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Acetoin and Diacetyl Production by *Lactobacillus plantarum* Able to Use Citrate

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ABSTRACT

Whole-cell suspensions of *Lactobacillus plantarum* grown on lactose in the presence of citrate did not produce acetoin and diacetyl (AD) (D) from citrate in succinate buffer, pH 4.4 unless both a source of energy and nitrogen was present, but did from pyruvate. The total AD and the amount of D, produced by citrate-grown cells, from citrate were about two times the amounts formed from pyruvate, calculated on a molar basis. It appears, that AD are formed not only from pyruvate resulting from cleavage of citrate but also from acetyl-coenzyme A arising during a probable breakdown of citrate in a reversible reaction of citrate synthetase. Neither acetate nor acetaldehyde had any effect on the total AD or the amount of D produced from pyruvate by pyruvate-grown cells. The rates of AD production from pyruvate by whole-cell suspensions of pyruvate-grown *L. plantarum* and *Streptococcus* subsp. *diacetylactis* represented only 69.7 and 6.6%, respectively, of that produced by *Lactobacillus casei*. These were 0.075 μmoles/mg dry wt^{-1} ml^{-1} min^{-1} for *L. casei*, 0.053 for *L. plantarum* and 0.005 for *S. lactis* subsp. *diacetylactis*.

Many strains of *Lactobacillus casei* and *Lactobacillus plantarum* use citrate, presumably in the same way as *Streptococcus lactis* subsp. *diacetylactis (13)*. The two species most frequently share the responsibility of ripening many sorts of cheese, including white pickled cheese.

There have been few reports on the ability of lactobacilli to produce diacetyl. Bassette et al. (*1*) reported diacetyl production by *L. casei* when grown in milk cultures. Drinan et al. (*10*) studied acetoin production by *L. plantarum* in MRS broth. Christensen and Pederson (*7*), Radler (*18*) and Whiting (*19*) have shown that extensive growth of *Lactobacillus brevis* and *L. plantarum* in fruit juice causes spoilage by production of diacetyl from the citrate present in the juice. Since diacetyl is produced in Cheddar cheese and reportedly contributes to the typical Cheddar flavor in low levels (*4,5,16*), the research reported herein was designed to determine the qualitative and quantitative aspects of acetoin and diacetyl production by *L. plantarum* isolated from salted milk and common to white pickled cheese.

MATERIALS AND METHODS

L. plantarum and *L. casei* used in these experiments were isolated from salted raw milk and identified by Hegazi (*15*). The organisms were maintained by subculture every month at 30°C in deep MRS agar (*9*) and then stored them in a refrigerator. *S. lactis* subsp. *diacetylactis* was propagated routinely at 30°C in sterile litmus milk and subcultured in tryptone-yeast extract- lactose broth (TYL) of Broome et al. (*3*).

The materials used and the methods adopted are described elsewhere (*11,12*).

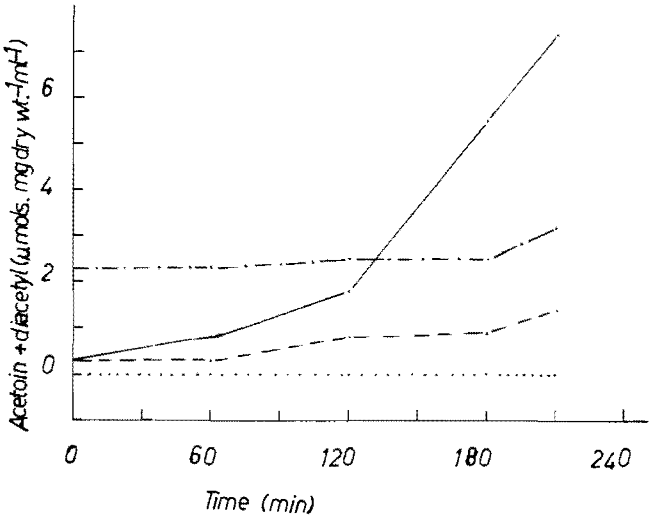

Figure 1. *Acetoin and diacetyl production by whole-cell suspensions of citrate-grown* L. plantarum *from: citrate + MRS broth* ———, *citrate + lactose - - - -, citrate alone ...., pyruvate alone-.-.-.-.-.*

RESULTS AND DISCUSSION

Effect of supplements and substrate on acetoin and diacetyl (AD) production

Whole-cell suspensions of *L. plantarum* which had been grown on lactose in the presence of citrate were unable to form acetoin and diacetyl from citrate when suspended in a succinate buffer, pH 4.4 (Fig. 1), resembling in this respect *S. lactis* subsp. *diacetylactis* DRC3 (*12*). Lactose only slightly stimulated AD production, whereas inclusion

TABLE 1. *Acetoin and diacetyl production (μmoles/mg dry wt^{-1} ml^{-1}) from pyruvate, in the presence of acetate and acetaldehyde, by whole-cell suspensions of pyruvate-grown* L. plantarum.

