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PRELIMINARY REPORT

APPEARANCE OF MORPHOLOGICALLY ABERRANT CELLS OF *MICROCOCCUS LYSODEIKTICUS* (*LUTEUS*) IN MEDIUM WITH PAPAIN

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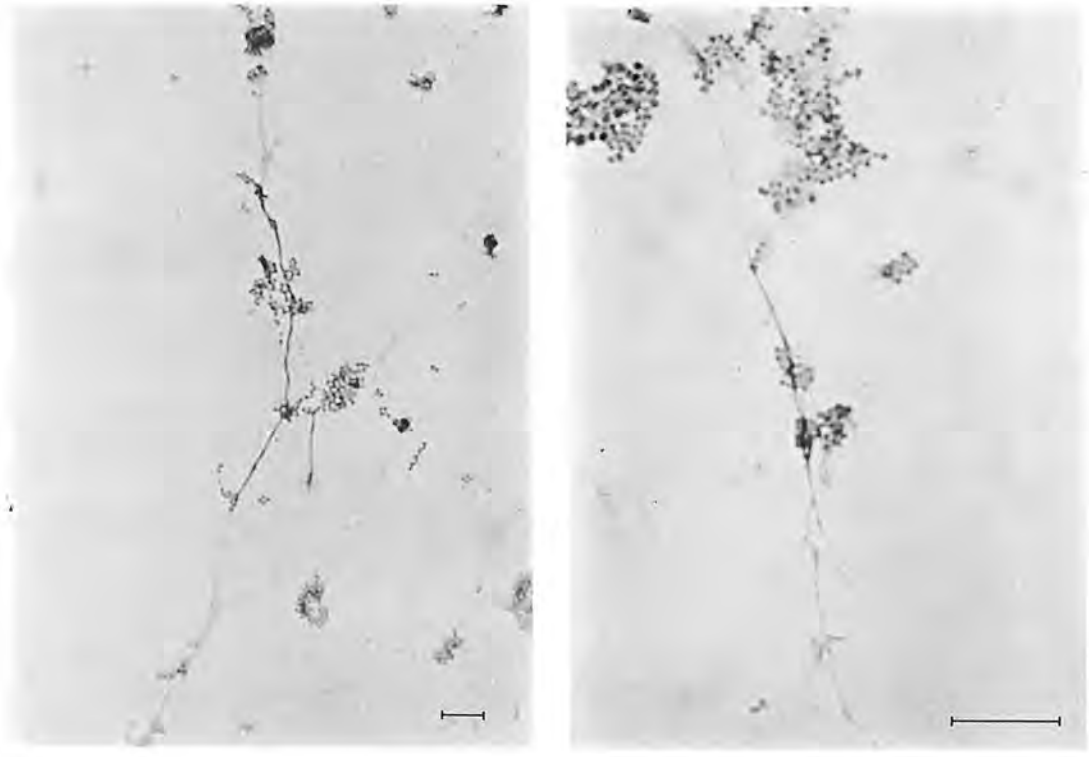
Previously (Monodane et al., 1973, 1975), we presented evidence that autolysis of the cell walls observed in the log phase cells of *Micrococcus lysodeikticus* (*luteus*) IFO 3333 was due to incomplete inactivation of an autolytic enzyme(s) which had finished some physiological function such as separation of dividing cells and consequently had become useless to the cells. This suggested that it might be possible to change the growth and/or morphology of cells by controlling some autolytic enzyme(s) from outside the cell.

In studies on this possibility, we found that the cells of a non-autolysable mutant of *M. lysodeikticus* IFO 3333, which are arranged in regular tetrads unlike those of the parent which are single or in irregular masses, displayed interesting morphological changes in the presence of various proteolytic enzymes. Formation of cell packets consisting of regularly arranged three dimensional "unit tetrads" occurred without any accompanying changes in the chemical composition of the cell walls (in preparation).

