Growth conditions for [abn-1] and [abn-2] in liquid media

R. W. Barratt

W. N. Ogata

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Abstract

Growth conditions for [*abn-1*] and [*abn-2*] in liquid media

Barratt, R.W. and W. N. Ogata, Growth conditions for [abn-1] and [abn-2] in liquid media.

The extrachromosomal (cytoplasmic) mutants [abn-1] and [abn-2] have a distinctive slow-growing, aconidial morphology as well as possessing abnormal mitochondria (Garnjobst, Wilson and Tatum 1965 J. Cell Biol. 26:413; Diacumakos, Garnjobst, Wilson and Tatum 1965 J. Cell Biol. 26:427). These authors recommend Difco Potato Dextrose Agar for the culturing of these strains. Using growth tube assays, they report that growth was not improved by a wide variety of environmental conditions. In routine culturing of [abn-1], inos (FGSC#1448) and [abn-2] (FGSC#1458) in our laboratory, these strains were found to grow quite satisfactorily on slants of Difco Neurospora Culture Agar, which consists of Bacto-Proteose Peptone #3, Bacto-Yeast Extract, Bacto-Maltose and agar. Initial experiments were carried out in liquid media containing various degraded proteins, yeast extract and carbon sources plus inositol (30 μ g/ml) in the case of [abn-1], inos. Difficulty was encountered in obtaining uniform inocula for such comparative growth studies. Uniform inocula could be obtained by

Table 1. Growth of [abn-1] , inos with various supplements at 25°C.

Supplement *	Yield"
None	18.2
Hydrolyzed yeast nucleic acid	26.2
Mixed B vitamin solution	32.5
Hydrolyzed yeast nucleic acid + mixed B vitamin solution	53.0
Bacto yeast extract (0.1%)	122.7
Bacto yeast extmct (0.5%)	208.2
Adenine, guanine, cytorine, uridine, thymidine (50 /ml)	0.0
B vitamin soln. + adenine, guanine, cytorine, thymidine	0.0

• in Vogel's medium N + 2% sucrose and 30/ml inositol.

● *mycelial dry weights (mg) are overager of duplicate flasks (40 ml medium in 125 ml flask) grown with aeration on a shaker for 65 hrs.

Table 2. Growth of [abn-2] in various media at 25°C.

Basal medium	Supplements	Carbon source	Yield*
Distilled water	Yeast extmct (0.5%) + tryptone (0.5%)	Maltose (4%)	55.4
"	"	Maltose (2%)	62.5
"	"	Sucrose (2%)	27.7
"	Yeast extract (0.5%)	"	20.0
"	Tryptone (0.5%)	"	15.0
"	Tryptone (0.2%)	"	10.6
Vogel's N	---	"	0.2
"	Yeast extract (0.5%)	"	17.5
"	Tryptone (0.5%)	"	10.5
"	Tryptone (0.2%)	"	17.2
"	Yeast extract (0.5%) + Tween 80 (0.1%)	"	15.5

*mycelial dry weights (mg) ore averages of duplicate flasks (40 ml medium in 125 ml flask) grown with oerotion on a shaker for 120 hr.

Growth of wild type controls were:

in distilled water + yeort extmct (0.5%) + tryptone (0.5%)

+ sucrose = 296 mg

in Vogel's medium N + sucrose = 259 mg.

yeort extmct ore stimulatory for growth, that Vogel's minimal N will not support growth of the strain, and that supplemented Vogel's is no better than the supplements alone. The total growth obtained for [abn-2] under the best conditions is approximately 20% that of wild type.

It can be concluded that [abn-1] , inos mycelio can be readily grown in large quantities in liquid oemted culture at 25°C in minimal medium supplemented with inositol, yeast extract and sucrose. Further, respectable yields of [abn-2] can be obtained in liquid medium consisting of yeast extract, tryptone and maltose. The active ingradient in yeort extract which promoter growth of these strains has not been identified.

• • • Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

homogenizing mycelial pellets from a 2-3 day old shoke culture with the aid of a sterile Corning Ten Broeck tissue grinder (Corning 7725). Mycelial pellets were lightly ground with the homogenizer until uniform frag-ments were obtained. Such inocula could be employed as one uses conidial suspensions. Diacumakos et al prepared inocula for liquid cultures with the aid of a sterile blender. Initial experiments indicated that for [abn-1] : (a) sucrose was as effective a carbon source as maltose, (b) peptones or tryptone were not rtimulatory to growth, (c) yeast extract stimulated growth, especially in cultures oemted on a shaker.

The data in Table 1 summarize the growth of [abn-1], inos in shake cultures at 25°C in variously supplemented media for 65 hours. Agreement between the replicates was as good as replicates of conidiating strains employing conidial suspensions, with the exception of the cultures grown with 0.5% yeast extmct where unexplained variation occurred. The data confirm that yeast extract has a marked stimulatory effect on [abn-1] under these conditions, and that none of the known growth factors tested con substitute for yeast extract. In this and subsequent experiments, the growth habit of [abn-1] re-mained colonial, forming characteristic pellets in shake cultures and approximated 50% of the wild type growth when grown on yeort extract. The rtimulatory effect of yeast extract cannot be replaced by Tween 20 or 80, vitamin A or D, ergosterol, canavanine, sarcosine or sodium acetate. While the temperature optimum for [abn-1] under these experimental conditions has not been rigorously determined, growth at 32°C is less than at 25°C. Garnjobst et al. reported no effect of oxygen in growth tube assays, but our early experiments showed much better growth in aerated, compared to stationary, cultures.

The data in Table 2 summarize the growth of [abn-2] in shoke cultures at 25°C in variously supplemented media for 120 hours. The data show that maltose is a better carbon source than sucrose, that both tryptone and maltose are stimulatory for growth of the strain, and that supplemented [abn-2] under the best conditions is approxi-