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SHORT COMMUNICATIONS

Reversible Adhesion Protects the Thermophilic Bacterium *Bacillus licheniformis* 603 from *N*-Ethylmaleimide

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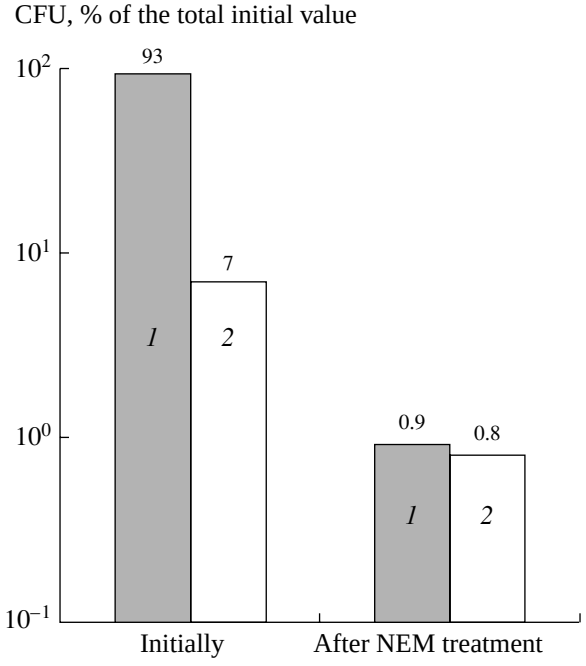
We have previously shown that the thermophilic bacterium *Bacillus licheniformis* and some other bacterial species display a pronounced capacity for reversible adhesion (RA), manifested as a temporary decrease in the number of suspended cells immediately after inoculation into fresh medium [1, 2]. RA is suppressed under favorable conditions and is most pronounced under conditions suboptimal for the growth of a given microbial species. These data suggested that RA is adaptive and could enhance microbial population survival. In the present work, we demonstrate directly that RA protects *B. licheniformis* cells from adverse effects, in particular, during treatment with *N*-ethylmaleimide (NEM), an agent that oxidizes –SH groups.

We have studied *Bacillus licheniformis* strain 603 isolated from a geodesic water well. Protocols for bacterial growth, quantitative RA determination, and composition of M9 and LB growth media are described in detail elsewhere [1–3].

Bacterial cultures were grown in M9 medium at 45°C until they entered the exponential growth phase, corresponding to A_{450} = 0.3–0.5. Turbidity of the culture was measured on a Specol spectrophotometer (Carl Zeiss, Germany). The cells were then washed with fresh M9 medium on 0.45-μm Millipore filters to remove traces of culture medium, resuspended in M9 medium to A_{450} = 0.1 and incubated in a new flask for 20 min at 30°C. During this incubation, the optical density decreased by 7–15% due to cell adhesion on the flask walls [1, 2]. The culture was thus divided into two subpopulations, adhered and suspended cells. To separate these subpopulations, the liquid was transferred to another flask, and the original flask was filled with fresh sterile M9 medium. NEM (Sigma) was then added to both cultures to a final concentration of 200 μM, and both flasks were incubated for 20 min as described above. The number of viable cells was determined in cultures of both subpopulations by plating serial tenfold dilutions onto solidified LB. Before plating, the cells in both cultures were desorbed from the flask walls by vigorous shaking (1 min) with glass beads (1–2 mm in diameter) sterilized by dry heat. This procedure also promoted breakage of aggregates formed by suspended cells under adverse conditions (i.e., in the presence of the toxic substance). The lack of cell aggregates was a prerequisite for accurate determination of the number of viable cells by plating. The degree of homogenization was followed microscopically using a Docuval microscope (Carl Zeiss, Germany)

The effect of NEM on cell viability is illustrated in the figure. Treatment by NEM caused the death of more than 97% of the bacterial population. Such a high lethality was used intentionally, since we have earlier shown that the apparent efficiency of adaptive factors parallels the degree of inhibition of microorganisms [4]. The fraction of survivors was tenfold higher among adhered cells than among suspended cells (10% vs 1%, see figure). The selective survival led to a change in the



Protective effect of adhesion of *Bacillus licheniformis* 603 cells during NEM treatment. Number of colony-forming units are shown as % of the total initial number in the population. *1*, suspended cells; *2*, adhered cells.

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population structure: before the NEM treatment, 90% of the cells were suspended, whereas afterwards, the numbers of viable cells were equal in suspended and adhered subpopulations.

Therefore, the data presented here suggest that reversible adhesion to solid surfaces protects bacterial cells from toxic compounds.

The comparative analysis of bacterial cell resistance to NEM in the adhered and suspended state supports our recent data on the adaptive nature of RA [1] and the conventional view that adhesion in general is adaptive. In comparison with free suspension, the adhered state doubtlessly has numerous advantages for cells, such as higher concentrations of nutrients adsorbed on the same surface, protection from the possibility of drying or being carried away with currents in the liquid phase, protection from toxic enzymes, and better cell-to-cell communication through extracellular metabolites [5–8]. The results presented here allow us to expand this list with higher resistance to chemical biocides.

Mechanisms of protective effects of adhesion are poorly studied. One possible reason may lie in the partial shielding of cells by the surface, which hinders the access of a toxic agent to the cells. Cell adhesion to the surface could also trigger general conformational changes in membrane structures, which would in turn influence their stability and functional activity [9].

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