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Full Length Research Paper

The chloride ion been responsible for filament formation and inhibitory effect on cell division in *Zymomonas mobilis* 232B growth

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To elucidate respective effects of sodium and chloride ion on filament formation in *Zymomonas mobilis*232B culture induced by NaCl, the morphological changes of cell grown in medium containing 0.2 M $(NH_4)_2SO_4$, Na^+(as Na_2SO_4) and Cl^-(as NH_4Cl) were observed using a scanning electronic nicroscope (SEM). The occurrence of filament formation began as early as 4 h after exposuring to NaCl and Cl^- and the length was approximate 10 to 15 µm after exposuring for 20 h, which was 3 to 5 fold longer compared to normal cell, while Na^+ and $(NH_4)_2SO_4$ treated cells kept normal morphology, indicating that filament formation in NaCl-inhibited *Z. mobilis* cultures is attributable to chloride ion. In the presence of 0.2 M chloride ion, increase in cell number stop almost immediately, whereas cells continued to elongate slowly, suggesting that chloride ion has a direct inhibitory effect on cell division.

Key words: *Zymomonas mobilis*, chloride ion, filament formation, cell division.

INTRODUCTION

A disadvantage of all *Zymomonas mobilis*, strain is their low tolerance to inorganic ion. Since Swings and De Ley (1977) first reported that only 71% of the strains tested grew in the presence of 1% (w/v) NaCl, whereas none could grow at 2% (w/v), many researchers have reported filament formation was observed in the presence of calcium and sodium salts (Ranatunga et al., 2000; Bajpai and Margaritis, 1984; Kirk and Doelle, 1992). However, the cause of filament formation is poorly understood (Fein et al., 1984; Spangler and Emert, 1986).

In our preliminary work, it had been found that *Z. mobilis* 232B was sensitive to NaCl and filament formation was produced by NaCl but the relative contributions of cation and anion to morphological changes of *Z. mobilis* and cause of forming filaments have not been elucidated clearly. In this work, the effects of sodium and chloride ion on cell morphology were studied separately by comparing NaCl with sodium and chloride ion at similar concentrations in from of Na_2SO_4 or NH_4Cl and cause of forming filaments was also investigated.

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MATERIALS AND METHODS

Media and cell growth conditions

The strain *Z. mobilis* 232B was supplied generously by Center for Applied and Environmental microbiology, Chengdu Institute of Biology, Chinese Academy of Sciences. The strain was anaerobically cultivated at pH 5.5 and 30°C in flasks as described by Vriesekoop et al. (2002). The medium contained (g/L): Glucose, 100; $MgSO_4$, 0.5; KH_2PO_4, 1; $(NH_4)_2SO_4$, 1 and yeast extract, 5. The salinity of the medium was adjusted with 0.2 M of NaCl, $(NH_4)_2SO_4$, Na^+ (as Na_2SO_4) and Cl^- (as NH_4Cl).

