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Mg^{++} POSITIVELY REGULATES FERMENTATION EFFICIENCY IN *ZYMOMONAS MOBILIS*

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EARLIER reports have suggested that increased initial glucose concentration in the fermentation medium decreases ethanol production[1,2] in *Zymomonas mobilis* by destabilizing the cell wall[3]. In addition, it is also known that the addition of Mg^{++} restores the cell wall[4–6] and improves the glucose uptake rate[7–10]. Our studies on the fermentation pattern of a flocculent, a non-flocculent and two spontaneous Tet^r mutants (GK 101 and GK 104, 50 μg/ml) of a flocculent strain of *Zymomonas mobilis* using sucrose as the substrate, suggests that Mg^{++} addition enhances the fermentation efficiency in the flocculent strain and its Tet^r derivatives.

The fermentation medium contained 1% yeast extract, 1% peptone and 15% (w/v) sucrose (15% w/v initial sugar concentration was found to be optimum for ethanol production at 72 hr in all our strains[11]). Sterile $MgSO_4 . 7H_2O$ was added to the fermentation medium whenever required. Exponentially growing cells (10% v/v) were inoculated into the fermentation medium and the fermentation carried out at 30°C. The amount of ethanol produced was analyzed with gas chromatography (Model 5830A Hewlett Packard, USA) as reported[12]. The residual sugar was determined by phenol-sulphuric acid method[13].

The fermentation kinetics of the two *Zymomonas mobilis* strains (ATCC 10988 and ATCC 12526) was studied with and without Mg^{++}, to determine its effect on the fermentation process. From the data presented in table 1, it is clear that 0.05 M magnesium sulphate in the fermentation medium, increases the yield of ethanol and substrate utilization by the flocculent strain (ATCC 12526) compared to the control. Although the same was true with the non-flocculent strain (ATCC 10988), the difference is not significant. More than 0.05 M concentration of magnesium sulphate decreased the substrate utilization and fermentation efficiency of both the strains (data not shown). It is thus evident that 0.05 M Mg^{++} is the optimum concentration to achieve the increased ethanol production.

The ethanol production being highly significant with 0.05 M magnesium sulphate in flocculent strain, we studied the effect of using some mutants in the fermentation process. Two spontaneous Tet^r mutants GK 101 and GK 104 of the flocculent strain were isolated and the fermentation pattern studied in comparison with the wild type with and without 0.05 M Mg^{++}. The results are shown in figures 1a and b.

As shown in figure 1b, without Mg^{++}, the rate of ethanol production and yield is low in both the mutants as compared to the wild type. This could be due to the fact that both the mutants by themselves were slow growers. The reason for the slow growth might be due to the chelation of Mg^{++} available in the fermentation medium by the formation of Tet-Mg chelation complex[14], with intracellularly accumulated Tet molecules in the mutants, which reduces the availability of Mg^{++} for good growth of Tet^r mutants.

The ethanol yield was almost similar in both flocculent and non-flocculent strains without Mg^{++}. It is interesting that with 0.05 M Mg^{++}, the amount of ethanol produced in 72 hr increased considerably in the flocculent strain and its Tet^r derivatives (figures 1a and b). As can be seen in figure 1a, no significant change in ethanol producing ability of the non-flocculent strain with 0.05 M Mg^{++} was observed.

