MANGANESE REQUIREMENT OF THE TRANSCRIPTION PROCESSES IN *LACTOBACILLUS CURVATUS*

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1. Introduction

The requirement of lactobacilli for manganese has been known for a long time [1]. Media for lactobacilli therefore contain about 10^{-4} M Mn^{2+}. The site of action of manganese, however, is not known although it was recently found [2] that *Lactobacillus casei* possesses a lactic acid dehydrogenase activated by Mn^{2+} *in vitro*.

In studies on the induction of lactic acid racemase and of ribose fermentation in *Lactobacillus curvatus*, it was found that both processes are dependent on Mn^{2+}. From the kind of interaction between the inductor, actinomycin D and Mn^{2+} described in this paper, we conclude that Mn^{2+} is involved in the transcription process of *L. curvatus* possibly via a manganese-dependent RNA polymerase.

2. Material and methods

L. curvatus DSM* 20010 isolated and described by Abo-Elnaga and Kandler [3] was grown in MRS-medium [4] at 30°C without aeration.

Quantitative determination of manganese was carried out by means of atomic absorption spectrometry. L(+)– and D(–)-lactate were determined enzymatically [5].

* Deutsche Sammlung für Mikroorganismen.

3. Results and discussion

L. curvatus forms only L-lactic acid in the early logarithmic growth phase. When about 0.1% L-lactic acid is produced, lactic acid racemase is induced resulting in a complete racemisation of lactic acid [7]. In MRS-medium without manganese, growth stops when about half of the normal density is reached. No racemisation occurs although fermentation continues until 0.3–0.8% of L(+)-lactic acid is produced. Only very little D(–)-lactic acid is formed due to a very weak D(–)-lactic acid dehydrogenase [6].

Table 1 shows the response of growth and racemisation to various concentrations of Mn^{2+}. When the original Mn^{2+} concentration of the medium, 5.5×10^{-7} M, is increased by about 50%, a significant increase of growth and racemisation occurs. This effect is rather specific for Mn^{2+} (table 2). None of the other divalent cations tested shows any effect. Mg^{2+} which is added to the MRS-medium routinely at a concentration of 8×10^{-4} M, has no influence of racemisation. To decide if manganese is a cofactor or an effector of lactic acid racemase or if it is necessary for the induction process, glucose fermentation was carried out in buffer solution using cells grown in MRS-medium with or without manganese. As shown in table 3 no racemisation occurred although Mn^{2+} was present during fermentation in the buffer solution. This excludes an activation of the racemase by Mn^{2+} but speaks for its participation in the induction process. This assumption is also in agreement with the finding (table 1) that racemisation occurs, if the inductor (at least 0.1% of L(+)-lactic acid) is added to the manganese-deficient medium together with the inoculum. Due to the very

Table 1
Formation and racemisation of lactic acid by *L. curvatus* at different concentrations in MRS-basal medium containing 8×10^{-4} M Mg^{2+}.

Mn^{2+} added	Final concentration of Mn^{2+} (M)	Growth* ΔA_{578}	Lactic acid		
			Total mg/ml	L(+)– %	D(–)– %
0.0	5.50×10^{-7}	0.70	4.90	95	5
6×10^{-4}	6×10^{-4}	1.85	10.39	53	47
3×10^{-4}	3×10^{-4}	1.85	7.84	53	47
3×10^{-5}	3.1×10^{-5}	1.75	8.06	48	52
3×10^{-6}	3.6×10^{-6}	1.48	6.85	51	49
2×10^{-6}	2.6×10^{-6}	1.40	6.29	51	49
1×10^{-6}	1.6×10^{-6}	1.26	5.55	59	41
6×10^{-7}	1.2×10^{-6}	1.17	5.15	72	28
3×10^{-7}	8.5×10^{-7}	1.04	4.82	85	15
3×10^{-8}	5.8×10^{-7}	0.75	4.76	99	1
3×10^{-9}	5.53×10^{-7}	0.75	4.62	92	8
$0.0 + 10^{-2}$ M L(+)-lactic acid	5.50×10^{-7}	0.72	4.66	55	45

* Turbidity in stationary phase after 30 hr incubation.

low unavoidable manganese content of the medium, growth and induction occur, but growth stops at the same density as in other manganese-deficient samples when manganese becomes limiting. When no L(+)-lactic acid is added, induction cannot occur although the concentration of L(+)-lactic acid reaches 0.3–0.8% during the stationary phase. This discrepancy can easily be explained by the assumption that the manganese supply is already exhausted before the inducing concentration of 0.1% L(+)-lactic acid is reached and that the further increase of lactic acid is due to fermentation without growth.