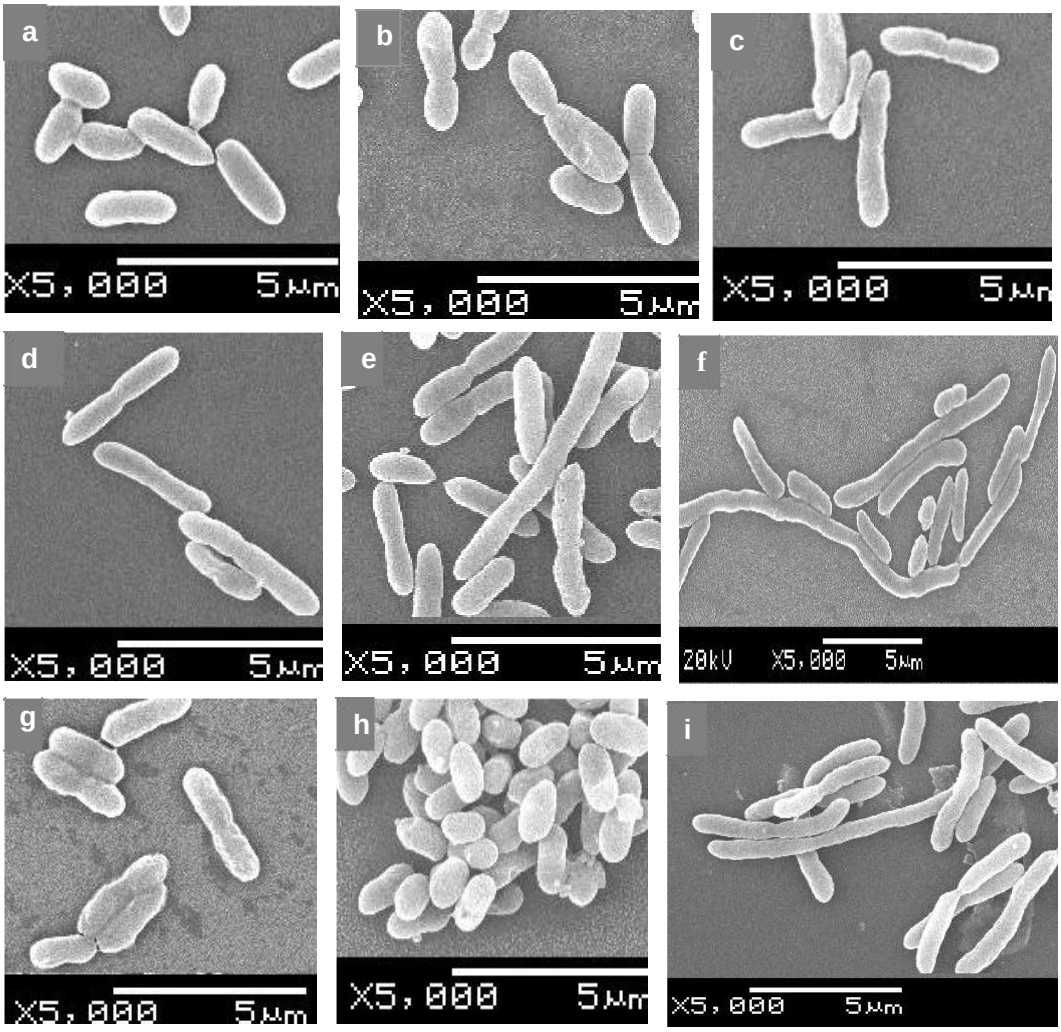

Figure 1. Time course of morphological changes of *Z. mobilis* 232B following exposure to 0.2 M Cl^- (as NH_4Cl) for 0, 4, 8, 12, 16 and 20 h (a-f).The effects of exposure for 20 h to 0.2 M Na^+(as Na_2SO_4)(g), $(NH_4)_2SO_4$(h) and NaCl(i) were also shown.

Measurement of cell growth

The population density was estimated using a Coulter counter. Changes in cell biomass were measured by monitoring the optical density of the medium at absorbance 550 nm and calculated from the absorbance values using predetermined correlation factor, with an optical density of 1.0 which corresponds with a cell dry weight of 232 mg/L.

Measurement of DNA synthesis

DNA synthesis was measured as described by Zhidong et al. (2006).

Cell photography

Cells were harvested and pretreated as described by Jiajun et al. (2005), then they were photographed using a scanning electronic microscope (SEM).

Statistical analysis

Every experiment was conducted in triplicate. All results were analyzed using Statistical Package for the Social Sciences (SPSS) 1.20 software version.

RESULTS AND DISCUSSION

Cell morphology changes in response to saline stress

It was shown that the occurrence of filamentous growth began as early as 4 h after exposure to the 0.2 M NaCl or Cl^- (as NH_4Cl) (Figure 1b) and the cell length was approximate 10 to 15 μm after exposure for 20 h, which was 3 to 5 fold longer compared to normal cell (Figure 1f), while there was no observable difference in cell size between normal cells and Na^+ (as Na_2SO_4) (Figure 1g) or