We also observed aberrant morphological changes of cells of the parent IFO 3333 strain in the presence of papain. Figure 1 depicts the aberrant morphological appearance under a light microscope of cells grown overnight in liquid medium supplemented with papain (a non-crystalline preparation, Type N. F. VIII, E. Merck Co., Darmstadt, Germany) at a final concentration of 5 μg/ml. The filamentous structures originating from coccal cells were gram-negative, but the cocci themselves were either gram-positive or -negative. About 5% of the cells had filamentous structures. Scanning electron microscopy (Fig. 2) showed that most of the cells were no longer round, but had become distorted or had a knobly surface. The filamentous structures appeared to protrude from the cells, elongate and branch, indicating that they were part of the cells but had a defective cell wall. It is impossible for technical reasons to decide whether the cells showing aberrant morphology are alive. The frequency of occurrence of morphologically aberrant cells and the extent of aberrance increased with the external concentration of papain. *M. lysodeikticus* IFO 3333 grew poorly in medium containing a final concentration of 25 μg papain per ml. A crystalline pre-

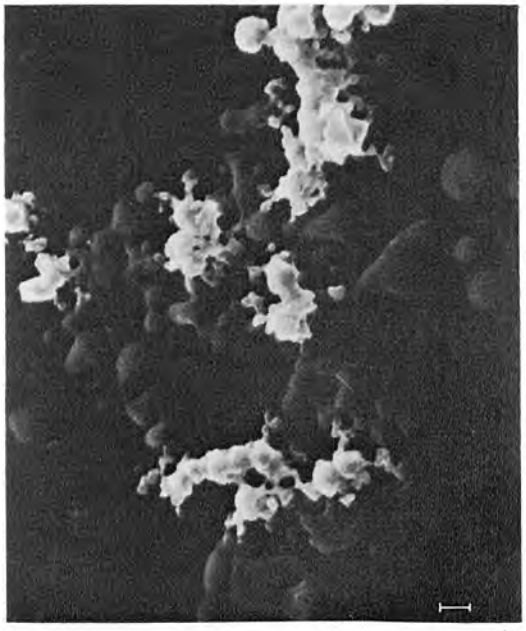


a b

FIGURE 1. *M. lysodeikticus* IFO 3333 cultivated overnight in liquid medium containing papain at a final concentration of a) 5 μg and b) 25 μg/ml medium. The liquid medium consisted of 10 g peptone (Polypepton, Daigo Eiyo Kagaku Co., Osaka, Japan), 5 g yeast extract (Dried Extract of Yeast, Daigo Eiyo Kagaku Co.) and 3 g sodium chloride per liter, and was adjusted to pH 7 with 1 N sodium hydroxide solution. Papain which had been sterilized by passage through a filter (MF-Millipore, Type HA, 0.45 μm, Millipore Filter Co., Mass., USA) was added to the autoclaved medium just before inoculation. Cells were cultured with shaking at 37 C in an L-shaped tube. The cells were stained with 0.25% aqueous safranin solution. Scale: 10 μm.

paration of papain (Sigma Chemical Co., Mo., USA) also caused morphological changes detectable by light microscopy. However, trypsin (crystalline, Trypsilin, Mochida Pharmaceutical Co., Tokyo, Japan), α-chymotrypsin (crystalline, Type II, Sigma Chemical Co.), pronase P (a non-crystalline material, Kaken Kagaku Co., Tokyo, Japan), ficin (non-crystalline, Wako Pure Chemical Co., Osaka, Japan and crystalline, Sigma Chemical Co.) or heat-treated (100 C for 15 min) papain at final concentrations of 5 and 25 μg/ml medium, did not induce aberrant morphological changes of IFO 3333 cells. Very occasionally cells with short filamentous structures were found in the absence of added papain.

It is well known that when bacteria are incubated in appropriate medium with cell wall lytic enzymes or antibiotics that inhibit cell wall biosynthesis, or when readily autolysable bacteria are incubated without any additions, they form spheroplasts or protoplasts. When the cell wall is damaged during spheroplast formation, part of the cytoplasmic membrane protrudes from through the wall. However, the morphological changes described above induced by papain were not due to a cell wall lytic enzyme contaminating the en-