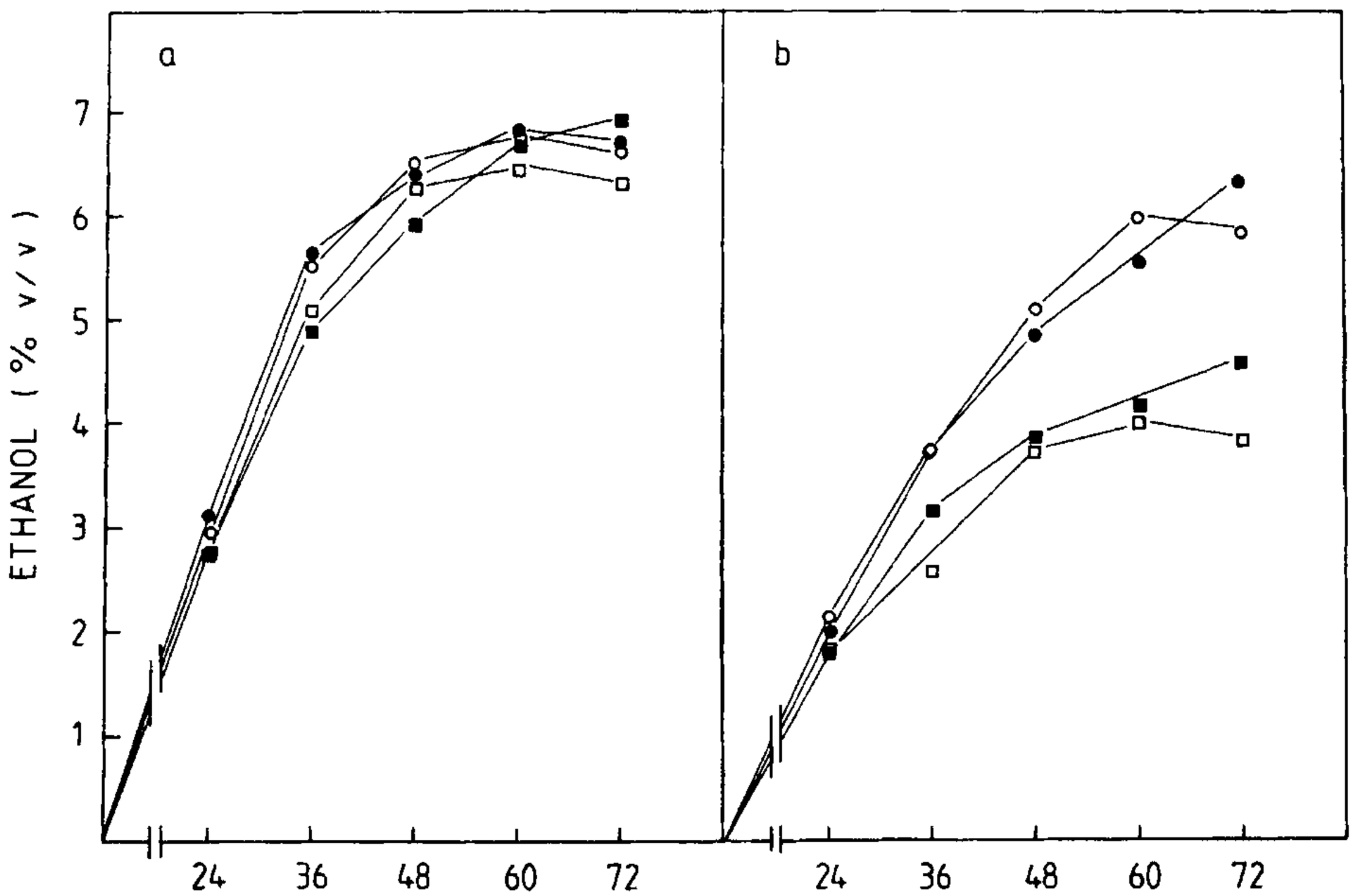
It could be concluded from the above that the fermentation efficiency of the flocculent strain of *Zymomonas mobilis* ATCC 12526 and the Tet^r derivatives of the same increased with 0.05 M magnesium sulphate in the fermentation medium. It is interesting to note that the ethanol production increased also in mutants (the Tet^r mutants by themselves are low ethanol producers, see figure 1b) with 0.05 M Mg^{++}.

