Yeast Extract as a Supplement to Chemically Defined Medium for Axenic Culture of *Caenorhabditis briggsae*

Caenorhabditis briggsae, a free living soil nematode, can be grown axenically in a chemically defined medium[1] if this is supplemented with proteinaceous materials[2]. Only a few such materials, derived from chick embryo, bacteria, and liver[3] have been found to be effective supplements. Since yeast cultures on agar support nematodes in vitro[4] it seemed probable that an effective supplement could also be made from extracts of yeast.

Extracts were first prepared in small quantity from a culture of *Saccharomycopsis guttulata* and from baker's yeast (Fleishman's). The cells were broken in a Braun homogenizer, model MSK (Bronwill Scientific) and the walls removed by centrifugation. Supernatants were sterilized by filtration and added to chemically defined medium at a 10% level. Both extracts supported reproduction.

Larger quantities of extract were then prepared from baker's yeast by suspending 900 g of washed cells in 800 ml of 0.05 *M* potassium phosphate buffer, pH 7, adding 800 ml of 120 μ Superbrite beads (Minnesota Mining and Mftr. Co.), and running this mixture through a colloid mill for 1 h. After removal of cell walls by centrifugation at 10,000 × *g*, the supernatant was collected and stored at −23 °C.

The extract was treated by dialysis to 0.15 *M* potassium phosphate buffer, pH 7, or by precipitation of the protein with 70% ammonium sulfate, the precipitate being then resuspended in the phosphate buffer and dialyzed to remove the ammonium sulfate. Preparations were sterilized by filtration through 0.3 μ membranes (Millipore-PH) and portions were lyophilized aseptically. Biological activity was determined by addition of the preparations to chemically defined medium at levels of 10% and 1%. Aliquots of 0.25 ml of supplemented medium were inoculated with 3 newly hatched larvae each and the subsequent development of the cultures at 20 °C was observed[1].

The biological activity is shown in the Table as measured by the relative maturation response[1]. All preparations were active at 10% (2–3 mg/ml). Activity was increased by the several treatments. Stock solutions retained activity during repeated freezing and thawing.

Maturation of *C. briggsae* in chemically defined medium supplemented with preparations of baker's yeast extract at 10% and 1%

Supplement		Relative maturation response	
Treatment	Protein (mg/ml)	10%	1%
Untreated	38	48	nm[a]
Lyophilyzed	38	74	nm[a]
Heated	38	86	89
Dialyzed	21	66	57
Ammoniumsulfate precipitated	20	63	57

The extract was assayed untreated and after the following treatments: dialysis to 0.15 *M* potassium phosphate buffer, pH 7; precipitation with 70% ammonium sulfate followed by dialysis to the above buffer; heating at 37 °C for 5 h. Maturation response is shown relative to maturation time of *C. briggsae* when cultured in association with *Escherichia coli* on agar (2.8 days = 100). Protein was determined using the folin phenol method of Lowry et al.[8]. [a] Non maturing.

Liver growth factor supplements have the interesting property of being 'activated', that is, certain treatments such as freezing[5], mild heating[6], and addition of Ficoll[1] greatly increase their biological activity. The yeast preparations were activated by freezing and heating but not by Ficoll. For example, after heating at 37 °C for 5 h, the relative maturation response with 1% supplement was increased to 89.

Yeast preparations were active in media in which the pH ranged from 6.0–7.5. Cultures were characterized by adults 20% larger than found in media containing growth factor from liver[2]. Young adult worms ranged in length from 1.1–1.3 mm and were 40–50 μ wide. Populations exceeded 1000 worms at 10% supplement levels, comprising the progeny of 2 generations. Continuous cultures are now being maintained[7].

Yeast extract was tested as supplement for 3 other species in axenic culture, *C. elegans*, *Neoplectana glaseri*, and *Pelodera strongyloides*. Maturation was supported in each case. The response of parasitic nematodes is under study.

Commercially available yeast autolysate (Albimi Lab., N.Y.) and fresh yeast extract (Grand Island Biological Co., N.Y.) did not make effective supplements to the defined medium.

The availability of baker's yeast, the ease of preparation of the extract, its apparent stability, and its high biological activity over a wide pH range make this a useful supplement for culture of free living nematodes, and of possible value in the culture of other organisms. Fractionation of the extract is under study[9,10].

Zusammenfassung. Es wird der Nachweis erbracht, dass Hefenextrakte die Neubildung der *C. briggsae* in bakterienfreien (axenischen) Kulturen fördern. Durch Zufügung dieser Extrakte in einen chemisch definierten Nährboden werden grössere Nematodenpopulationen erzielt. Die biologische Aktivität wird durch Dialyse, Ammoniumsulfatniederschlag oder durch leichtes Erwärmen verstärkt.

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[1] E. J. Buecher, E. L. Hansen and E. A. Yarwood, Proc. exp. Biol. Med. *121*, 390 (1966). Available from Grand Island Biological Company, Grand Island, New York 14072 (USA).
[2] E. L. Hansen, E. J. Buecher and E. A. Yarwood, Nematologica *10*, 623 (1964).
[3] E. C. Dougherty, E. L. Hansen, W. S. Nicholas, J. A. Mollett and E. A. Yarwood, Ann. N.Y. Acad. Sci. *77*, 176 (1959). – W. L. Nicholas, E. C. Dougherty and E. L. Hansen, Ann. N.Y. Acad. Sci. *77*, 218 (1959). – F. W. Sayre, E. L. Hansen, T. J. Starr and E. A. Yarwood, Nature *190*, 1116 (1961).
[4] V. Nigon and E. C. Dougherty, J. exp. Zool. *112*, 485 (1949).
[5] E. L. Hansen, F. W. Sayre and E. A. Yarwood, Experientia *17*, 32 (1961).
[6] E. L. Hansen, P. H. Silverman and E. J. Buecher, J. Parasit. *52*, 137 (1966).
[7] E. L. Hansen and W. S. Cryan, Nematologica *12*, 138 (1966).
[8] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).
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