Eberhart, B. M. and B. S. Madden.

A new ß-glucosidase.

We have observed an unreported and electrophoretically distinct ß-glucosidase that exists in a majority of Neurospora stocks now in common laboratory use. Comparisons have been made of the physical properties of the new ß-glucosidase with cellobiase and aryl-ß-glucosidase, which have already been described in some detail (Eberhart and Beck 1970 J. Bacteriol. 101:408). The new enzyme is usually a minor (<10%) component of the ß-glucosidase system and only reaches high levels in exotic strains (such as P-212).

The detection of the new enzyme resulted from changes in our standard electrophoretic technique by the addition of the following improvements: (a) The use of Gelman's Sepratek apparatus was combined with 15-minute runs of Sepraphore III to give clearly separated bands; (b) Umbelliferone-ß-D-glucoside was used as the enzyme substrate. The product umbelliferone is fluorescent and can be detected at much lower levels than can the product of the usual substrate, para-nitrophenyl-ß-D-glucoside (PNP-G); (c) Since the new enzyme is usually at a low concentration, the extracts examined were concentrated prior to examination by dialysis against carbowax-20.

The new ß-glucosidase has several distinctive properties that are shown in Table 1. The new enzyme is the least mobile of the ß-glucosidases in an electrophoretic field. Its presence in conidial washes and its absence from mycelia is opposite to the occurrence of cellobiase. The molecular weight (as determined by gel filtration) is much less than that of aryl-ß-glucosidase, more similar to that of cellobiase. General studies of pH optima indicate that aryl-ß-glucosidase and the new ß-glucosidase have optimal activities at pH 5.0. The new ß-glucosidase does not attack cellobiose. Enzyme incubated with cellobiose (1%) produces neither glucose nor transglucosidation products that are characteristic of the other two ß-glucosidases.

Table 1. Properties of ß-glucosidases.

	New ß-glucosidase	Aryl-ß-glucosidase	Cellobiase
Relative electrophoretic mobility toward anode	0.4	1.0	0.8
Presence in induced mycelia	Trace (if any)	+	+
Presence in conidial washes	+	+	-
Molecular weight (approx)	53,000	168,000	43,000
pH optimum	5.0	5.0	6.0
Transglucosidation of cellobiose	-	+	+
Presence in gluc-2 stocks	-	-	+

Strains used were: 74-OR8-1a, 74-OR23-1A and P-212.

Purified preparations of aryl-ß-glucosidase yield several new electrophoretic bands when treated with urea or guanidine hydrochloride. The principal band resulting from this treatment corresponds to the new, naturally occurring ß-glucosidase. This suggests that the new ß-glucosidase is at least in part a sub-unit of aryl-ß-glucosidase. The reason for its presence in some strains and not in others is still not clear. The proposed relationship between aryl-ß-glucosidase and the new ß-glucosidase is also strengthened by the fact that gluc-1 and gluc-2 strains do not possess either aryl-ß-glucosidase or the new ß-glucosidase, while cellobiase activity remains normal in these strains.

In the several strains that have been studied to date, the new ß-glucosidase is less thermostable than its corresponding aryl-ß-glucosidase. Substrate affinity studies (Km) suggest that the new enzyme has a greater affinity for PNP-G than does the corresponding aryl-ß-glucosidase in the same strains. The presence of multiple forms of carbohydrases in Neurospora is common (Metzenberg 1964 Biochim. Biophys. Acta 89: 291; Bates, Hedman and Woodward 1967 J. Bacteriol. 93: 1631; Yu, Garvell and Sussman 1971 Genetics 68: 473). The natural occurrence of the new ß-glucosidase in most Neurospora strains suggests that there is some selective advantage to the flexibility that the variety of physical properties of these enzymes possess in ß-glucosidase activity.

This work was supported by National Science Foundation Grant No. 8453. - - - Department of Biology, University of North Carolina at Greensboro, Greensboro, North Carolina, 27412.