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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 (30γ/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 year+ 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 overoger 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 hrr.

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.

homogenizing mycelial pellets from a 2-3 doy old rhoke culture with the aid of a sterile Corning Ten Broeck tissue grinder (Coming 7725). Mycelial pellets were lightly ground with the homogenizer until uniform fragments were obtained. Such inocula could be employed as one uses conidiol suspensions. Diacumakos et al prepared inocula for liquid cultures with the aid of a sterile blendor. Initial experiments indicated that for [abn-1] : (a)sucrose was as effective a carbon source as maltose, (b) peptones or tryptone were not rtimulotory 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 conidiol 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 yeost extract. In this and subsequent experiments, the growth habit of [abn-1] remained colonial, forming characteristic pellets in shake cultures and approximated 50% of the wild type growth when grown on yeort extract. The rtimulotory 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 there 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 rhoke 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 yeort extmct ore rtimulotory 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% thot of wild type.

It can be concluded that [abn-1], inos mycelio con be readily grown in large quantities in liquid oemted culture at 25°C in minimal medium supplemented with inositol, yeast extract ond sucrose. Further, respectable yields of [abn-2] con be obtained in liquid medium consisting of yeast extract, tryptone ond maltose. The active ingradient in yeort extract which promoter growth of these strains has not been identified. • • • Deportment of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.