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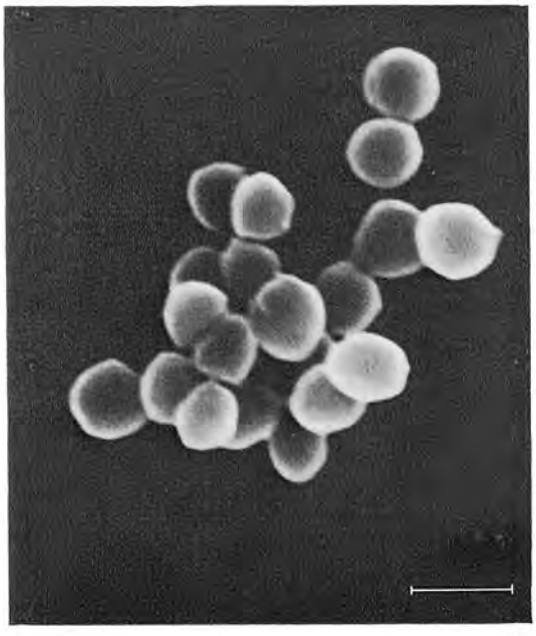

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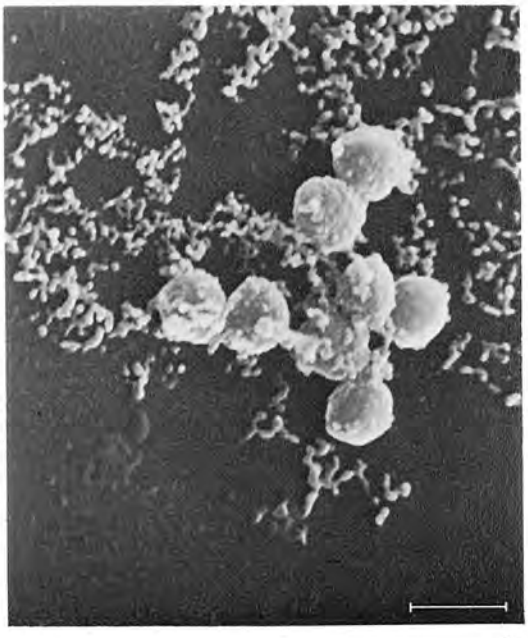
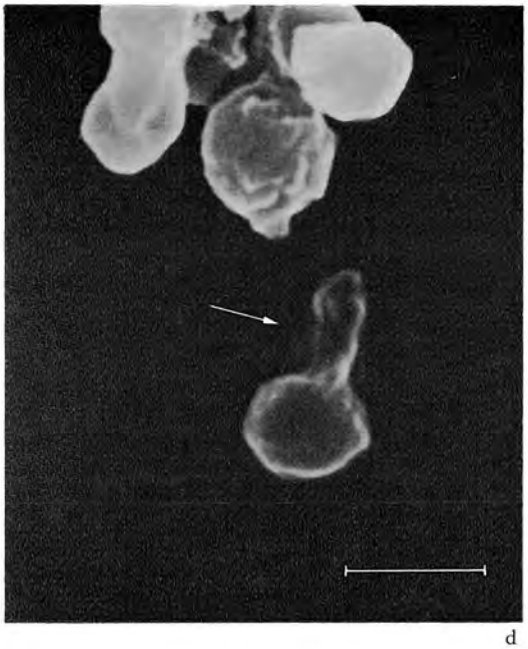


c

FIGURE 2. *M. lysodeikticus* IFO 3333 cultivated in the absence (a) or presence (b-g) of papain (final concentration, 5 μg/ml medium) under a scanning electron microscope. Cells were incubated with a final concentration of 4% glutaraldehyde for 3 hr at room temperature. Then the cells were washed twice or three times with deionized water, fixed and dehydrated in a graded series of concentrations of ethanol (50, 70, 90, 95 and 100%). The cells on a small cover glass were dried in an evacuated desiccator, coated with gold and observed in a scanning electron microscope (MINI-SEM, Type MSM-4S, Akashi Seisakusho Ltd., Tokyo, Japan). Fragments of branched filamentous structures are seen separated from cells (c). The arrows indicate the filamentous structures (d-g). Scale: 1 μm.

zyme specimen, because the papain preparation used (either crude or crystalline) had no activity to lyse the cell walls of *M. lysodeikticus* IFO 3333.

