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NOTES

Necessity of Calcium Ion for Cell Division in *Lactobacillus bifidus*

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Electron microscopy revealed that reversion of the bifid form to the bacilloid form of *Lactobacillus bifidus* takes place by cross wall formation, the process requiring available calcium ions.

In our previous reports (2, 3), we showed that the bifid form of *Lactobacillus bifidus* was induced in a medium in which calcium ions were deficient or not available due to the antagonistic action of monovalent cations; the necessity of calcium ions for cross wall formation in the organism was suggested. To clarify this pleomorphic process, the change in morphology of the bifid cells when they were transferred into a calcium-rich medium was examined by electron microscopy. The results are presented in this paper.

The bacilloid cells were harvested from an anaerobic culture in a medium containing 1% glucose and 0.04% cystine in nutrient broth enriched by addition of an equal volume of liver infusion; the bifid cells were cultivated in this medium which was either supplemented with 0.35 M NaCl or deprived of calcium ions by addition of oxalic acid.

Fixation and embedding of the cells for electron microscopy were carried out by a modification of the method of Kellenberger et al. (1). The organisms were fixed in 1% osmium tetraoxide in Veronal buffer containing 0.02 M sucrose (*p*H 6.2) for 15 hr at room temperature and then suspended in melted agar (1.5%). The small agar cubes were stained in a 2% (w/v) solution of uranyl acetate for 1.5 hr and subsequently dehydrated in increasing concentrations of alcohol. After treatment with propylene oxide, the blocks were left in 1:1 alcohol-Epon 812 mixture for 2 hr. The materials were then embedded in Epon 812 and sectioned by the ultratome. The thin sections were stained with lead citrate for 1.5 min and examined by an electron microscope (JEM 7A, Japan Electron Optics Laboratory Ltd.).

Figure 1 shows a section of the bifid form. One can see no cross wall in the cell. Figure 2 shows a section of the bacilloid form. A cross wall, probably in an early stage of formation, is indicated by the arrows. Figure 3 shows bifid cells harvested 5 hr after being transferred into basal medium; formation of cross walls (CW) can be seen. These observations further support an indispensable role of Ca ions for cytokinesis in *L. bifidus*.

LITERATURE CITED

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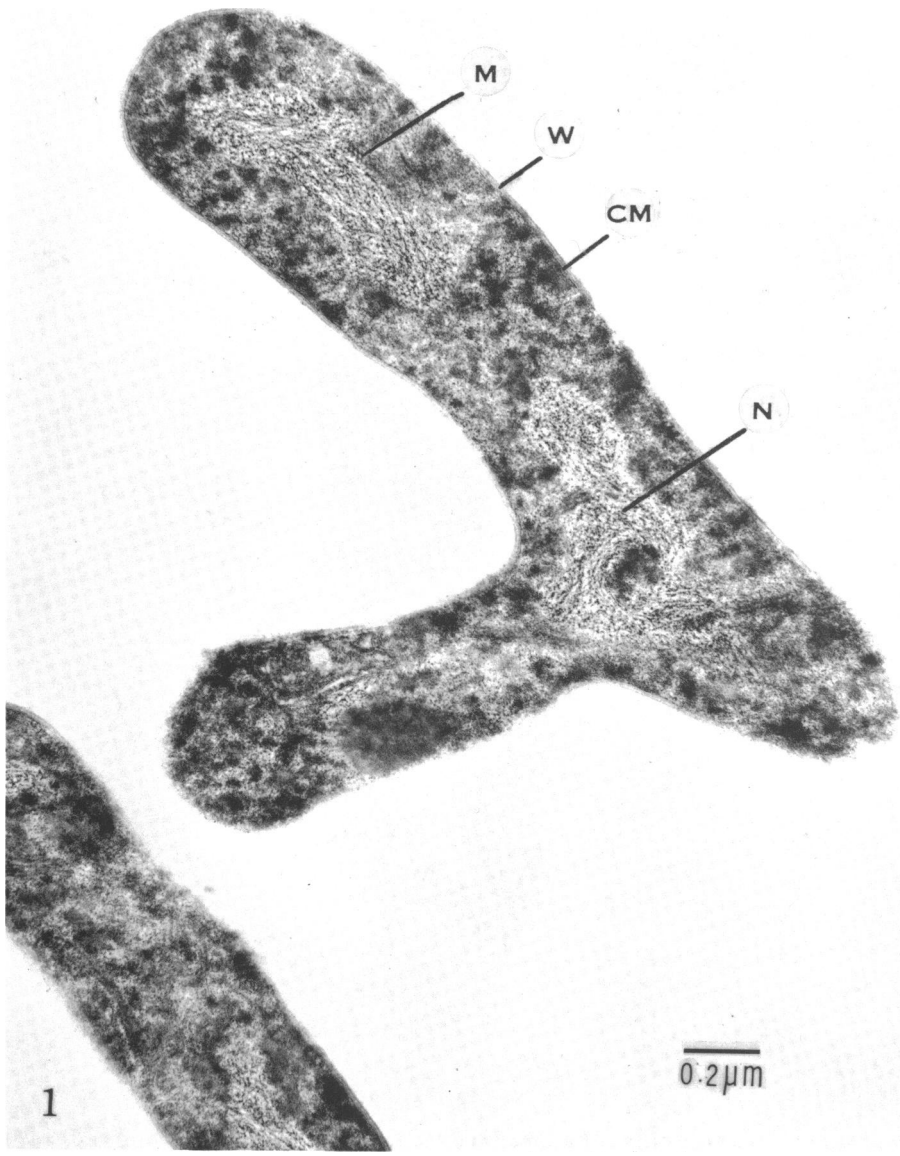

