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Production of L-lactic acid by *Rhizopus* species

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Of 19 *Rhizopus* spp. only four produced L-lactic acid in shake-flask culture. Aerobically and in the presence of a neutralizing agent, *Rhizopus oryzae* NRRL 395 produced the highest concentration of L-lactic acid (65 g/l) but with O_2-limited growth ethanol was produced instead.

Key words: Ethanol, fumaric acid, L-lactic acid, metabolism, *Rhizopus*.

Lactic acid can be produced by humans, other animals, plants and microorganisms. From the nutritional point of view, the L form is the most interesting for the food industry; the human body is only adapted to assimilate this form and only produces L-lactate dehydrogenase (Soccol 1992). Certain types of *Rhizopus* spp. are capable of producing only L-lactic acid in high concentrations (Lockwood *et al.* 1979; Hang *et al.* 1989). The present study was of the production of L-lactic acid by *Rhizopus* spp. under different cultural conditions.

Materials and Methods

Nineteen strains of *Rhizopus* from various international collections (Table 1), were grown at 30°C in 50-ml volumes of culture medium containing (g/l): glucose, 100; $(NH_4)_2SO_4$, 3.2; KH_2PO_4, 0.6; $MgSO_4.7H_2O$, 0.75; and $ZnSO_4.7H_2O$, 0.04. The medium, in Erlenmeyer flasks, was adjusted to pH 6.0 and incubated, with shaking at 140 rev/min, after inoculation with 2×10^7 sporangiospores/flask. Cultures were run under aerobic and O_2-limited conditions. The O_2-limited conditions were obtained by sealing the flasks. After 24 h, 4 g sterilized $CaCO_3$ was added to half the flasks and shaking was increased to 220 rev/min. After a further 48 h the fermented medium was strained through a 200-µm pore nylon mesh and the filtrate was centrifuged (6000 × *g*, 10 min). Lactic acid, ethanol, and fumaric acid were determined in the supernatant by HPLC using an Aminex HPX 87H column (Bio-Rad). A commercial kit (139.084; Boeringer) was used to determine the lactic acid isomers. The biomass retained by the sieve was washed with 2 M HCl and its dry weight determined. Glucose concentrations during the fermentation were determined using the dinitrosalicylic acid method.

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Results and Discussion

All 19 strains produced fumaric acid (Table 1) but particularly high concentrations were produced by *Rhizopus arrhizus* 16179, *R. delemar* 34612 and *R. microsporus* 46436, confirming the studies of Rhodes *et al.* (1959). *Rhizopus oryzae* 2710 and *R. oryzae* 28627 produced the highest concentrations of ethanol (25 and 16 g/l, respectively) (Table 1). Fujio *et al.* (1984) demonstrated that certain *Rhizopus* strains could also produce ethanol from raw cassava starch.

Only four of the strains studied, *R. oryzae* 395, *R. arrhizus* 16179, *R. delemar* 34612 and *R. arrhizus* 24425, produced significant amounts of L-lactic acid. Of these strains, *R. oryzae* 395 gave the highest acid concentration (65 g/l), confirming the observations of Hang *et al.* (1989).

The effect of different culture conditions on the metabolism of *Rhizopus oryzae* NRRL 395 is shown in Figure 1. In air and without $CaCO_3$ (Figure 1A) there was a little consumption of glucose because the accumulation of fumaric and lactic acids caused a rapid decrease in pH. When $CaCO_3$ was added to aerobic cultures (Figure 1B) glucose was completely consumed, a high concentration of L-lactic acid (65 g/l) was produced, and biomass was substantially increased. Other