Other workers have reported similar observations on gram-negative rods, which are



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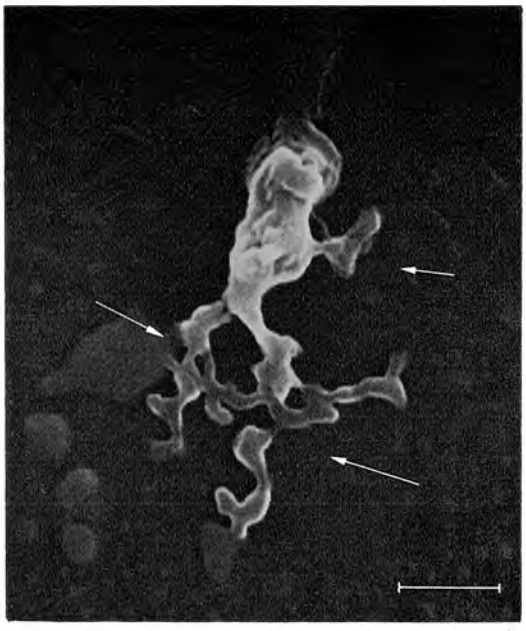

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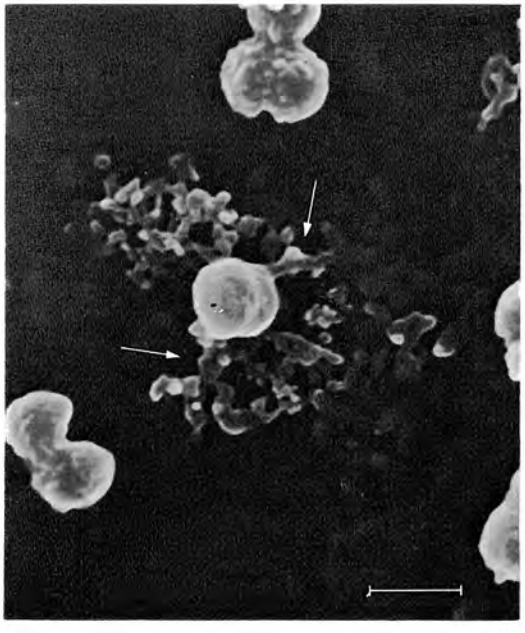

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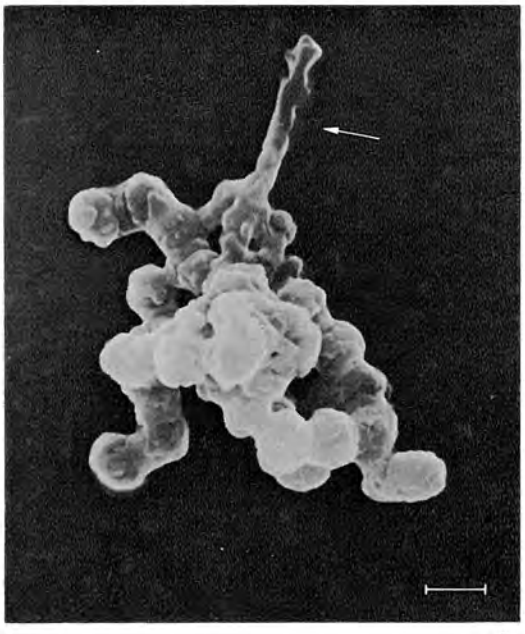

g

unlike the gram-positive coccus reported here. Greenwood and O'Grady (1973) observed that bulbous forms of *Proteus mirabilis* were produced by ampicillin at concentrations between those causing filament-production and spheroplast formation. Ellis et al. (1976) reported

that treatment of *Escherichia coli* EC 14 with a minimal inhibitory concentration of carbenicillin produced septal region swelling, short filaments, beaded chains, a rough surface and debris which increased with time. These morphological changes were probably due to disturbance of cell wall biosynthesis by β-lactam antibiotics. Thus the aberrant morphological changes with the appearance of monster forms reported here could be attributable to derangement of a physiological autolytic enzyme system which is located in the surface layers of the cell and which is accessible to extracellular papain. For some reason the autolytic enzyme system in *M. lysodeikticus* IFO 3333 cells seems to be more sensitive to papain than to the other four proteolytic enzymes tested.

Similar morphological changes have been observed in *E. coli* C on treatment with mitomycin C or deprivation of thymine (Suit et al., 1967), and in a conditional *rod* mutant (thymine-requiring) of *E. coli* K 12 when grown in a thymine-less medium at 30 C after growth at 42 C (Henning and Schwarz, 1973). However, these phenomena do not seem to have any direct connection with the physiology of the cell walls, so that their underlying mechanisms may be different from that of the phenomenon described here.

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