FIG. 1. *A section of the bifid form of Lactobacillus bifidus. No cross wall can be seen. N, nucleoid; W, cell wall; CM, cell membrane; M, mesosome.* × *50,000.*

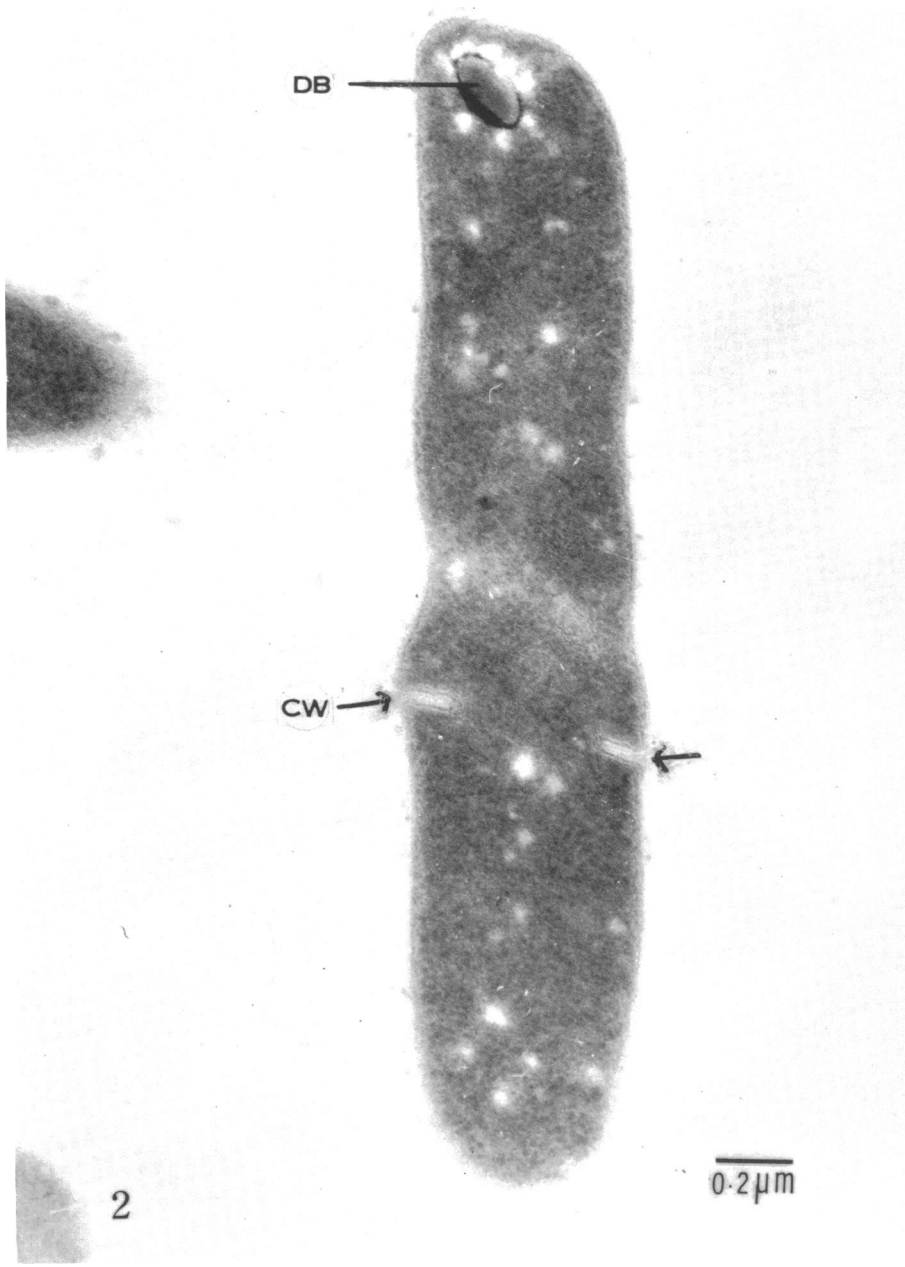

FIG. 2. *A section of the bacilloid form of Lactobacillus bifidus. Arrows show the beginning of cross wall formation. In the cytoplasm, a dense body can be seen, the real nature of which is uncertain. CW, cross wall; DB, dense body.* × *50,000.*

