Original Article

Extraction, Purification and Characterization of Lipopolysaccharide from Escherichia coli and Salmonella typhi

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Abstract

Lipopolysaccharide (LPS) is an important structural component of the outer cell membrane complex of gram negative microorganisms. Its causative role in gram negative bacteria-induced diseases and broad applications in different kinds of cell stimulation experiments provided a conceptual basis for studies directed at the isolation, purification, and detailed chemical characterization of LPS. The main problem with LPS purification protocols is the contamination of the end product with nucleic acids and proteins in variable proportions which could potentially interfere with downstream applications. In this study, a simple procedure for purification of LPS from Escherichia coli (E.coli) and Salmonella typhi (S.typhi) with high purity and very low contaminating nucleic acids and proteins based on the hot phenol-water extraction protocol has been introduced. The purity of extracted LPS was evaluated by silver and coomassie blue staining of SDS-PAGE gels and HPLC analysis. Limulus Amebocyte Lysate (LAL) coagulation activity and rabbit pyrogen assay were exploited to monitor the functionality of purified LPS. The results showed that DNase and RNase treatment of the sample is essential after the sonication step to eliminate nucleic acid contamination in the LPS fraction. Silver staining demonstrated ladder pattern which is characteristic of LPS. No contaminating protein was found as assessed by coomassie blue staining. HPLC fractionation revealed high degree of purity comparable with commercial LPS. Parenteral administration of purified LPS resulted in substantial increase of rabbits' body temperature (mean: 1.45°C). LAL coagulation assay confirmed the functional activity of the purified LPS. In conclusion, the protocol presented here could be employed for isolation of LPS with high purity and functional activity.

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Introduction

Lipopolysaccharide (LPS) is the main outer membrane component of gram negative bacteria which constitutes about 75% of the surface [(1)] and 5-10% of the total dry weight