β-LACTAMASE ACTIVITY IN YEAST

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β-Lactamase activity (E.C. 3.5, 2.8) has been detected in species belonging to most genera of bacteria and certain authors have speculated that all bacteria may produce at least one chromosomally mediated β-lactamase[2,4]. Recently, a β-lactamase enzyme was detected in several strains of eukaryotic algae[1]. In this paper, we report the presence of β-lactamase activity in certain yeast.

The cultures used in this study were obtained from the following sources: *Candida albicans* BC–759, a clinical isolate, and *Candida* sp. RM–1, a methanol-utilizing yeast isolated from soil, from the Smith Kline & French Culture Collection; *Candida utilis* ATCC–22023 from the American Type Culture Collection; *Saccharomyces cerevisiae* JW4–5Ccys–1 from Yest Genetic Stock Center, University of California, Berkeley, California; *Candida boidinii* NRRL Y–2332 and *Pichia pinus* NRRL YB–4025 from the Northern Regional Research Laboratories, Peoria, Illinois.

Cultures were stored as frozen cell suspensions at −70°C. The growth from a two-day-old SABOURAUD's agar slant (BBL, BioQuest, Cockeysville, Maryland) was used to inoculate SABOURAUD's broth medium (800 ml in 2-liter Erlenmeyer flask). The cultures were incubated at 28°C on a rotary shaker, 250 rpm (2-inch throw) for 40 hours. Cells were collected by centrifugation at 20,000×*g* for 15 minutes, washed twice with deionized water and stored at −20°C until used. Cell-free extracts were prepared by sonicating 5 g of cells with 5 g of superfine glass beads (Superbrite 3M Co., St. Paul, Minnesota) in 40 ml of deionized water. During sonication the temperature was maintained below 10°C. Cell debris were removed by centrifugation at 30,000×*g* for 15 minutes. The clear supernatant was used for the enzyme assays.

β-Lactamase activity was assayed in whole cells by the method of O'CALLAGHAN, *et al.*[3] using the chromogenic cephalosporin 87/312 as a substrate and by the method of ULLMANN[5] using cephacetrile as a substrate. In both methods the substrate is hydrolyzed to give a colored product. In these assays 2 μg of cephalosporin 87/312 or 1 μg of cephacetrile was added to 1 ml of 24-hour culture. This was incubated at 50°C and color formation observed over a 90-minute period. The appearance of red color with cephalosporin 87/312 or pink color with cephacetrile was considered a positive reaction. For pH and temperature studies, the color formation with cephalosporin 87/312 was monitored with a spectrophotometer. Activity was assayed in cell-free preparations by the method of O'CALLAGHAN, *et al.*[3] and by biological inactivation of penicillin G and cephalothin. Biological inactivation was determined by adding 0.3 ml of the enzyme preparation to discs containing 10 μg penicillin G or 30 μg cephalothin. The discs were incubated for 90 minutes and then transferred to Penase agar plates seeded with *Staphylococcus aureus* FDA209P. After incubation for 24 hours, the zone sizes of treated and non-treated discs were compared. A reduction of 6 mm or more in zone size was considered a positive reaction.

The detection of β-lactamase activity in yeast is shown in Table 1. β-Lactamase activity using the chromogenic cephalosporin substrate 87/312 and cephacetrile was detected with whole cell preparations of *C. albicans, C. boidinii* and *P. pinus* cultures but not with *S. cerevisiae, C. utilis* and *Candida* sp. RM–1 cultures. Further confirmation of β-lactamase activity was obtained using cell-free preparations of *C. albicans, C. boidinii* and *P. pinus*. β-Lactamase activity was detected in cell-free extracts of all three strains by hydrolysis of cephalosporin 87/312 and by biological inactivation of penicillin G and cephalothin. A cell-free preparation of *C. boidinii* shows a pH optimum of 7.0 and a temperature optimum of 50°C. The activity with cephalosporin 87/312 under optimal conditions for this preparation was 0.56 OD_{450} unit·hour^{-1}. The reaction was linear with respect to both time and enzyme concentration.

The β-lactamase enzymes appear to be widely

Table 1. Detection of β-lactamase activity in yeast

Culture	Assay method	β-Lactamase activity*	
		Whole cells	Cell-free
Candida albicans	Cephalosporin 87/312	+	+
	Biological	N. D.	+
	Cephacetrile	+	N. D.
Candida boidinii	Cephalosporin 87/312	+	+
	Biological	N. D.	+
	Cephacetrile	+	N. D.
Pichia pinus	Cephalosporin 87/312	+	+
	Biological	N. D.	+
	Cephacetrile	+	N. D.
Saccharomyces cerevisiae	Cephalosporin 87/312	−	N. D.
Candida utilis	〃 〃	−	N. D.
Candida sp.	〃 〃	−	N. D.

* N. D. refers to assays which were not done.
\+ indicates the appearance of red (cephalosporin 87/312) or pink (cephacetrile) color in the reaction mixture over a 90-minute period. Biological inactivation refers to at least a 6-mm reduction in zone size after treatment.
− no change in color.

distributed, if not universally distributed, amongst microbes. The data presented in this study demonstrates the presence of these enzymes in eukaryotic organisms. The function and possible evolutionary significance of β-lactamase in yeast will have to await further study. Considering the comparative cell-wall structure of yeast and bacteria, it is unlikely that β-lactamases function in cell-wall biosynthesis in both groups of organisms.

β-Lactamase activity was observed with whole cells and cell-free extracts of three yeast cultures, *Candida albicans* BC–759, *C. boidinii* NRRL Y–2332 and *Pichia pinus* NRRL YB–4025. Three other yeast cultures, *C. utilis* ATCC–22023, *Candida* sp. RM–1, and *Saccharomyces cerevisiae* JW4–56, had no detectable activity.

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