at inocula <200 nematodes per ml. This may be analogous to lag measured in bacterial cultures and may be due to a requirement for substances which accumulate in the medium, such as the large amounts of amino acids and metabolic intermediates which are excreted by adult *C. briggsae* (Rothstein, 1963 & 1965). A standard inoculum of 400 nematodes per ml is large enough to avoid lag and small enough to permit a long period of logarithmic growth.

The growth rate *k* of the population during logarithmic growth can be calculated from the expression