Time in minutes	Pyruvate alone		Pyruvate + acetate		Pyruvate + acetaldehyde		Citrate	
	AD[a]	D	AD	D	AD	D	AD	D
0	1.43	0.16	1.43	0.19	1.75	0.22	0.03	-[b]
30	1.62	0.19	1.62	0.19	2.19	0.25	0.05	-
60	2.13	0.35	1.83	0.25	2.37	0.37	0.05	-
90	3.02	0.47	2.69	0.43	3.18	0.47	0.05	-
120	3.58	0.69	3.50	0.63	3.50	0.66	0.03	-
150	4.07	0.80	4.07	0.69	4.07	0.78	0.05	-
180	4.80	1.09	4.63	0.78	4.23	0.85	0.05	-
210	5.06	1.17	5.15	0.85	4.90	0.94	0.05	-

[a]A = acetoin; D = diacetyl.
[b]- = not determined.

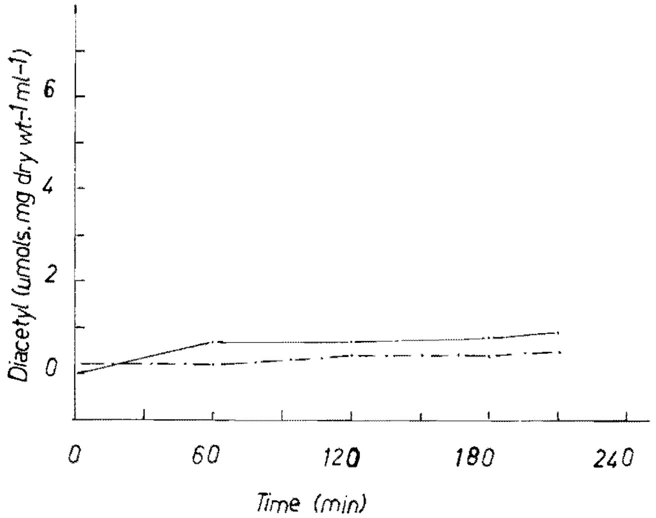

Figure 2. *Diacetyl production by whole cell suspensions of citrate grown* L. plantarum *from: citrate + MRS broth —; pyruvate alone -.-.-.-.-.*

of 0.5 ml of MRS broth in the buffer was essential for high AD production. Like *S. lactis* subsp. *diacetylactis*, failure to use citrate may be attributed to loss of the ability to transport citrate into the cells (*8*) due to rapid disintegration of an inducible citrate permease. Presence of both a source of energy and nitrogen was necessary for reformation of the enzyme. Pinsky and Stokes (*17*) found both types of substances to be quite essential for formation of the inducible formic hydrogenlyase of *Escherichia coli*. Cogan et al. (*6*) reported no detectable acetoin production in phosphate buffer at pH 6.1, whereas acetoin production occurred in acetate at pH 6.1. Species of buffer could be another reason for failure of *L. plantarum* to use citrate.

Both citrate- and pyruvate-grown cells produced AD from pyruvate immediately after its addition to the assay mixture (Fig. 1 and Table 1). The maximum amount, however, never reaching that produced from citrate, but it was greater in pyruvate- than in citrate-grown cells. The total AD and the amount of diacetyl (D), produced by citrate-grown cells, from citrate (Fig. 1 and 2) were about two times the amounts produced from pyruvate, calculated on a molar basis. It appears, therefore, that AD are formed not only from pyruvate resulting from cleavage of citrate, but acetyl-coenzyme A, arising during a probable breakdown of citrate in a reversible reaction of a citrate synthetase, may also be involved in their synthesis. Conversion of certain citric acid cycle intermediate compounds to citrate in a citrate synthetase reaction has been reported in the related species, *L. casei* (*2*).

Unlike *S. lactis* subsp. *diacetylactis* and *L. casei* subsp. *pseudoplantarum* (*11,12*), neither acetate nor acetaldehyde had any effect on either the total AD or the amount of D produced from pyruvate by pyruvate-grown cells (Table 1). This may be attributed to a capability of the strain to remove added aldehydes by their reduction to the corresponding alcohols (*14*). Pyruvate-grown cells produced only a very little amount of AD from citrate.

The abilities of whole-cell suspensions of pyruvate-grown cells of *L. casei*, *L. plantarum* and *S. lactis* subsp. *diacetylactis* to produce AD from pyruvate were compared. The results are shown in Table 2. *L. casei* showed considerable higher activity than either *L. plantarum* or *S. lactis* subsp. *diacetylactis*. The rates of AD production by *L. plantarum* and *S. lactis* subsp. *diacetylactis* represented only 69.7 and 6.6%, respectively, of that presented by *L. casei*. These were 0.075 μmoles/mg dry wt^{-1} ml^{-1} min^{-1}

TABLE 2. *The rate of acetoin and diacetyl production (μmoles/mg dry wt^{-1} ml^{-1} min^{-1}) from pyruvate by whole cell suspensions of pyruvate-grown* L. casei, L. plantarum *and* S. lactis *subsp.* diacetylactis.

Species	AD	Activity level
L. casei	0.075	100.0[a]
L. plantarum	0.053	69.7
S. lactis subsp. *diacetylactis*	0.005	6.6

[a]Set as arbitrary 100% standard.

for *L. casei*, 0.053 for *L. plantarum* and 0.005 for *S. lactis* subsp. *diacetylactis*.

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