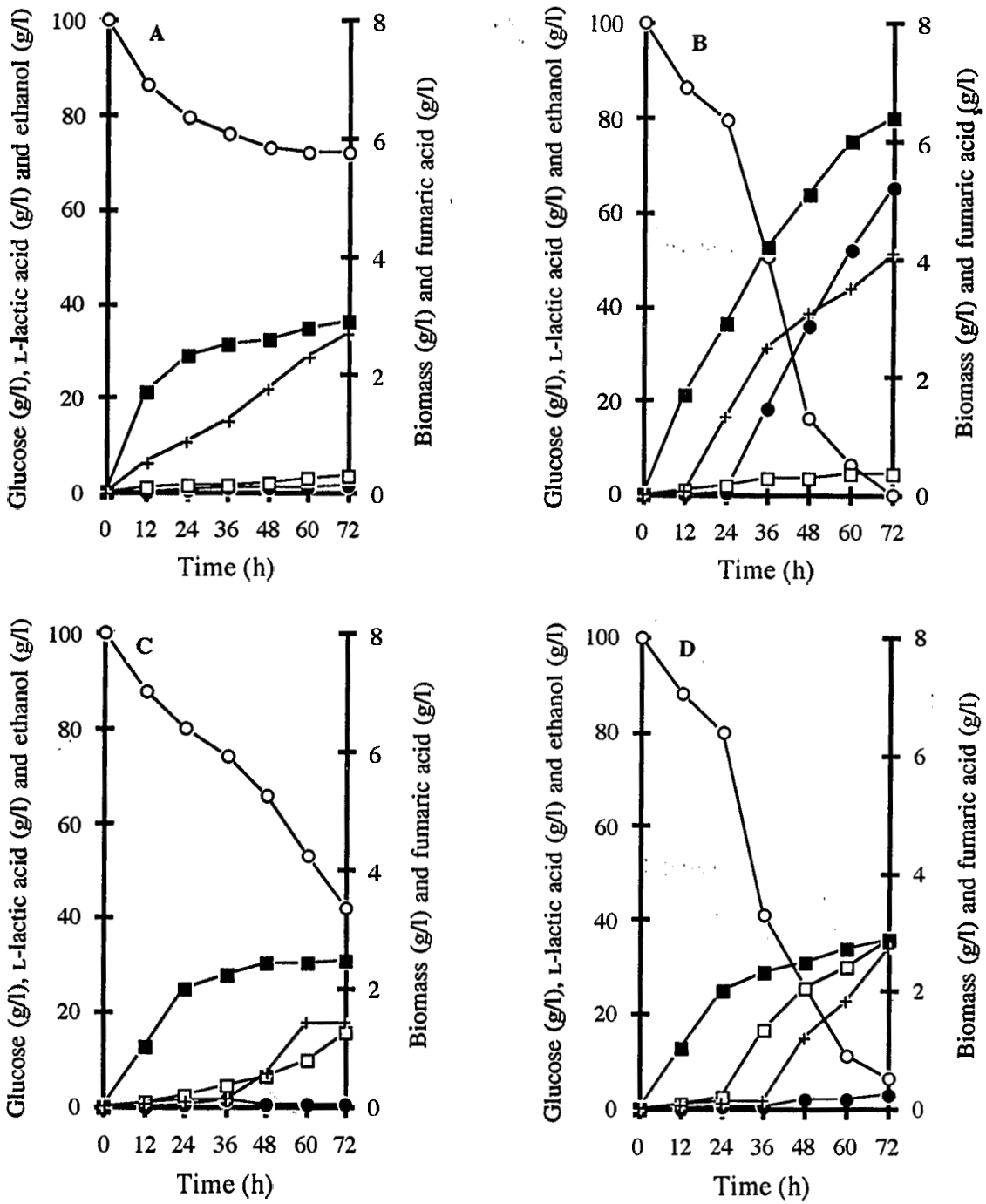

Figure 1. Effect of different culture conditions on *Rhizopus oryzae* NRRL 395. (A) Aerobiosis without $CaCO_3$ addition. (B) Aerobiosis with $CaCO_3$ addition. (C) O_2-limited without $CaCO_3$ addition. (D) O_2-limited with $CaCO_3$ addition. ○—Glucose; ●—L-lactic acid; □—Ethanol; ■—Biomass; + —Fumaric acid.

Table 1. Production of L-lactic acid and other metabolites by *Rhizopus* spp.*

Strain†	Residual glucose (g/l)	Biomass (g/l)	L-Lactic acid (g/l)	Fumaric acid (g/l)	Ethanol (g/l)	Final pH
R. arrhizus MUCL 16179	0	4	40	30	3	4.9
R. arrhizus MUCL 28425	18	4.9	33	24	5	4.9
R. arrhizus NRRL 1526	15	7.9	0	22	6	4.5
R. circicans NRRL 1475	31	8.6	0	12	0	4.9
R. delemar ATCC 34612	3	7.4	38	28	0	5
R. delemar NRRL 1472	18	6.5	0	17	8	4.6
R. formosa MUCL 28422	11	7.6	0	21	10	4.8
R. microsporus ATCC 46436	14	3.7	0	25	9	4.9
R. microsporus MUCL 9667	47	3.5	1	3	13	6.1
R. oryzae ATCC 22580	54	4.4	0	16	0	4.4
R. oligosporus ATCC 6203	19	3	0	20	8	4.7
R. oligosporus NRRL 2710	18	9.7	1	5	25	5.7
R. oryzae MUCL 28168	6	7.8	0	20	7	4.8
R. oryzae MUCL 28627	5	4.8	0	24	16	4.6
R. oryzae NRRL 395	0	6.4	65	4	4	5
R. oryzae NRRL 25976	21	7.9	0	21	8	4.6
R. sp. NRRL 25975	11	5.6	0	16	10	4.6
R. stolonifer MUCL 28169	6	5	0	15	13	4.9
R. stolonifer MUCL 28181	58	7.1	0	27	1	5.7

* Cultures were incubated at 30°C in aerobiosis with shaking at 140 rev/min for 24 h and then 220 rev/min for 48 h with $CaCO_3$ (80 g/l) in all flasks. All determinations were made in triplicate.

† ATCC—American Type Culture Collection, Rockville, Maryland, USA; MUCL—Mycology Collection, Catholic University of Leuven, Belgium; NRRL—Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Illinois, USA.

metabolites, such as ethanol and fumaric acid, were also present but in relatively low concentrations (Figure 1B).

In O_2-limited conditions and without $CaCO_3$ (Figure 1C) glucose consumption and metabolite production were again limited due to a rapid reduction in pH. A metabolic diversion to ethanol production was also noticed (Figure 1C). When $CaCO_3$ was added to O_2-limited cultures the microorganism was capable of consuming practically all the glucose present and its metabolism was directed exclusively to ethanol production (36 g/l). However, final biomass was considerably lower in the presence of $CaCO_3$ (Figure 1D) than in aerobic cultures (Figure 1B).

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