Table 2
Influence of various divalent cations on racemisation of lactic acid by *L. curvatus* in MRS basal medium, containing 8×10^{-4} M Mg^{2+}.

Cation 3×10^{-4} M	Lactate mg/ml	L(+)– %	D(–)– %
no	2.33	97	3
Ca^{2+}	1.96	97	3
Co^{2+}	1.97	96	4
Fe^{2+}	1.97	96	4
Cu^{2+}	1.93	96	4
Mn^{2+}	8.85	52	48
Zn^{2+}	1.84	97	3

To test this assumption, cells were grown in MRS-medium without manganese for 42 hr. The L(+)-lactic acid concentration was far above the necessary concentration for induction, namely 0.3%, but no induction took place. Then the pH was adjusted to 6.4 by NaOH and 3×10^{-4} M Mn^{2+} was added. As shown in fig. 1 the formation of L(+)-lactic acid was immediately enhanced; however, racemisation indicated by the formation of D(–)-lactic acid and a temporary decrease of L(+)-lactic acid started only after 2 hr. The immediate initiation of fermentation is due to the adjustment of the pH and most likely also to the

Table 3
Formation of L(+)– and D(–)-lactic acid in phosphate buffer (pH 6.4; 30°C; 1% glucose; 14 hr) by washed cells of *L. curvatus* after growth with ot without Mn^{2+}.

Mn^{2+} during		Lactic acid formed	
Growth	Fermentation	L(+)– mg/ml	D(–)– mg/ml
0.0	0.0	2.7	0.05
	3×10^{-4} M	2.9	0.1
3×10^{-4} M	0.0	3.0	2.6
	3×10^{-4} M	2.3	1.8

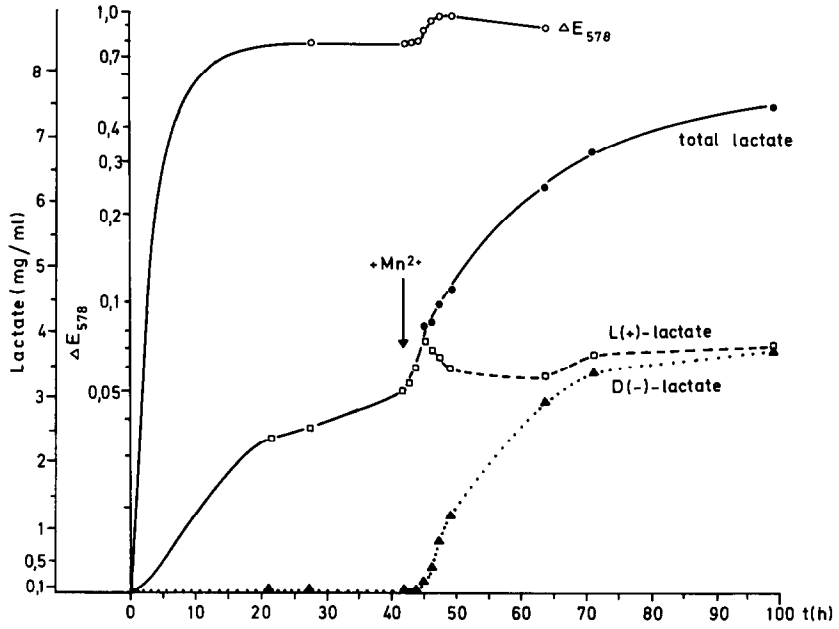

Fig. 1. The effect of the addition of Mn^{2+} (3×10^{-4} M) on the formation of lactic acid by *L. curvatus*, grown in a manganese-deficient medium: (○—○—○) Growth (ΔA_{578}); (□—□—□) L(+)-lactate; (△—△—△) D(−)-lactate; (●—●—●) total lactate.

activation of L-lactic acid dehydrogenase by Mn^{2+}, whereas the delay of racemisation may indicate the necessity of an induction. The lag period is relatively long, since stationary cells were used.

When chloramphenicol (50 μg/ml) was added simultaneously with manganese, no racemisation occurred, but the increase of L(+)-lactic acid was the same. Also actinomycin D inhibits racemase induction completely, when this specific inhibitor of DNA-dependent RNA biosynthesis is added together with manganese. Actinomycin D strongly inhibits even if it is added 1 hr after manganese whereas the addition after 2 hr has almost no effect (fig. 2). These data are explained best by the assumption, that Mn^{2+} is involved in the induction process, most likely on the level of transcription.

To investigate whether the manganese requirement is restricted to the induction of racemase, we have

Table 4

Ribose fermentation of glucose grown cells during and after incubation with ribose in the presence or absence of Mn^{2+} or actinomycin D.