In view of this, we suggest that the added 0.05 M Mg^{++} to the fermentation medium might act in two ways. It might restore the cell wall without being destabilized by the increased initial sugar concentration. Secondly, the increased leakage through cell wall allowing loss of co-factors (majority Mg^{++}) required

Table 1 *Effect of Mg^{++} ion on the fermentation pattern of Zymomonas mobilis strains ATCC* 10988 *(non-flocculent) and ATCC* 12526 *(flocculent)*

Strain used	$MgSO_4.7H_2O$ used (Molar)	Parameters studied				
		IS(g/l)	P(v/v)	Su	g/gs	E
ATCC 10988 (non-flocculent)	Control	150	6.6	82.77	0.43	68.75
	0.01	150	6.6	82.77	0.43	68.75
	0.05	150	6.7	84.03	0.43	69.79
ATCC 12526 (flocculent)	Control	150	6.3	79.0	0.44	65.63
	0.01	150	6.6	82.77	0.43	68.75
	0.05	150	6.9	86.54	0.43	71.88

IS (g/l)—initial sugar concentration (g/l); P—ethanol productivity (% v/v); Su—substrate utilized; g/gs—unit ethanol yield per gram of substrate utilized; E—percentage of theoretical yield.



Figures 1a, b. a. Effect of 0.05 M Mg^{++} on the fermentation pattern of wild type strains, control: O, ATCC 10988 (non-flocculent); □, ATCC 12526 (flocculent); after adding 0.05 M Mg^{++}, ●, ATCC 10988; ■, ATCC 12526. **b.** Effect of 0.05 M Mg^{++} on the fermentation pattern of Tet^r derivatives of ATCC 12526, control: O, GK 101; □, GK 104, after adding 0.05 M Mg^{++} ●, GK 101; ■, GK 104.

for the enzymes involved in E-D and glycolytic pathways such as gluco-kinase, glu-6-PO_4 dehydrogenase, phospho glycerate kinase and enolase, due to the accumulated ethanol concentration in the medium[15], is relieved by the addition of 0.05 M magnesium sulphate. Thus Mg^{++} ions satisfy the need of co-factors which are lost due to leakage through cell wall. The added Mg^{++} can also have a role in Tet^r derivatives of the flocculent strain of *Zymomonas mobilis* by the formation of Tet-Mg complex, so, Mg^{++} saturates the available Tet molecules apart from the above two functions.

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EFFECT OF URANYL ACETATE ON THE REPRODUCTIVE ORGANS OF ADULT BAT, *RHINOPOMA MICROPHYLLUM KINNEARI*

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MANY of the metals used in industries, produce toxic effect on the different body organs[1]. Uranium is frequently used as it has great importance in nuclear explosives, excavations, mining, power and isotope production. Uranyl nitrate has been studied for its toxicity[2]. Uranium and other metals have been screened for their toxic and antifertility activity in rats and mice[3]. Uranyl acetate is known to exert effect on skin, kidney and liver[2]. The present paper deals with the effect of uranyl acetate on the reproductive organs of adult bat, *Rhinopoma microphyllum kinneari.*

Freshly captured male and female bats (weighing about 30 g) were allowed to acclimatize to laboratory conditions for 7 days and were divided into six groups of five bats each. Three groups of bats received daily treatment of 1 ml of 1 mM, 0.8 mM and 0.4 mM soln/100 g of body wt of uranyl acetate (in distilled water) through subcutaneous route for 7 days. Other three groups served as controls which similarly received distilled water. All the bats were given 1 ml of 5% glucose solution twice a day through intragastic catheter for the entire period of experimentation. All the bats were sacrificed after 48 hr of the last injection. The uterus of females and testes of the males was perfused with saline followed by perfusion with aqueous Bouin's fluid and then fixed in Bouin's fluid. Paraffin blocks of these materials were sectioned at 5 μ and were stained with haematoxylin and eosin. The stained slides were studied microscopically. Figure 1 shows the histological structure of the testis of a control bat showing all normal structures.

The administration of 1 ml of 1mM solution of uranyl acetate induces shrinkage and wrinkles in the testes. The spermatozoa are absent from the lumen of the tubules and the number of spermatocytes reduced and arranged in only one or two rows (figure 2). The muscle layer around tubules becomes thicker and some show fibrosis. The central tubules show much fibrosis where most of the spermatids have degenerated and some spermatocytes also show necrosis (figure 2). Chromolysis is also observed in some resting spermatocytes. In most of the tubules there are some larger cells with large and dark nuclei which are found