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REVIEW ARTICLE

INDUCED LYSIS OF BACTERIAL CELLS USED FOR SYNTHESIS OF SENSITIVE PRODUCTS

***Frank Mayer**

Seerosenweg 1a DE- 26160 Bad Zwischenahn, Germany

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ABSTRACT

Bacterial cells that synthesize sensible products should be lysed with techniques that do not damage the products. We describe such an approach; we call it "induced lysis".

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INTRODUCTION

Synthesis of various products (enzymes, other proteins, nucleic acids, various other kinds of macromolecules) by bacteria is an approach that is used when products with high purity and in substantial amounts are needed. Parallel to the development of this approach, techniques have to be optimized for setting free these products. One of the common goals of all these techniques is preservation of the functional properties of the products. In most of the techniques used for setting free products synthesized by production bacteria, mechanical shear forces or chemicals of various kinds are used. As a consequence, sensitive products such as covalently closed circular DNA suffer damage (in case of DNA: broken strands), or products such as proteins can be denatured or even totally destroyed.

RESULTS AND DISCUSSION

In our patent WO 2004005506 A2 we described a procedure of "induced lysis" of production bacteria. This approach has the following basis: in the interplay of the structural and functional cellular components, the cells stay only alive when all the components are in perfect structural and functional state. As soon as one of the components is not in this state, the interplay does not work. In the case of our patent mentioned above, we used the following approach: we had obtained indications (Mayer 2003) for the assumption that the bacterial elongation factor EF-Tu has a double function: one is its role in protein synthesis, but a second function appeared to play a substantial role in the formation of a bacterial cytoskeleton. For further insights into our assumption, we performed experiments that should show what happens when, together with full-size EF-Tu, a truncated EF-Tu moiety is also present in the cell. We expected that, in this case, the formation and preservation of a bacterial cytoskeleton would be disturbed because truncated EF-Tu would compete with full-size EF-Tu as building blocks. To achieve such a situation, we introduced the genetic information for such a truncated EF-Tu into cells that contained the information for full-size EF-Tu. The synthesis of truncated EF-Tu was only switched on, by a slight variation of the growth temperature, after the bacteria had grown properly and had synthesized the product in question (the truncated EF-Tu was not synthesized during these growth phases). One to two hours after switching-on of the truncated EF-Tu synthesis, we observed, by electron microscopy, more and more cells that had lost their intact state: first, they lost their cytoplasmic membrane and their cell envelope; in fact, after one more hour, the cell culture did only contain bacterial cells with the typical state of total lysis. In fact, we had achieved an "induced lysis". Over the years, our assumption that EF-Tu is a major component of the bacterial cytoskeleton was confirmed (Defeu Souto *et al*., 2015, Defeu Souto *et al*., 2010). Without these data obtained by other research groups, one could have speculated that the presence of truncated EF-Tu in the cell could have its damaging effect on the structurally and

****Corresponding author: Frank Mayer,***
Seerosenweg 1a DE- 26160 Bad Zwischenahn, Germany.

functionally intact state of the cell by a negative influence on the role of EF-Tu as a component in protein synthesis. We suggest that our findings, together with our patent WO 2002087554 A2 , might induce further work on the application of "induced lysis" of production bacteria and, on the long run, on the development of antibacterial agents that, given from the outside, simulate presence of truncated components of substantial cell components inside the cell. The aim should be the availability of such a new class of antibacterial agents.

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