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Potassium Acetate Inhibition of *Lactobacillus casei*. II. Strain Differences. (23761)

M. N. CAMIEN AND M. S. DUNN

Chemical Laboratory, University of California, Los Angeles

It was reported in the first paper of this series(1) that *Lactobacillus casei* 7469 is markedly inhibited by the dual but not separate action of potassium and acetate ions. The inhibition was completely reversed by either lithium or sodium ions and by various fatty acids(1). Occasional failures of potassium acetate to inhibit *L. casei* growth were encountered during the course of this work but were not reported(1) because it appeared then that they were due merely to technical difficulties. It has now been found, however, that strains of *L. casei* differing markedly among themselves in sensitivity to potassium acetate are derivable from our stock culture, and it seems likely on the basis of these findings that our earlier variable results were caused by fortuitous population differences in the experimental inocula.

Methods. The stock culture of *L. casei* 7469 employed in these studies has been maintained in this laboratory since 1943 as described previously(2), with the exception that since 1948 fresh cultures have been prepared only every 3 weeks (originally this was done every 2 weeks). The possibility of accidental contamination during this period was kept at a minimum by preparing 2 or more stock cultures at each transfer time. Successive stock transfers were made only from the original, unused culture in each case, whereas transfers to experimental media were made exclusively from duplicates of this culture. The stock culture was never purified because it seemed likely that frequent and random selection of single cells for this purpose might lead to early divergence of originally identical strains in different laboratories. The *population characteristics* of this *L casei* culture with respect to potassium acetate inhibition were determined as follows. Cell suspensions prepared by transferring growth from the stock culture (using a sterile wire) to sterile saline solution were diluted appropriately and plated out in the same medium as that used in maintaining the stock culture (Yeast Dextrose Agar-Difco). The plates were incubated 40 hours at 35° under carbon dioxide, and growth from isolated colonies was transferred either directly to liquid inoculum medium or (by stab) to Yeast Dextrose Agar. Inocula prepared either directly from colony growth or from the stab cultures derived from such growth were tested by the procedures employed previously(1).

Results. Screening of approximately 1000 freshly isolated cultures in several independent experiments over a 3 months period revealed that from 6% to 45% of the isolates in a given series were resistant to inhibition by potassium acetate. The remaining isolates were not only highly sensitive to potassium acetate, but failed to grow normally even at the usual relatively low levels of potassium and acetate unless provided with a supplement of DL-lactate (the effects of sodium and lithium ion(3) were not determined in

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TABLE I. Concentrations of DL-Lactate (C_3) and DL-α-Hydroxycaprylate (C_8) Yielding Half Maximal Response with Different Strains of *L. casei* in Media with Different Potassium and Acetate Concentrations.*

	mμmols/ml					
	Medium 1		Medium 2		Medium 3	
Strain No.	C_3	C_8	C_3	C_8	C_3	C_8
686–21	85	31	186	68	324	>102
22	81	23	186	68	269	"
25	96	28	186	76	251	"
27	96	35	186	96	349	"
29	101	32	186	71	251	"
30	85	28	166	68	288	"
31	71	20	138	59	224	"
33	76	20	178	50	209	"
53	76	23	155	59	178	"
55	96	32	186	81	339	"

* Media 1-3 were the same as the medium used previously(1) except for variations in potassium and acetate content. Potassium ion and acetate ion concentrations in the 3 media, respectively, were: Medium 1, 9.4 and 146 μmols/ml; Medium 2, 80 and 146 μmols/ml, and Medium 3, 130 and 296 μmols/ml. Medium 1 contained more sodium ion by 70.6 μmols/ml than media 2 and 3.

these experiments, and acids other than DL-lactic acid were not extensively investigated). The amounts of either DL-lactate or DL-α-hydroxycaprylate required to effect half maximal growth of ten typical sensitive strains in media containing different concentrations of potassium and acetate ions are listed in Table I. It may be seen (Table I) that the inhibitory effects of potassium and acetate on these strains were reversed by elevating the supply of α-hydroxy acid.

Since it had now become evident that potassium acetate resistant forms abounded in the experimental inocula used previously (1), it seemed likely that their development was seriously impeded in the presence of the potassium acetate sensitive forms. This conclusion was verified experimentally by introducing varying numbers of cells of a typical resistant strain (strain 686-35) into a test medium with and without the addition of a constant number of cells of a typical sensitive strain (strain 686-30) with the results shown in Fig. 1. It may be seen (Fig. 1) that in the test medium containing 6 x 10^6 cells of strain 686-30 per ml, 40 hour growth of strain 686-35 inocula of less than 4 x 10^5 cells per ml was markedly inhibited. When potassium acetate was added to the test medium this effect was augmented as shown in Fig. 2. In this case it may be seen (Fig. 2) that 40 hour growth of strain 686-35 from an inoculum of 8 x 10^5 cells per ml was reduced from maximal to nearly negligible by the presence of 6 x 10^6 cells of strain 686-30 per ml.

