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ORIGINAL ARTICLE

Purification of heat labile toxin from *Bordetella pertussis* vaccine strain 134 employed indigenous technology

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KEYWORDS

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Abstract *Aim and objective:* The aim of the study was the evaluation of purified HLT from *B. pertussis* vaccine strain 134 by employing indigenous technology and examining the immuno-biochemical aspects of the purified protein.
Materials and methods: Shaker cultivation of *B. pertussis* strain 134, sterility, opacity confirmation, TCA precipitation of cellular proteins, G50 purification subsequent DEAE purification, purity analysis, specific activity of HLT, and immune response analysis.
Results: The shaker cultivated *B. pertussis* strain 134 passed its quality attributes such as sterility, opacity and purity. During TCA precipitation the *B. pertussis* desired proteins were precipitated and confirmed. The indigenous bed height was optimized and recovery was also calculated in G50 purification. The fractions were analyzed for OD, the total protein concentration and the results were 0.074–0.214, and the total protein content was found between 12.33 μg/ml and 35.67 μg/ml. Subsequent DEAE purification of selected G50 fractions was done and the fractions 9 and 14 had higher OD values of 0.675 and 0.397. Furthermore the DEAE purified samples were structurally analyzed through SDS PAGE and it was found that HLT in the single polypeptide band was around 140 kDa. The qualitative immune response analysis of DEAE purified selected fractions pool showed positive immune response in ODD assay, and in the case of guinea pig antisera it led to the development of diagnostic kits such as ELISA and other vital techniques.

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Here, in case of guinea pig experiment, the hemorrhagic necrosis analysis showed the necrosis on the skin at the injection site.

Conclusion: The *B. pertussis* HLT could be purified through two phase with G50 and DEAE, cost effective techniques, the G50 purification has reduced the bioburden problems during DEAE purification and at the same time the quality of the product was high.

1. Introduction

Pertussis, also known as whooping cough, is a highly infectious and communicable disease caused by a gram-negative bacterium *Bordetella pertussis*. Being an important cause of death in infants and adolescents worldwide, it still continues to be a public health concern, even in countries with high vaccine coverage. Estimates from WHO suggest that, there are 16 million cases reported about 195,000 deaths per year, 95% of these cases are prevalent mostly in the developing nations.[1]

The disease is characterized by the intense paroxysmal coughing, which terminates in an inspiratory ''whoop sound''.[2] Whole cell pertussis vaccine (Wcpv) is a suspension of killed *B. pertussis* organisms and has been used worldwide for nearly 90 years for the universal immunization of children. To control whooping cough disease, it is used in combination with diphtheria and tetanus vaccines, commonly known as DTP vaccine. *B. pertussis* organism produces a wide range of biologically active components called virulence factors, which contributes to the ability of the organism to cause the disease. These virulence factors are Pertussis Toxin (PT), Adenylate Cyclase Toxin (ACT), Filamentous Haemagglutinin (FHA), Lipopolysaccharide (LPS), Pertactin (PRN) (69 kDa), Agglutinogens and Heat Labile Toxin (HLT).

Each of the virulence factors has its own role in immunogenicity and the biological property of the final pertussis vaccine component. The Heat Labile Toxin (HLT) is one among the virulence factors of *B. pertussis*, which may play an important role in Bordetellosis, since this toxin is one of only two toxins known to be produced by all virulent Bordetella species. Bordet and Gengou[3] who were the first to describe the action of this toxin, noted that *B. pertussis* cells were dermonecrotizing and lethal, when injected into animals.[4,5] The HLT is a cytoplasmic protein present in all Bordetella species. The activity of the toxin is detoxified by heat inactivation at 56 °C for 30 min during Wcpv preparation. Therefore, the aim of the study was the evaluation of purified HLT from *B. pertussis* vaccine strain 134 by employing indigenous technology and examining the immunobiochemical aspects of the purified protein.

2. Materials and methods

2.1. Strain of *B. pertussis*

B. pertussis vaccine strain 134 (Rijks institute, Bilthoven, Holland) was used in this study. The strain was maintained in lyophilized state at 4 °C and is an important strain used for the production of routine Wcpv.

2.2. Bordet-Gengou (BG) medium

Bordet-Gengou (BG) Medium was prepared as per Cruikshank[6] with the significant modifications as prescribed by WHO.[7]

2.3. B2 culture medium

The B2 culture medium was prepared with following compositions: Bacto casamino acid (BCA) 1800 g, L-glutamic acid 1500 g, NaCl 750 g, KH_2PO_4 150 g, $MgSO_4$ 30 g, $CaCl_2$ 3 g, $FeSO_4$ 3.74 g, $CuSO_4$ 0.15 g, Glutathione 3.05 g, yeast extract 1500 g, and soluble starch 450 g. Starch solution was prepared by dissolving starch in cold water. The suspension was then added to 20 l of hot distilled water and steamed in autoclave at 118 °C for 20 min separately. The remaining chemicals were dissolved in serial order in 50 l of warmed distilled water in separate vessel. L-glutamic acid solution was prepared by dissolving in 50% NaOH to get amorphous solution in warm distilled water. BCA was dissolved in 10 l of distilled water and yeast extract was added to this solution. Finally L-glutamic acid solution and other chemicals were added, made up to 300 l and mixed properly, then it was transferred to the fermentor and the medium was sterilized at 121 °C for 30 min.[8]

2.4. Sterility media

Nutrient agar medium, Soyabean Caesin Digest Medium (SCDM) and Alternate Thio glycolate medium (ATGM) were used to study the purity and sterility of the culture at the appropriate stage to ensure its purity and safety.

2.5. Preparation of seed culture

One ampoule freeze dried working seed stock (134 strain) was opened under sterile environment and resuspended in 2 ml of sterile B2 medium. The suspension was then inoculated in BG slope and incubated at 35 °C ± 1 °C for 72 h, and the purity was ensured by Gram staining. Furthermore, the culture was scraped at aseptic conditions and inoculated into a 1 l flask containing 400 ml of B2 medium. The flasks were loaded on seed shaker for 24-h at 35 °C ± 1 °C. Further flasks were loaded on shakers (140 ± 10 RPM) and sterile filters were applied over the surface of the medium (3–5 Lpm). Cultivation was allowed to continue for 30–36 h until the maximum yield of organism was achieved. Samples were collected and checked for purity, opacity and pH. The pH should range from 7.8 to 8.2.