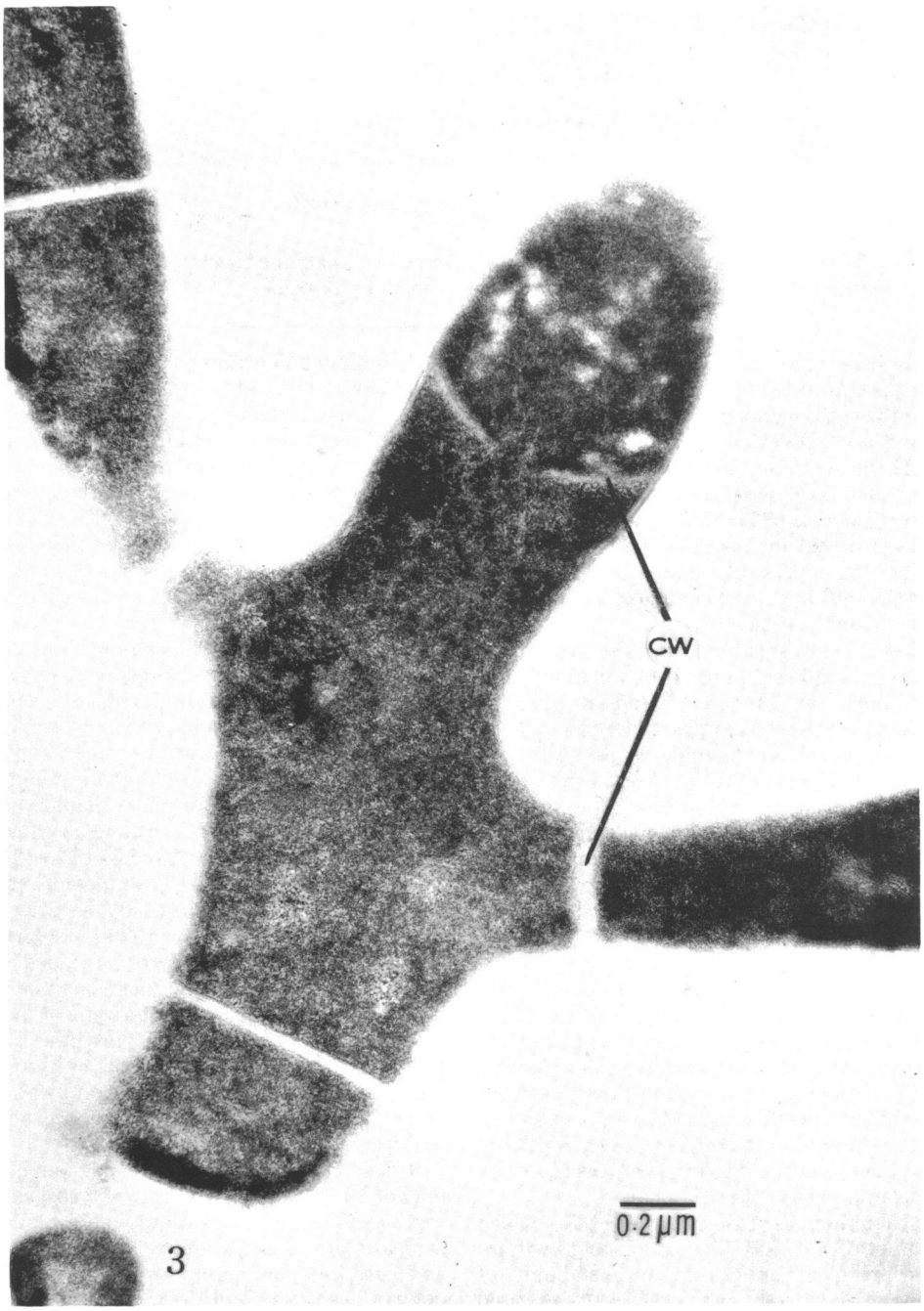

FIG. 3. *A section of the bifid form of Lactobacillus bifidus transferred into a medium without NaCl. One can see many cross walls (CW).* × 50,000.