Note

Effects of Cellular Calcium and Magnesium Contents on Growth of Ionophore A23187-Treated *Lactobacillus lactis*

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In this work the physiology of the divalent cations calcium (Ca^{2+}) and magnesium (Mg^{2+}) has been studied in the Gram-positive lactic acid bacteria *Lactobacillus lactis*, for this bacterium is well-adapted to high external calcium content, namely milk, and because many bacteriophages of lactic acid bacteria have been shown to require calcium for proliferation.^{1,2} On the other hand, magnesium is the major intracellular divalent cation required for the growth of all cells.³

Cellular Ca^{2+} and Mg^{2+} contents of *L. lactis* were manipulated with the divalent cation-specific ionophore A23187⁴) and $\text{CaCl}_2/\text{MgCl}_2$ supplements in the growth medium Lactobacilli MRS Broth (Difco), and the effects of these treatment on *L. lactis* growth were revealed. It is known that A23187 induced Ca^{2+} and Mg^{2+} fluxes through the cell membrane via two proton exchanges.⁵) The magnitude and direction of cation fluxes and consequently their effects on bacterial growth are affected by several factors such as ionophore concentration and magnitudes of divalent cation and proton (H^+) gradients across the cell membrane.⁶)

Figure 1 shows a summary of the growth test experiments. As shown, it was possible to restore the growth of A23187-treated *L. lactis* cells, if enough MgCl_2 (10 mm or more) was present in the growth medium. The dualistic behaviour of a CaCl_2 supplement with MgCl_2 on *L. lactis* growth is obvious: at lower external Ca^{2+} concentrations (less than 20 mm), Ca^{2+} showed synergism with Mg^{2+} , but it was toxic when more than 20 mm CaCl_2 was present in the growth medium (Fig. 1). To confirm this unexpected feature of Ca^{2+} and further to try to understand its basis, a set of experiments including cellular Ca^{2+} and Mg^{2+} content measurements was done (Table I). As shown (control 2 and exp. 1 in Table I), an A23187-mediated reduction of cellular Mg^{2+} content to one third of the control level completely prevented bacterial growth. This occurred even in the presence of 20 mm MgCl_2 in the growth medium (the Ca^{2+} concentration of unsupplemented MRS medium is about 1 mm). With higher concentrations of MgCl_2 (40~60 mm), it was possible to

diminish (but not to prevent) A23187-induced Mg^{2+} leakage by almost a half (about 60% of control value) and bacterial growth was then no longer considerably affected (control 2 and exps. 2 and 3 in Table I). When the growth medium for A23187-treated cells was supplemented with varying concentrations of CaCl_2 (up to 40 mm) together with 20 mm MgCl_2 , the cellular Ca^{2+} content increased linearly with increasing external CaCl_2 concentrations, but the cellular Mg^{2+} content was constantly about one third of the control Mg^{2+} level, as might be expected (controls 1 and 2 and exps. 4~7 in Table I). Again the dualistic feature of Ca^{2+} on bacterial growth after A23187 treatment was evident: when Ca^{2+} was present at concentrations of 20 mm or less in the growth medium, bacterial growth was promoted (exps. 4 and 5), but at higher external concentrations (more than 20 mm CaCl_2), strong growth inhibition was observed (exps. 6 and 7). The growth of *L. lactis* continued if about half of the original Mg^{2+} content was preserved after A23187-induced Mg^{2+} leakage. Even one third of the control Mg^{2+} content was enough for bacterial growth, if an appropriate Ca^{2+} influx accompanied A23187 treatment (Table I).

The results show that Ca^{2+} cannot completely substitute for the functions of Mg^{2+} in *L. lactis*, although it shows some synergism with Mg^{2+} (Fig. 1 and Table I). It

FIG. 1. The Dualistic Effects of CaCl_2 Supplement with MgCl_2 in Growth Medium on the Growth of A23187-Treated *Lactobacillus lactis*.

100 μl of *L. lactis* culture in logarithmic growth phase in MRS medium were added to 5 ml MRS medium supplemented with different concentrations of CaCl_2 and MgCl_2 and with 1.5 $\mu\text{g}/\text{ml}$ A23187 (solid line). In controls (dotted line), an appropriate volume of ethanol (ionophore solvent) was used. Cultures were incubated for 24 hr at 37°C and the turbidities of the cultures were measured at 600 nm (OD_{600}). Symbols for MgCl_2 concentrations in MRS growth medium: (●), no MgCl_2 ; (○), 2 mm MgCl_2 ; (Δ), 10 mm MgCl_2 ; (▽), 20 mm MgCl_2 ; (□), 40 mm MgCl_2 .

TABLE I. EFFECTS OF EXTERNAL MAGNESIUM AND CALCIUM CONCENTRATIONS ON CELLULAR MAGNESIUM AND CALCIUM CONTENTS AND ON THE GROWTH OF *Lactobacillus lactis* CULTURES AFTER A23187 TREATMENT

Lactobacillus lactis was grown in MRS medium supplemented with 20 mM MgCl₂ or CaCl₂ at 37°C. After an OD₆₀₀ of 0.3 was reached, 3 µg/ml A23187 (exps. 1~8) and different concentrations of MgCl₂ or CaCl₂ were added. In controls, instead of A23187 an equal amount of ionophore solvent ethanol was added. After 40 minutes, samples were withdrawn for cellular Mg and Ca measurement. The growth of bacterial cultures was followed by measuring an increase in OD₆₀₀ 24 hr after A23187 addition. Identification of cellular cation content of *L. lactis* has been described elsewhere.⁷⁾

Experiment	External concentration (mM)			Cellular cation content (nmol/mg dw)			Increase of OD ₆₀₀
	Mg	Ca	Sum	Mg	Ca	Sum	
Control 1	2	20	22	195	25	220	0.9
Control 2	20	1	21	160	<10	160~170	0.9
Exp. 1	20	1	21	55	<10	55~65	0.1
Exp. 2	40	1	41	90	<10	90~100	0.8
Exp. 3	60	1	61	100	<10	100~110	0.8
Exp. 4	20	10	30	60	20	80	0.45
Exp. 5	20	20	40	45	50	95	0.55
Exp. 6	20	30	50	50	60	110	0.2
Exp. 7	20	70	60	55	100	155	0.15
Exp. 8	2	20	22	20	200	220	0.05

is possible that Ca²⁺ is able to directly take care of some of the functions originally carried out by Mg²⁺ in *L. lactis*. However, we cannot exclude the possibility that, at least partially, the synergistic effect of Ca²⁺ is caused by the A23187-mediated Ca²⁺/2H⁺ exchange,⁴⁾ which might help the cell in the re-balance of intracellular pH, disturbed by Mg²⁺ efflux concomitant with H⁺ influx. The results show that it is possible to increase cellular Ca²⁺ content to a considerable extent (up to 40~50 nmol/mg dry weight, Table I) without any drastic effect on *L. lactis* growth. The toxic effects of high cellular Ca²⁺ contents (more than 50 nmol/mg dry weight, Table I) may be a result of the limited solubility of Ca²⁺ in cellular sap. Possibly the formation of intracellular Ca²⁺ phosphate precipitate may be involved.

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