Adaptation medium contained	Lactic acid formed					
	During adaptation			During fermentation		
	Total mg/ml	L(+)- %	D(−)- %	Total ng/ml	L(+)- %	D(−)- %
Glucose + Mn^{2+} (control)	4.7	65	35	Trace	–	–
Ribose, no Mn^{2+}	0.4	96	4	Trace	–	–
Ribose + Mn^{2+}	1.1	82	18	0.75	47	53
Ribose + Mn^{2+} + actinomycin D	0.5	96	4	Trace	–	–

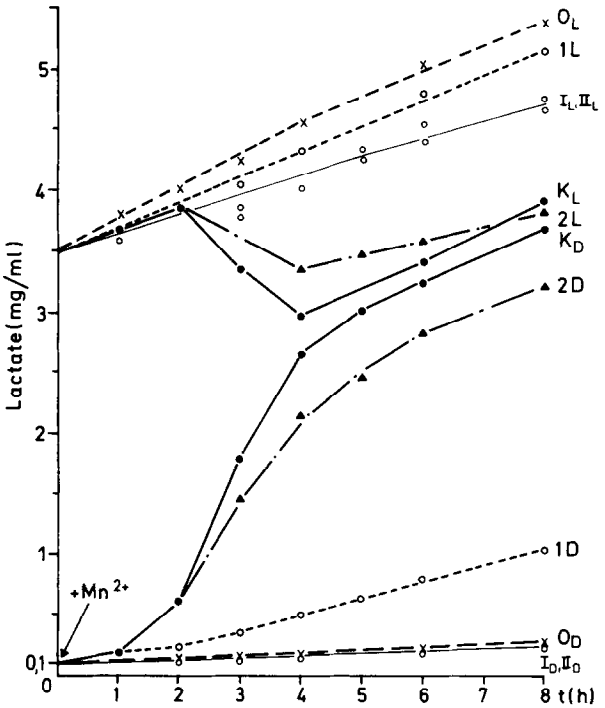

Fig. 2. The effect of the addition of Mn^{2+} (3×10^{-4} M) and of actinomycin D (1 μg/ml) on the formation of lactic acid by *L. curvatus*: I = without Mn^{2+} without actinomycin D; II = without Mn^{2+} with actinomycin D; K = with actinomycin D; 0 = with Mn^{2+} with actinomycin D added at zero time; 1 = with Mn^{2+} with actinomycin D added 1 hr after Mn^{2+}; 2 = with Mn^{2+} with actinomycin D added 2 hr after Mn^{2+}; L = L(+)-lactate; D = D(−)-lactate.

studied the effect of manganese on ribose fermentation, which was known from previous unpublished work to be an adaptive property of *L. curvatus*. Cells grown in MRS-medium without manganese were harvested in the stationary phase and inoculated after 5-fold concentration in a ribose-containing medium completely depleted of manganese** (adapt medium). After 6 hr the cells were harvested by centrifugation and transferred in phosphate buffer pH 6.4 which contained 0.5% ribose. Lactic acid was determined in the 'adaptation medium' as well as in the buffer solution after 2 hr incubation. As shown in table 4 only the sample which contained Mn^{2+} in the adaptation medium fermented ribose in the buffer and formed both isomers in both the adaptation medium and the buffer solution. When actinomycin D was added to the adaptation medium in addition to Mn^{2+}, no adaptation to ribose occurred. Therefore the manganese requirement for induction is not restricted to the induction of lactic acid racemase but may be a general phenomenon in *L. curvatus* or even in all the lactobacilli. Considering the site of action of manganese one may suggest either a manganese requirement of the repression mechanism or of the RNA polymerase. The latter seems more likely since it is the more universal factor common to all induction processes, whereas the various repressor proteins should not have the same property. In general, RNA polymerases do not require manganese but magnesium. Only in eucaryotic cells [7] Mg^{2+} can be replaced by Mn^{2+}. Within the bacteria a manganese-dependent and magnesium-independent RNA polymerase has never been found. It would be a new character which may be even unique for the lactobacilli. Preliminary experiments of Stetter and Zillig (unpublished) with enzyme preparations from *L. curvatus* indicate in fact such an unusual cation requirement of the RNA polymerase. If this can be further substantiated and extended to the other lactobacilli, the molecular basis of the well known nutritional requirement for manganese in the genus *Lactobacillus* would be elucidated.

** The manganese depletion was achieved by growing *L. curvatus* in MRS-medium without manganese and with ribose instead of glucose. After growth has ceased the suspension was centrifuged, the cells were discarded the pH was adjusted to 6.4 and the medium was autoclaved.

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