

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, cytosine, uridine, thymidine (50 /ml)	0.0
B vitamin soln. + adenine, guanine, cytosine, thymidine	0.0

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

● *mycelial dry weights (mg) are overages 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) are averages of duplicate flasks (40 ml medium in 125 ml flask) grown with aeration on a shaker for 120 hrs.

Growth of wild type controls were:

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

+ sucrose = 296 mg

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

yeast 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 ingredient in yeast extract which promoter growth of these strains has not been identified.

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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 fragments 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 stimulatory 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 can substitute for yeast 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 yeast extract. The stimulatory 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 yeast extract stimulated growth of the strain, and that supplemented [abn-2] under the best conditions is approximately