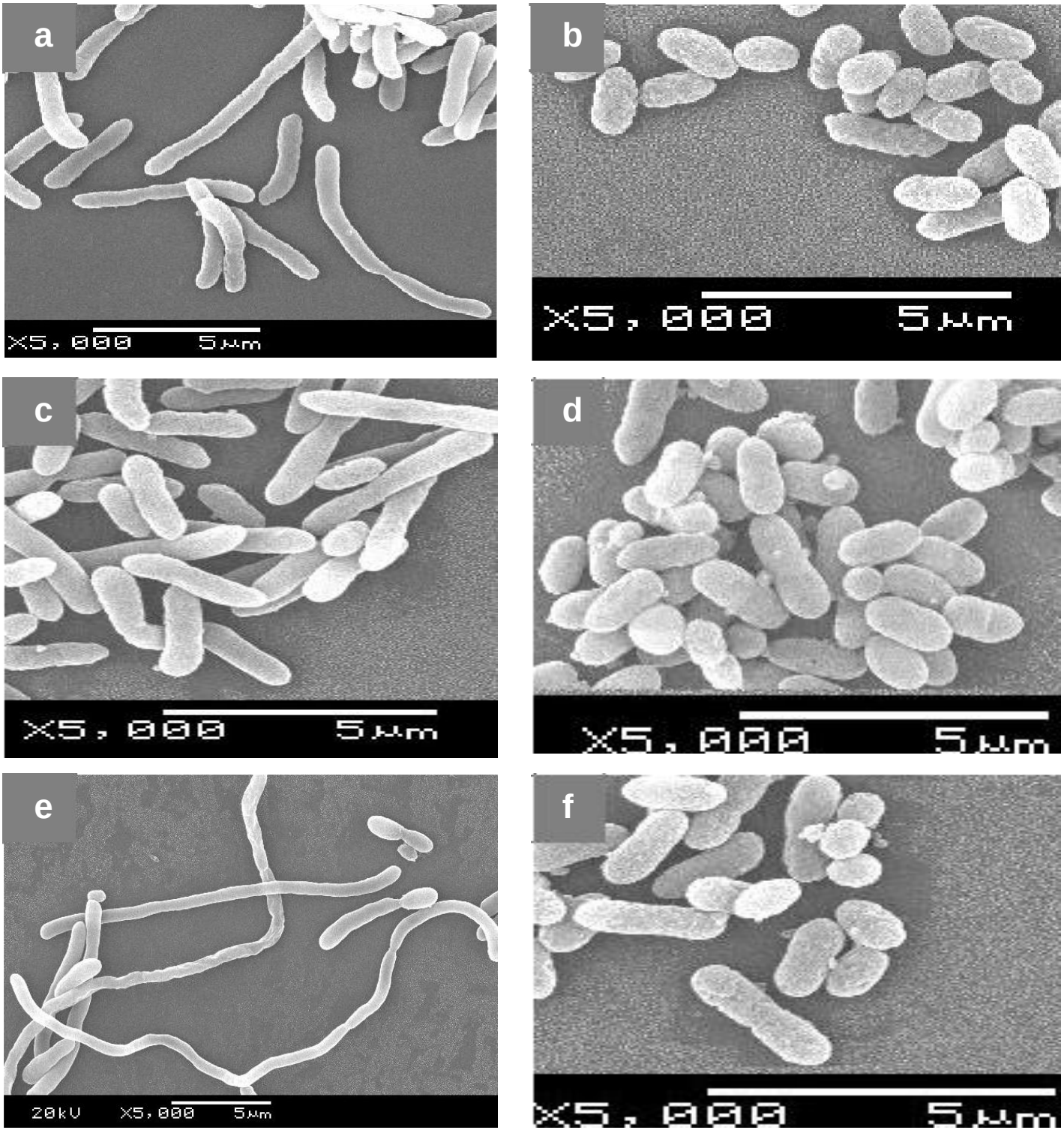

Figure 2. The filament formation of *Z. mobilis* 232B after exposure to 0.1 M $CaCl_2$ (a), $MgCl_2$ (c) and 0.2 M KCl (e) for 20 h. The morphology of cell after exposure to 0.2 M $Ca(NO_3)_2$ (b), $MgSO_4$(d) and 0.1 M K_2SO_4 (f) for 20 h, as a control, was also presented.

$(NH_4)_2SO_4$ treated cells (Figure 1h). It is indicated that filament formation in NaCl-inhibited *Z. mobilis* cultures is attributable to chloride ion. Further evidence was obtained from $CaCl_2$, KCl and $MgCl_2$ treated cells (Figure 2). Spangler and Emert reported that filament formation during the simultaneous saccharification and fermentation of cellulose to ethanol by *Z. mobilis* is associated with $CaCl_2$ and they assume that the calcium ion is the key factor (Spangler and Emert, 1986), while the present data revealed that the chloride ion, rather than calcium ion, was responsible for the case.

Furthermore, filamentous cells could recover to normal size when the cells were transferred to normal medium (Figure 3).

