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Endotoxin masking: A kinetically controlled reaction mechanism

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Lipopolysaccharides are the major components of the outer membrane of Gram-negative bacteria. Due to their toxic effects after administration into the bloodstream, lipopolysaccharides are also called endotoxins. They are ubiquitously found, often in low concentration, in nearly all natural products, including pharmaceutical products. Thus, it is mandatory to control parenteral drug products in order to avoid endotoxin contaminations. In quality control of biopharmaceutical products, "Low Endotoxin Recovery" (LER) has been observed while using common detection methods, like the Limulus Amebocyte Lysate (LAL) assay. This is of particular importance when surfactants and other ingredients such as salt, urea and other organic substances are present, all molecules representing parameters strongly influencing the critical micelle concentration (CMC). Micelles and other aggregates are assumed to represent the major physicochemical mechanism on the LER effect by masking the toxin, thus precluding correct determination in bacterial endotoxin testing. As a consequence, once the LER effect appears underestimation of potential endotoxin contaminations and therefore, false negative results in the Limulus-based test methods may appear. In pharmaceutical quality control units, such false negative results have to be strictly avoided. Our work was guided by the assumption, that the root cause of LER effect is the interplay of endotoxin with complex forming agents and surfactants, resulting in diverse aggregates of highly complex molecular structures. However, the physicochemical mechanism and principles of LER are still a matter of debate. Due to the time dependency of the LER phenomenon, time dependent reactions of endotoxin recovery have been analyzed in more detail. The results presented here, demonstrate that dilution of a sample delays or avoids the identification of LER but also indicate predominant effects of complex forming agents. Within the used model system, a minimum concentration of citrate to serve as complex forming buffer system could be determined. Furthermore, we could demonstrate that the presence of surfactants is a strong prerequisite but does not determine kinetics of the LER effect. Interestingly, the endotoxin concentration itself had no significant impact on masking kinetics. Based on our results we propose a new theoretical model for simulating masking kinetics, leading to a better understanding of endotoxin masking in aqueous solutions.

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Suppression of rice blast and sheath blight diseases by Alcaligenes faecalis, a new biocontrol agent with multiple modes of action

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Five rhizobacteria from rice rhizosphere were isolated and examined to find novel competent biocontrol agents for rice blast fungus Magnaporthe grisea and sheath blight fungus Rhizoctonia solani, the two devastating pathogens of rice. Results from in vitro trials showed that three isolates identified as *Alcaligenes faecalis* strain Bk1, *Alcaligenes faecalis* strain Kp6 and *Bacillus amyloliquifaciens* strain Bk9 showed tremendous antifungal activity against *M. grisea, R. solani, Fusarium graminearum and Botrytis cinerea*, while, two *Brevibacillus laterosporus* strains B9 and S6 showed significant antagonism towards *Xanthomonas oryzae pv. oryzae*. After 5 days of co-cultivation in the two compartment petri-dishes, the mycelium growth of *R. solani* was significantly inhibited by fungi static volatiles produced by *A. faecalis* strains Bk1 and Kp6. The application of Bk1 and Kp6 strains as soil treatments respectively suppressed the rice blast disease by 72.9%, sheath blight diseases by 71%, compared to control. In addition, *A. faecalis* strains significantly improved plant growth, enriched mineral nutrients and enhanced the expression of defense related genes. The bioactivity mechanisms revealed that these strains were able to produce biofilm, along with other PGPR-associated traits and comprise lipopeptide biosynthetic genes in their genomes, which helped *A. faecalis* to serve as potential biocontrol agents.

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