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Induction of Pleomorphism in *Lactobacillus bifidus*

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It is well known that *Lactobacillus bifidus* often shows branched forms (R. F. Norris, T. Flanders, R. M. Tomarelli, and P. György, J. Bacteriol. **60**:681, 1950; V. Sundman, K. Björkesten, and H. G. Gyllenberg, J. Gen. Microbiol. **21**:371, 1959), but the mechanism underlying this phenomenon has not yet been clarified. Although it has been reported that the bifid form of the organisms is induced by certain carbonates [N. Homma, J. Ota, and S. Arai, Japan. J. Pediat. **131**:274, 1960 (in Japanese)], it remained to be seen whether CO_3^{-2} or cations were responsible for the induction. In this paper, the induction of pleomorphism by cations, especially by univalent alkali metallic ones, is described.

The basal culture medium consisted of 1% glucose and 0.04% cystine in nutrient broth enriched by an equal volume of beef liver infusion. Chemicals to be tested were added at sub-bacteriostatic concentrations. After being adjusted to *p*H 6.8, the final medium was sterilized by filtration. Approximately 4×10^8 cells were inoculated into 20 ml of medium in a test tube, and the culture was grown at 37 C under anaerobic conditions by the conventional alkali-pyrogallol method. At intervals, one loopful of culture fluid was removed to prepare smears which were stained with methylene blue and observed under a microscope (oil immersion).

The earlier report (N. Homma, J. Ota, and S. Arai, Japan. J. Pediat. **13**:1274, 1960) that 0.2 M sodium carbonate induced the bifid form was confirmed by us. Moreover, it was proved in our experiments that sodium chloride (0.25 to 0.35 M), sodium sulfate (0.20 to 0.35 M), sodium nitrate (0.20 to 0.35 M), tribasic sodium phosphate (0.10 to 0.15 M), or sodium acetate (0.20 to 0.30 M) produced a similar effect. It was further observed that the pleomorphic response occurred in the presence of chlorides of other univalent cations. Such activities, however, differed quantitatively among the cations tested. The order (from strong to weak) was apparently $K^+ \fallingdotseq Na^+ > NH_4^+ > Li^+ \geqq Rb^+ > Cs^+$, which almost follows the lyotropic series. Typical appearances are shown in Fig. 1.

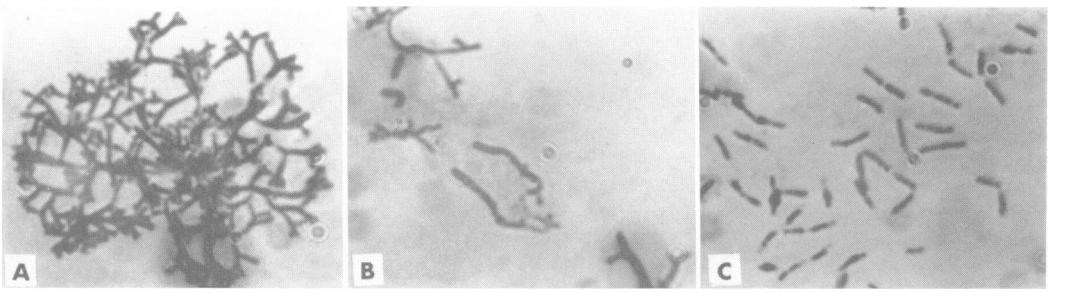

FIG. 1. *Branched or rhizoid growth induced by univalent cations. Organisms grown in medium containing 0.35 M KCl (A) or 0.35 M RbCl (B) are compared with those in the control medium with no salts (C). × 1,500.*

When chlorides of Ca^{++} (0.175 M), Ba^{++} (0.087 M), Mg^{++} (0.25 M), Mn^{++} (0.065 M), or Zn^{++} (0.005 M), at the highest sub-bacteriostatic concentrations which produce no precipitation in the medium, were used, no similar pleomorphic effect was noted, except swelling or slight branching of the polar parts of cells. In a medium containing 0.35 M NaCl plus 0.01 M $CaCl_2$, or 0.35 M NaCl plus 0.033 M $MgCl_2$, there was a slight (but no complete) inhibition of pleomorphism. The presence of 12.7% glucose or 24.2% sucrose in the medium also caused pleomorphism, but to a significantly lesser extent than with NaCl. In a 15-hr culture, in which the organism began to show

