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Microbiological Reduction of Quininone to Quinidine

LALITAGAURI RAY, CHAITALI DAS GUPTA, AND S. K. MAJUMDAR*
Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-700 032, India

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A yeast identified as *Hansenula anomala* var. *schneggii* was found capable of reducing quininone to quinidine in 50% yields in 7 days of fermentation.

Cinchona bark contains a number of alkaloids, of which quinine, quinidine, cinchonine, cinchonidine, epiquinine, and epiquinidine are important. The stem bark of *Cinchona ledgeriana* contains a high percentage (up to 14%) of quinine, which constitutes 90% of the total alkaloid present. Demand for quinidine sulfate, a minor constituent of cinchona alkaloid, has increased largely for its use against cardiac ailments. Since its content is less than 10% of the total alkaloid (1), the demand for quinidine from natural resources has been difficult to meet. The microbiological preparation of quinidine (Fig. 1) from quininone, an oxidation product of quinine, has been achieved, and this paper describes preliminary data on the reduction of quininone to quinidine by a yeast identified as *Hansenula anomala* var. *schneggii*.

Of about 450 organisms isolated from different soil samples, two isolates were able to convert quininone to quinidine, and one of them gave good conversion under the conditions of the experiment. The particular organism was isolated from the soil, mixed with modified Czapek-Dox medium and quinidine sulfate, and incubated for 2 months. Identification of the isolate was done by following the standard method for yeasts (2). It was maintained on a glucose-peptone-yeast extract-malt extract-agar slant at 28°C and was subcultured at monthly intervals.

Microbial transformation of quininone to quinidine was studied in the medium consisting of (wt/vol) 1.0% glucose, 0.1% potassium dihydrogen phosphate, 0.5% ammonium sulfate, 0.05% magnesium sulfate, and 0.1% yeast extract (pH 4.5). Fermentation was carried out in 50-ml Erlenmeyer flasks, each containing 10 ml of medium, on a rotary shaker (120 rpm) at 28°C. The optimum conditions for reduction of quininone to quinidine by the yeast species are: initial pH, 4.5; temperature of incubation, 28°C; inoculum (24-h cell growth) volume, 10% (vol/vol); substrate concentration, 0.05% (wt/vol). Quininone was prepared by the method of Woodward et al. (4). It was added at different time intervals at a final concentration of 0.05%. At different time periods, the samples were centrifuged and assayed for quinidine.

The compound was separated by thin-layer chromatography on Silica Gel G with the solvent system benzene-diethyl ether-diethyl amine (60:36:15) and compared with the standard sample (3). The plate was then heated at 110°C for 10 min to remove solvents. On cooling it was sprayed with 10% sulfuric acid. Quinidine gave blue fluorescence, and quininone gave yellow-green fluorescence, under UV light. The standard preparation of quininone, quinine, and quinidine showed R_f values of 0.63, 0.19, and 0.26, respectively, in the solvent system used. Quinidine was extracted from the adsorbent by 0.02 N sulfuric acid. The recovery of quinidine from the thin-layer chromatography plate was 96%. The fluorescence of the resulting quinidine solution was measured fluorimetrically with a Beckman Ratio Fluorimeter (primary filter, 450 nm; secondary filter, 360 nm). The amount of quinidine was determined by using the standard curve. The results are shown in Table 1.

Table 1 shows that the quinidine concentration reached a maximum value (0.25 mg/ml) on day 7 of fermentation, when quininone was added at the concentration of 0.5 mg/ml at 24 h of fermentation and the maximum conversion of quininone to quinidine was 50%.

Quinidine was recovered from the fermentation broth (pH adjusted to 10.0 with NaOH) with diethyl ether and then was purified further by column chromatography with Silica Gel G. Benzene-diethyl ether-diethylamine (70:36:15) was used as the eluant. Finally, it was crystallized from chloroform solution. The compound was identified on the basis of its characteristic physicochemical properties, viz., specific rotation, UV and infrared absorption spectra, fluorescence, melting point, and elemental analysis.

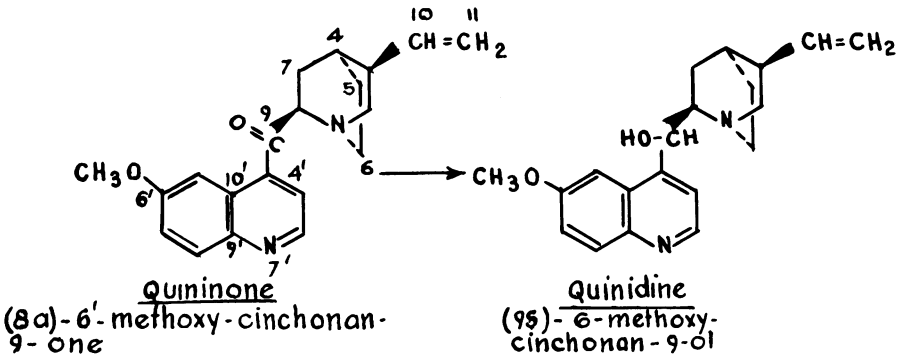

FIG. 1. Structures of quinone and guinidine.

TABLE 1. Quinidine production from quininone by *Hansenula anomala* var. *schneggii*

Time of addition of quininone (0.5 mg/ml) (h)[a]	Quinidine concn (mg/ml) on day:						Conversion of quininone to quinidine (%) on day:					
	3	4	5	6	7	8	3	4	5	6	7	8
0	0.05	0.18	0.19	0.22	0.22		10	36.0	38.0	44.0	44.0	
24		0.14	0.20	0.24	0.25	0.25		28.0	40.0	48.0	50.0	50.0
48			0.13	0.24	0.24	0.24			26.0	48.0	48.0	48.0

[a] Quininone initially added, 5 mg/ml.

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