

Bacterial Polysaccharides - Potential Candidate for Vaccine Development

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Abstract

Capsular polysaccharides are important surface components of bacteria. These are virulence factors. *Pasteurella multocida* (DP1) was isolated from ducklings. The polysaccharide components were isolated from *P. multocida* (DP1) strain. The structural elucidations were done by Infra-Red (IR) and nuclear magnetic resonance (NMR) spectrum. The polysaccharides are potential candidates for development of vaccines.

Keywords: *P. multocida*; DP1; Polysaccharides; Vaccines; IR; NMR

Introduction

P. multocida is the etiological agent of fowl cholera, an extremely contagious disease of wild and domestic birds that inflicts serious economic losses on the poultry industry [1]. Recognised by Louis Pasteur as one of the important infectious diseases [2]. The etiological agent of fowl cholera in avian species like chicken, turkey and waterfowls. It causes septicemia in birds and rapidly fatal. Carrier birds play a major role in the transmission of fowl cholera. Based on capsule antigens, they are 5 serogroups - A, B, D, E and F. Fowl cholera is generally caused by A:1, A:3 or A4 strains. In this study we isolated the capsular PS and the structure was studied.

Materials and Methods

The cultures of DP1 were maintained in brain heart infusion agar. For production of capsular polysaccharides the cultures were grown in Brain heart infusion broth with chitin flakes, with occasional shaking. Chitin flakes enhances the production of capsule. The cells were removed by centrifugation at 6000 rpm. The supernatant containing the polymeric material was concentrated and precipitated with 2.5 volumes of ethanol. The resulting pellet was dissolved in water and treated with Sevag's reagent [CHCl₃:CH₃OH (3:1)] proportion to remove the protein. The solution containing the capsular material was treated with equal volume of cetyl trimethyl ammonium hydroxide and refrigerated for 12 hours. The supernatant obtained was treated with 2.5 times of ethanol and refrigerated for 48 hours. The precipitate is centrifuged and dried to obtain light brown powder, the capsular polysaccharide. The compound was completely soluble in DDW and the dissolved solution is used for Infra red (IR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy. The IR and NMR spectroscopy was done at IIT, Bombay, Mumbai, India.

Sugar Analysis

Sugar analysis was done by Anthrone test [3].

Results

The sugar analysis confirmed that the compound was carbohydrate. In the IR spectrum, absorption peaks were observed at 3451 cm⁻¹, 2926 cm⁻¹ and 1642 cm⁻¹ indicating OH stretching, a C-H bond and a C=O bond respectively [3]. The peak at 920 cm⁻¹ corresponds to β-(1,6) linkage [4] α-D specific peaks for glucan at 850 cm⁻¹ were not found in DP-1 PS spectrum. NMR analysis showed that, H chemical shift of anomeric region was observed around 4 ~ 6 ppm [5]. Anomeric signals for β-1,-6 linkage appear at 4.5 ~ 4.6 ppm [6]. The absence of peak in the range 0.5 ~ 3.0 ppm implied that DP1 polysaccharide is pure glucan (Figures 1 and 2).

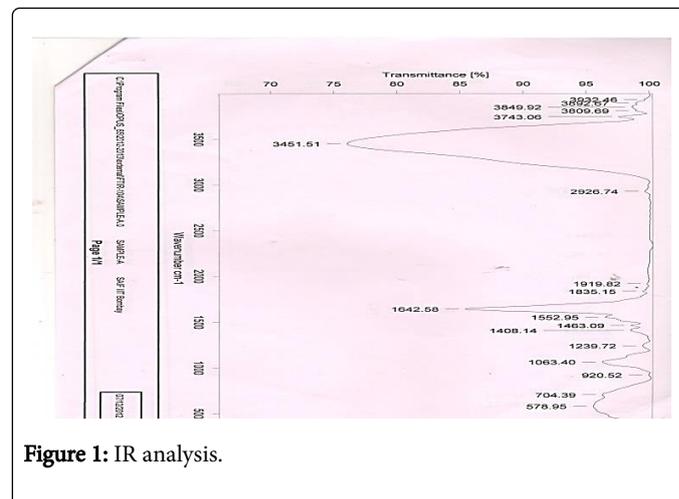


Figure 1: IR analysis.

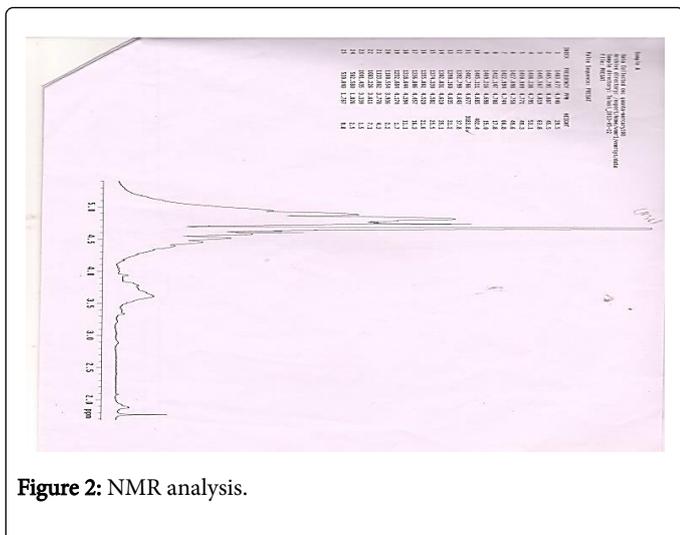


Figure 2: NMR analysis.

Discussion

Bacteria possess variety of mechanisms to host defenses and capsule is one of the virulence factors. The potential roles of capsular *Pasteurella multocida* during life cycle include avoiding phagocytosis, defeating complement, modulating host responses of physiology and resisting desiccation [7,8]. Polysaccharides have lot of applications now-a-days. Considering *Pasteurella multocida* as antigens, immunity against these antigens can confer protection against disease. *Pasteurella*

multocida was the target of several investigators in 1920s and 1930s. Development of vaccines based on capsular polysaccharides is an effective way to fight against disease. From the present study, we arrive at the conclusion that the *Pasteurella multocida* obtained from DP1 is β (1-6) glucan. The antigenic variation possessed by carbohydrate antigens is a limiting factor for vaccine production. *Pasteurella multocida* utilisation in vaccine production has been partially successful. Further studies will reveal the potential of using DP1-PS for vaccine production.

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