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Difference Between Manganese Ion Requirements of Pediococci and Enterococci

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For maximal turbidity and dissimilatory activity, three strains of pediococci required the addition of Mn^{2+} to Rogosa basal medium. Twenty-two strains of enterococci proved indifferent to this supplement.

In a comparative investigation of the physiological properties of a collection of enterococci and pediococci, we obtained excellent growth for both bacterial groups by using the Rogosa broth medium (RBM) (2, 3). Our enterococci included twenty-two strains representing various types. The pediococci consisted of three strains of *Pediococcus cerevisiae*.

The omission from RBM of added Mg, Fe, and Mn salts and of cysteine did not affect significantly the amount of growth of all the enterococci. It did cause, however, scanty growth in the pediococci.

We pursued an analysis of this nutritional difference by employing various modifications of the RBM. This was done to establish the effect of a single component on the overall metabolism of the strains.

Inocula were grown in unmodified RMB (Table 1). Cells from 24-hr single tube cultures were harvested by centrifugation and were suspended in 100 ml of phosphate-buffered (*p*H 7.4) saline. One drop of the suspension from a sterile Pasteur pipette was dispensed into 10 ml of each of the media listed in Table 1. Metabolic activity was assessed at 2, 4, 6, 8, 12, and 24 hr, by turbidimetric measurements and by determination of the *p*H of the cultures. The 24-hr results for the three pediococci and one of the tested strains of enterococci (*Streptococcus faecium* ATCC 6057) are given in Table 2.

The omission of added Mg, Fe, and Mn salts, although drastically reducing the growth of the pediococci, had no appreciable effect on the *S. faecium* (Table 2; modified medium 1). acid (EDTA) caused a marked reduction of growth in the enterococcus (Table 2; medium 5), indicating that the basal RBM contained sufficient concentrations of cations for maximal growth. Selective omissions and additions (Table 2; media 2 through 7) traced the observed effect on the pediococci primarily to a single component, Mn. The addition of either Mg (Table 2; medium 6) or Fe salt (Table 2; medium 7) to the basal RBM had little or no effect on the extent of growth of the strains. The addition of Mn proved essential for comparable levels of growth (Table 2; medium 2 versus control). In the absence of cysteine (Table 2; medium 3), Mn could maintain only a moderate level of development. The requirement for added Mn could not be replaced by Zn (Table 2; medium 4). The addition of EDTA (Table 2; medium 5) sharply reduced the final optical density of the cultures, especially of strain 25, which probably had much higher requirements for cations (including Mn) than the other pediococci.

The Mn^{2+} requirement may indicate a specific mineral deficiency which could arise from the presence of citrate acting as a chelating agent. MacLeod and Snell (5) showed in *Lactobacillus arabinosus* that much larger concentrations of Mn^{2+} are required to permit growth when citrate is present. The same workers showed that *S. faecalis* did not have this requirement. Niven and Evans (6) described a Mn requirement for *L. viridescens*.

The nature of the stimulatory effect of Mn^{2+} on pediococci may merit further examination for two utilitarian reasons. One is the difficulty, on primary isolation, to distinguish between strains of pediococci and *S. faecium*; both these groups have many characteristics in common (1), and the criteria of their differentiation, presently in use, are limited in number and scope (7). The second is the possibility of improving the selectivity of enterococcus media (4) by controlling the Mn content in the

TABLE 1. *Composition of various modifications of Rogosa broth medium for the growth of pediococci and enterococci*

Component	Concn	Unmodified control	Modification[a]						
			1	2	3	4	5	6	7
Trypticase	10 g	+	+	+	+	+	+	+	+
Yeast extract, dehydrated	5 g	+	+	+	+	+	+	+	+
Tryptose	3 g	+	+	+	+	+	+	+	+
K_2HPO_4	3 g	+	+	+	+	+	+	+	+
KH_2PO_4	3 g	+	+	+	+	+	+	+	+
NH_4 citrate	2 g	+	+	+	+	+	+	+	+
Salt solution:	5 ml								
Tween 80	1 g	+	+	+	+	+	+	+	+
Na acetate	1 g	+	+	+	+	+	+	+	+
Glucose	20 g	+	+	+	+	+	+	+	+
Cysteine	0.2 g	+	+	+	−	+	+	+	+
Water[b]	to 1,000 ml	+	+	+	+	+	+	+	+
Salt solution:									
$MgSO_4 \cdot 7H_2O$	11.5 g	+	−	−	−	−	−	+	−
$FeSO_4 \cdot 7H_2O$	0.68 g	+	−	−	−	−	−	−	+
$MnSO_4 \cdot 2H_2O$	2.4 g	+	−	+	+	−	+	−	−
Water[b]	to 100 ml	+	+	+	+	+	+	+	+
Other additives:									
EDTA[c]	0.064 mM	−	−	−	−	−	+	−	−
Zn^{2+}	0.064 mM	−	−	−	−	+	−	−	−

[a] +, Component added; −, component omitted; in all cases the *p*H of the medium was adjusted to 6.8.
[b] Deionized, all-glass-distilled water.
[c] Ethylenediaminetetraacetic acid; disodium salt.

TABLE 2. *Metabolic response of pediococci and enterococci to various modifications of Rogosa broth medium*[a]

Strain	Unmodified control[b]		Modification[b]										
			1		2		3	4	5	6		7	
	OD	*p*H	OD	*p*H	OD	*p*H	OD	OD	OD	OD	*p*H	OD	*p*H
P. cerevisiae:													
ATCC8081	331	3.9	47	5.2	336	3.9	270	65	210	41	5.1	81	4.7
ATCC10791	384	4.0	71	5.0	374	4.0	290	75	240	66	4.9	80	4.7
25	197	4.0	68	4.9	219	4.0	145	60	10	60	4.9	87	4.7
S. faecium:													
ATCC6057	126	4.3	112	4.4	126	4.4	135	130	90	116	4.4	115	4.4

[a] Optical density (OD) at 600 nm: × 100 × dilution factor (1 or 10, using uninoculated RBM as diluent). Averages of quadruplicate determinations obtained after 24 hr of incubation at 37 C for the enterococci and 32 C for the pediococci.
[b] Refers to the same medium listed in Table 2.

presence of citrate. In this manner, growth of pediococci and related lactic acid bacteria may be effectively suppressed without adversely affecting the growth of enterococci.

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