It is evident from the foregoing results that growth of the sensitive and resistant strains is markedly interdependent in α-hydroxy acid-free media. The former organisms depend on the latter to produce growth promoting material,† but under unfavorable conditions growth of the latter is inhibited by the presence of the former. A reasonable hypothesis based on these considerations is that

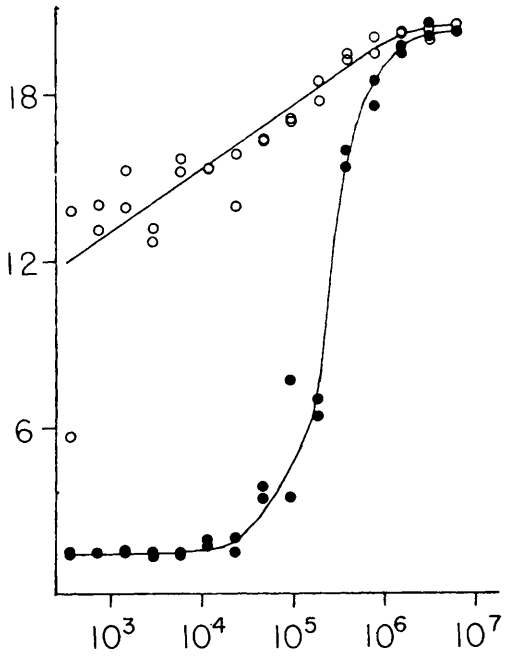

FIG. 1. Production of acid by graded inocula of strain 686–35 after 40 hr incubation in Medium 2 (see Table I) alone (○) and with the addition of 6 × 10^6 cells of strain 686–30/ml (●). Values on vertical scale represent acidity calculated as ml of 0.01 *N* sodium hydroxide required to titrate 1 ml of culture. Values on horizontal scale represent the numbers of cells of strain 686–35 introduced per ml.

† That *L. casei* normally produces a substance with hydroxy acid-like growth promoting activity has been recognized for several years(4). This substance was originally thought to be D-lactic acid, but it is now known that D-lactic acid is not formed in measurable amounts by *L. casei* and that the growth promoting substance is a more lipophylic acid(5).

the sensitive strains elaborate toxic material when incubated in α-hydroxy acid-free media but not when a source of this growth promoting material is provided. Potassium acetate inhibition of mixed cultures, according to this hypothesis, could result from the increased α-hydroxy acid demand of the sensitive strains in high potassium acetate media (Table I). It also seemed possible that production of growth promoting material by the resistant strains might be impeded at elevated potassium acetate concentrations. The latter hypothesis was tested by culturing three freshly isolated resistant strains (strains 697-7, 697-16 and 697-32) in a) Medium 1 (Table I), b) Medium 3 (Table I), and c) Medium 3 with the addition of 10 mμ mol of linoleic acid per ml. The 40 hour cultures were clarified by centrifugation and assayed for growth promoting activity with strain 280-16A(6). The results shown in Table II indicate not only an appreciable inhibitory effect of potassium acetate on proluction of growth promoting activity (growth and total acid production are apparently not affected),

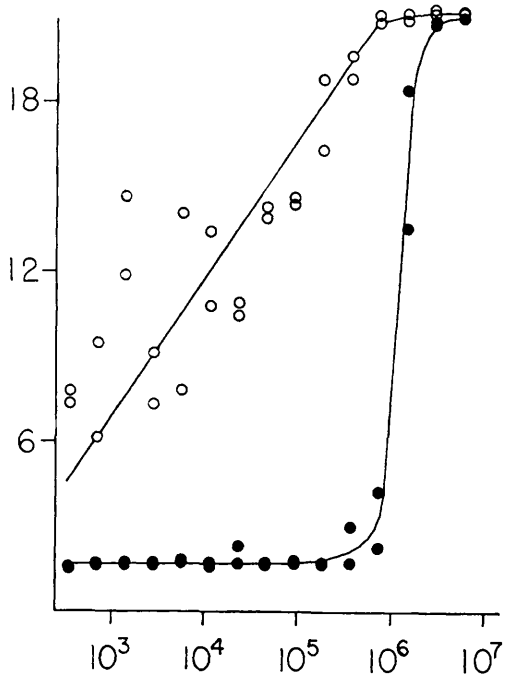

FIG. 2. Same as Fig. 1 with the exception that Medium 3 (Table I) was used in place of Medium 2.