DNA synthesis and cell division in response to Cl^-

Cell cycle is a complex periodic event containing nuclear segregation, cell elongation and cell division, resulting that daughter cells are identical to the parent cell. DNA

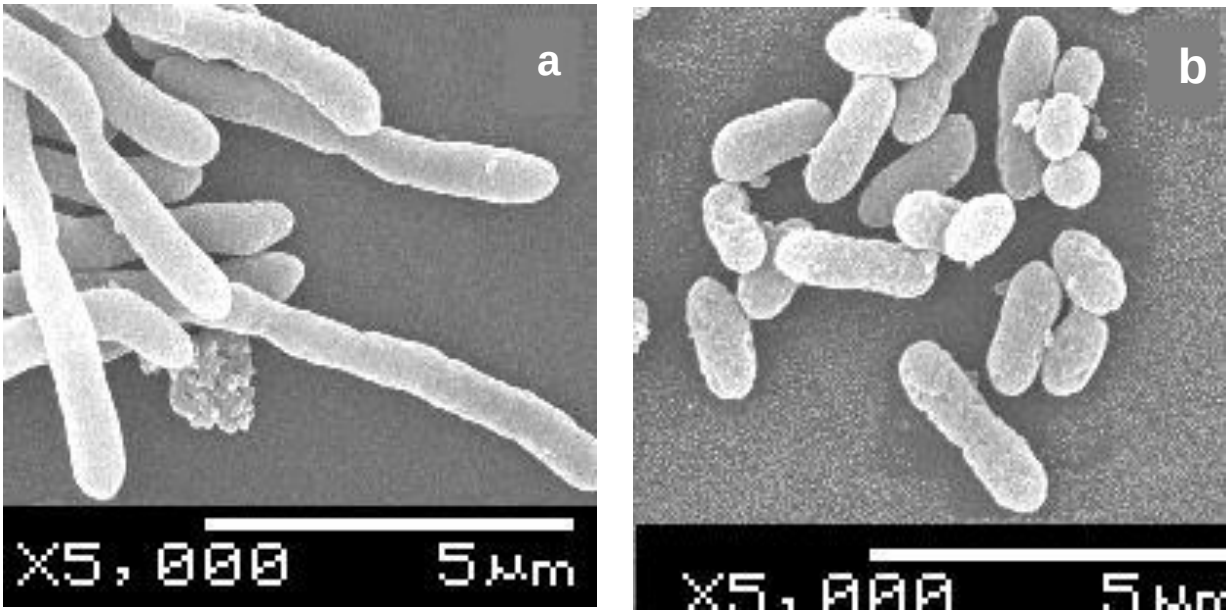

Figure 3. The changes in morphology of *Z. mobilis* 232B during recovery. Filaments (a) were inoculated into normal medium and cells (b) were examined by SEM after growing for 20 h.

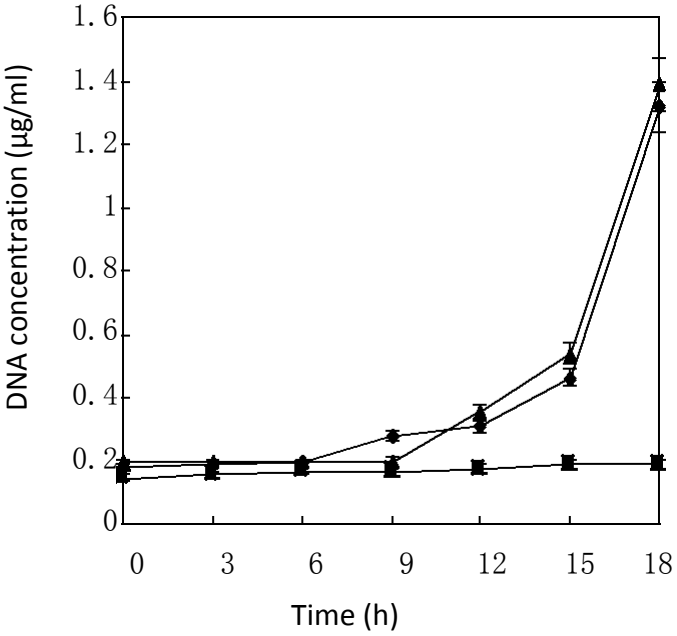

Figure 4. The yield of DNA synthesis of *Z. mobilis* 232B grown in 0.2 M chloride ion (■) and during the recovery period (▲). The DNA yield of cell grown in normal medium (◆), as a control, was also presented.

synthesis continued after cell exposure to chloride ion (Figure 4) and the recovery of the cells after incubation in normal culture can be seen to yield normal cells in a burst while there was little increase in the DNA yield. Thus, the strain appeared to be normal for the DNA synthesis but was blocked in a vital stage in cell cycle.