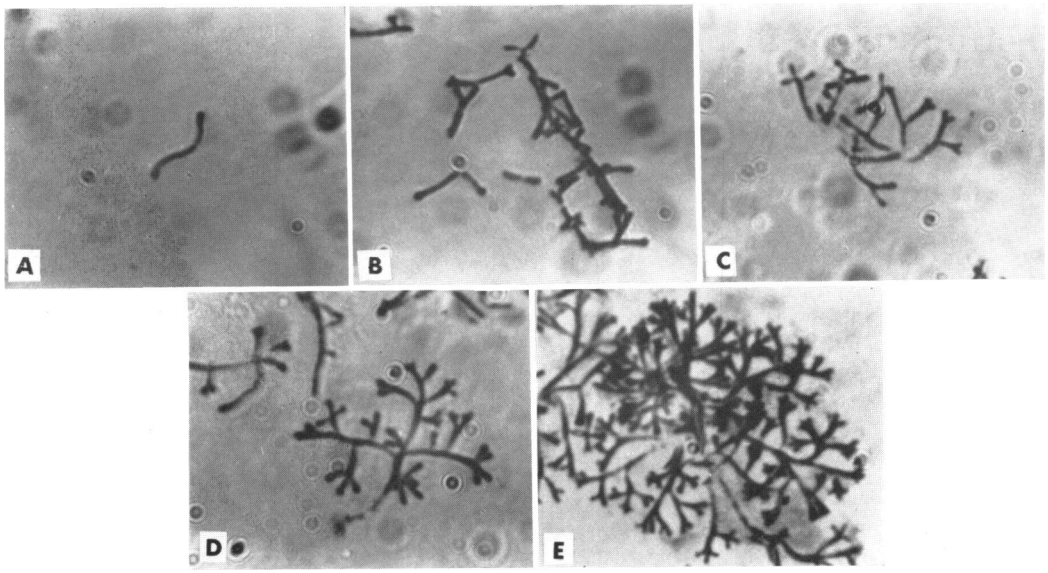

FIG. 2. *Development of cellular branching induced by NaCl at 12 hr (A), 14 hr (B), 16 hr (C), 18 hr (D), and 22 hr (E) after the beginning of incubation.* × *1,500.*

the bifid growth, the consumption of glucose was less than 15% of the initial content, and the osmotic value of the medium after the cultivation showed only a slight increase over that at the beginning of cultivation. Rhamnose and polyethylene glycol, neither of which is utilized by the organism, were ineffective. The pleomorphic effects of univalent cations, therefore, cannot be explained solely on the basis of osmotic pressure.

The branching was recognized about 12 hr after the beginning of incubation and developed extensively for the subsequent 8 to 10 hr. An example of this developmental process is shown in Fig. 2.

Based on these observations, the relationship between the growth and the cellular population was examined. As illustrated in Fig. 3, the viable bacterial population increased to about 1.5 times that of the original inoculum during 20 hr, whereas the packed cell volume, as determined by hematocrit, increased by as much as 25 times, corresponding to about 28% (an average of three experiments) of control growth in the medium without NaCl. Thus, the presence of Na^+ caused a much greater increase in the cell mass than in the viable count. Further studies on induction of pleomorphism are now in progress.

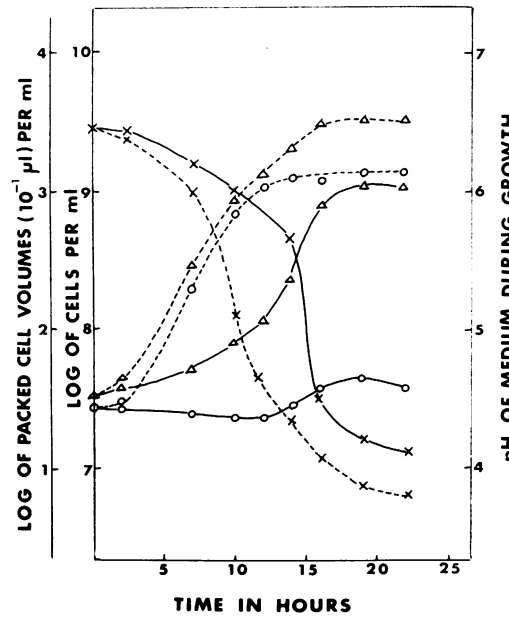

FIG. 3. *Effects of NaCl on the growth of Lactobacillus bifidus. Symbols:* ○, *viable count;* △, *packed cell volume;* ×, *pH. Solid lines refer to the medium containing 0.35* M *NaCl; broken lines refer to the medium without NaCl.*