TABLE II. Growth Promoting Activity Produced by Different Strains of *L. casei* in Different Media.*

Strain No.	μeq†/ml Medium 1	Medium 3	Medium 4
697- 7	8.3	5.4	7.0
16	7.8	5.4	5.6
32	7.6	5.1	5.6

* Media 1 and 3 were the same as those described in Table I. Medium 4 contained 10 mμmols of linoleic acid/ml but was otherwise the same as Medium 3.

† Growth promoting activity for strain 280–16A equivalent to that per μeq of D-lactic acid.

but also a considerable reversal of this effect in one of the strains (strain 697-7) by linoleic acid.

Discussion. It is clear from the experimental results that potassium acetate inhibition and its reversal by various fatty acids as described previouly(1) is not characteristic of any single variety of *L. casei,* but rather is characteristic of a somewhat narrowly defined natural mixture of *L. casei* varieties. When the ratio of sensitive cells to resistant cells in the mixed inoculum is less than approximately 8:1 (Fig. 2), little or no inhibition due to potassium acetate is apparent. Mixed inocula with higher ratios of sensitive to resistant cells are markedly inhibited by potassium acetate, and reversal of the inhibition by either fatty acids or lithium and sodium ions(1) is evidently dependent on complex interactions of the different *L. casei* varieties.

It may be concluded from the present data that α-hydroxy acid-dependent varieties of *L. casei* occur naturally and differ only in minor respects, if at all, from strain 280-16 (4), which has previously been supposed to have resulted from an ultraviolet irradiation-induced mutation. The relatively high proportion of dependent varieties characteristic of our stock culture of *L. casei* is not, however, characteristic of 2 other stock cultures of *L. casei* which we have investigated recently in this regard. One of the latter, obtained through the courtesy of Dr. S. Shankman,‡ originated from our stock in 1946, but has subsequently been carried in Yeast Dextrose Agar modified by the addition

‡ Shankman Laboratories, Los Angeles.

of 0.1% sodium acetate. The other was obtained as a lyophilized preparation (catalog number 7469) from the American Type Culture Collection. No dependent varieties were found among 60 isolates from each of these cultures. The question of whether our stock of *L. casei* contained a high proportion of dependent varieties in 1946 (when the Shankman stock originated from it) is apparently unanswerable, but it seems likely that this was true of our stock in 1952 on the basis of published data(4). It was reported(4) that 15 hour growth (but not later growth) of the "parent culture" was markedly stimulated by lactic acid, whereas we now know (unpublished data) that even the early growth of purified independent strains is not stimulated by α-hydroxy acids, and it may be inferred from this difference that the "parent culture"(4) contained a considerable proportion of dependent forms.

Summary. The stock culture of *L. casei* 7469 carried in this laboratory contained 6 to 45% (in different experiments) of forms resistant to inhibition by potassium acetate, yet inocula containing up to 12% of the resistant forms were markedly inhibited by potassium acetate apparently because of complex interactions between the resistant and sensitive varieties. Sensitive strains were found to be α-hydroxy acid-dependent (formerly this characteristic has been ascribed only to "mutant" strains). Sensitive strains, although predominating in the authors' stock culture, were not found among 60 isolates from either of two outside cultures of *L. casei* 7469.

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Renal Response to Non-Shocking Hemorrhage. Sodium Retention at Constant Perfusion Pressure.* (23762)

ALLAN V. N. GOODYER,† LOUIS R. MATTIE,‡ AND ALLEN CHETRICK§
(Introduced by Philip K. Bondy)
Department of Internal Medicine, Yale University School of Medicine, New Haven, Conn.

Non-shocking hemorrhage (20 cc/kg for 35 minutes) decreases renal excretion of sodium despite autonomic blockade or renal denervation(1). Excretion of sodium increases again *during retransfusion* of the shed blood even when this is accomplished at a constant renal arterial blood pressure by inducing extrarenal vasodilatation with hexamethonium. The present experiments were carried out in order to study renal excretion of sodium *during hemorrhage* at constant arterial pressure, under conditions where direct effects on the renal tubules of pharmacologic agents, or of the shed blood itself, were avoided.

Methods. Acute experiments were performed in dogs weighing from 10 to 20 kg anesthetized with 60 mg/kg of Dial intravenously. Inulin and para-aminohippurate in a solution of isotonic saline were administered via one femoral vein by means of a constant infusion pump. Renal arterial pressure was continuously recorded and monitored via a catheter inserted to the level of the renal vessels through the femoral artery. In some experiments the brachial artery pressure was recorded as an index of changes of the superior aortic blood pressure. A Sanborn Twin-Viso recorder and amplifiers were used with a Hathaway pressure capsule. Urine was col-

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