The growth of *Z. mobilis* 232B in the presence of 0.2 M chloride ion was illustrated during the growth period. As shown in Figure 5, there was an abrupt cessation in cell numbers almost immediate after the shift from normal medium to Cl^--inhibited medium. On the other hand, the biomass continued to increase slowly and cells elongated continuously. Furthermore, the recovery of filaments was very rapid after a shift-back to the normal medium and the cell number increase was in a burst but growth increased slowly (Figure 6). It is indicated that the Cl^--senstitive step is required very late in the cell cycle and chloride ion has a direct inhibitory effect on cell division. When the cell divided, the septum formation was observed clearly (Figure 7), suggesting that chloride ions may have an inhibitory impact on septum formation or septum separation.

In present work, it is strongly suggested that reversal of inhibition of *Z. mobilis* cell division caused by chloride ion, resulting the filament formation. To avoid filamentous growth, chloride ion should be excluded from lignocellulose-to-ethanol fermentation by *Z. mobilis*, for example, sulfuric acid is preferable to hydrochloric acid to be used for the hydrolysis.

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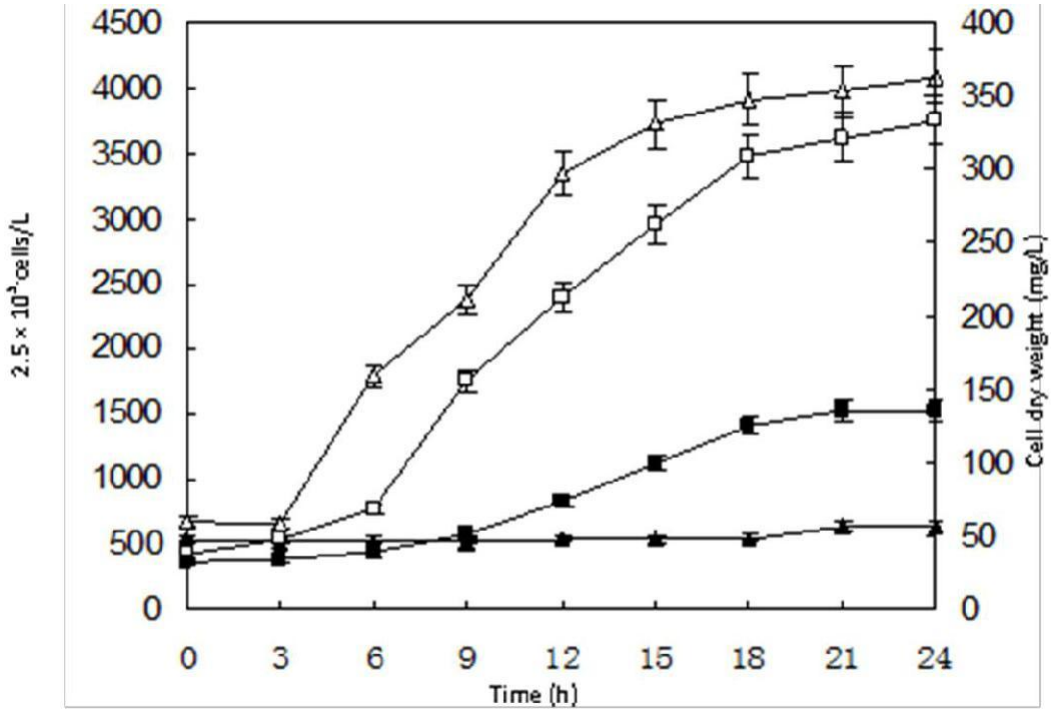

Figure 5. Cell number and growth of *Z. mobilis* 232B were measured. Increase in cell number (▲) and growth (■) of cell grown in the presence of 0.2 M chloride ion was shown. Increase in cell number (△) and growth (□) of cell grown in normal medium, as a control, was also presented.

