GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Separation, purification, and identification of flagellin, and preparation of its antisera

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Genet. Mol. Res. 13 (4): 9161-9170 (2014)
Received May 23, 2014
Accepted October 30, 2014
Published November 7, 2014
DOI http://dx.doi.org/10.4238/2014.November.7.3

ABSTRACT. The aim of this study was to separate, purify, and identify *Salmonella paratyphi* A flagellin, and to prepare its antisera. Primary flagellin was isolated from *S. paratyphi* A using the acid lysis method. The flagellin was purified with weak anion exchange chromatography and the protein was identified with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blot, and negative staining with phosphotungstic acid with scanning electron microscopy (SEM). The production of the obtained flagellin was then quantified. New Zealand white rabbits were then immunized with the isolated flagellin, the presence of serum anti-flagellin antibodies was assessed with the immunoblot test, and its potency was determined with the double immunodiffusion test. The results of SDS-PAGE showed that the molecular weight (m.w.) of the purified flagellin was 52 x 10^3. The immunoblot test also showed a band at 52 x 10^3 m.w. The SEM results

showed that the flagellin was filamentous. These three results showed that the protein was homogeneous. The protein quantification analysis found that 4.8 ± 0.5 mg flagellin could be extracted per 1 g wet weight bacteria. The titer of the anti-flagellin antiserum was 1:64. Through this method, we obtained high productions of flagellin, which could be easily purified, identified, and prepared into high titer antiserum.

Key words: *Salmonella paratyphi* A flagellin; Purification; Antisera