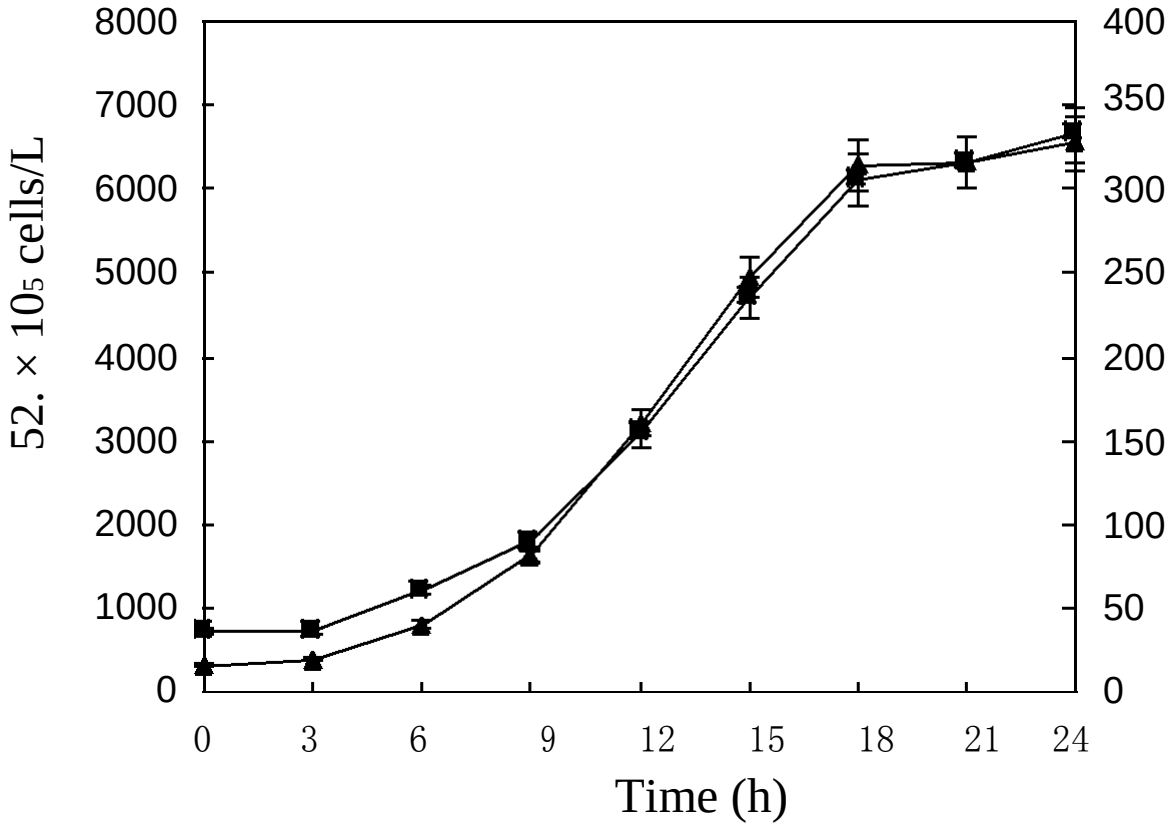

Figure 6. Cell number (■) and growth (▲) of *Z. mobilis* 232B was measured during the recovery period.

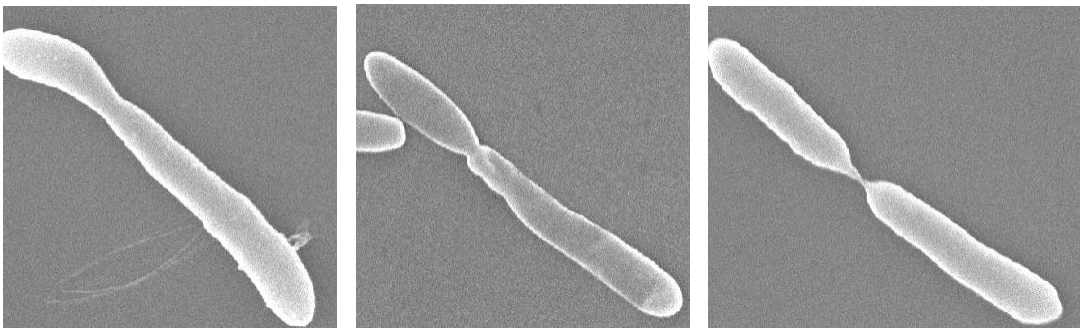

Figure 7. Septum formation of *Z. mobilis* 232B during the recovery of filaments produced by chloride ion, at a magnification of 15,000.

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