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# Synthesis, Characterization and Immunological Properties of LPS-Based Conjugate Vaccine Composed of O-Polysaccharide from *Pseudomonas aeruginosa* IATS 10 Bound to Recombinant Exoprotein A.

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## ABSTRACT

*Pseudomonas aeruginosa* is an important opportunistic pathogen that can cause infection in immunocompromised patient. Lipopolysaccharide (LPS), the major surface antigen of P. aeruginosa, is immunogenic and elicits protective antibodies in animals. The O-polysaccharide (O-PS) from International Antigenic Typing Scheme (IATS) 10, the antigenic determinant of LPS, was coupled to recombinant exoprotein A (rEPA) through adipic acid dihydrazide (ADH) mediated by carbodiimide condensation reaction. Mice were immunized with the conjugate emulsified with monophosphoryl lipid A-trehalose dicorynomycolate (MPL-T) and Freund's adjuvants. The conjugate emulsified with MPL-T adjuvant elicited the highest level of IgG and IgM followed by Freund's adjuvant. IgG titers using both MPL-T and Freund's adjuvants were recorded to be higher than IgM titers after the second post of the immunization. Immunization of mice with the prepared conjugates emulsified with MPL-T and Freund's adjuvants provided high level of protection (100%) against ten times the LD50 of homologous strain of P. aeruginsoa. The elicited high IgG level and the in vivo protection test results provided good evidences for the possible protection of the conjugate against subsequent infection with the pathogen. These findings will enable us to use it as protective vaccine candidate.

**Keywords:** Adjuvant, Conjugate Vaccine, Lipopolysaccharide, *Pseudomonas aeruginosa*.

## INTRODUCTION

*Pseudomonas aeruginosa* is an important opportunistic pathogen that can cause a significant number of nosocomial infections, especially in individuals whose immune systems are severely compromised (Bodey *et al*., 1983). Once established, *P. aeruginosa* can cause a wide variety of acute or chronic infections. Infection with *P. aeruginosa* remains one of the most difficult to treat because of their intrinsic resistance to many antimicrobial agents, including the β-lactams. The intrinsic antibiotic resistance of *P. aeruginosa* is largely due to the presence of multiple drug efflux pumps and the low permeability of the outer membrane (Yagel *et al*., 1996; and Li *et al.,* 1998). Therefore, immunotherapy of active immunization with vaccines or passive immunization with immunoglobulin preparations is an alternative for the treatment of *P. aeruginosa* infection.

The pathogenesis of *P. aeruginosa* infection is complex because it includes a number of bacterial virulence factors, involving both extracellular (e.g., exotoxin A, exoenzyme S and elastase) (George *et al*., 1993) and somatic (e.g., lipopolysaccharide (LPS)) antigen (Cryz *et al*., 1984). LPS is the virulence factor of *P. aeruginosa* (Rocchetta *et al*., 1999). It is an integral part of the bacterial cell wall and exposed on the cell surface playing an important role in interaction of the bacterium with immune system, so it can elicit bactericidal antibodies (Rocchetta *et al*., 1999).

Structurally, the LPS is composed of into three regions: the lipid A, the core oligosaccharide and the O-antigen polysaccharide (O-PS) (Rivera *et al*., 1989). Because of its toxicity, whole LPS is not suitable to be used as a vaccine, and its isolated O-PS is usually poor immunogen especially in infants (Schneerson *et al*., 1987; Jennings, 1990; and Chu *et al*., 1991). Nevertheless, conjugation of the antigenic O-PS or capsular polysaccharide to protein leads to enhance their

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immunogenicity (Schneerson *et al*., 1987; Jennings, 1990; Szu *et al*., 1991; Gupta *et al*., 1995; and Johnson *et al.,* 1995). Such enhancement was observed after conjugation of O-PS or capsular antigens from various strains of *Streptococcus pneumonia*, *Neisseria meningitides*, *Haemophilus influenzae*, *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, and *Vibrio cholera* to carrier proteins (Schneerson *et al*., 1987; Chu *et al*.; 1991; Szu et al., 1991; and Johnson *et al*., 1995).

Each serologically distinguishable strain of *P. aeruginosa* produces a unique O-antigenic O-PS having a specific composition and structure. Based on chemical and serological variations, the O-PSs of *P. aeruginosa* are currently classified into 20 serogroups according to the International Antigenic Typing Scheme (IATS), with many serogroups possessing subtype strains having subtle variations in the O-antigen (Knirel, 1990).

*P. aeruginosa* produces a large number of extracellular products contributing to its virulence. Exotoxin A is the most toxic pathogenic factor (Shang *et al*., 1996). It is produced by 90% of *P. aeruginosa* clinical isolates (Cross *et al*., 1980), and considered as a common antigen between different *P. aeruginosa* serogroups. Thus, development of multivalent LPS based vaccine coupled with the rEPA common antigen of *P. aeruginosa* may have potential cross protection against subsequent infections with the antigenically related pathogens.

Increased levels of antibody directed against LPS and exotoxin A have been associated with an improved prognosis in patients with *P. aeruginosa* septicemia (Pollack *et al*., 1976; and Cryz *et al*., 1983). Robbins *et al*. (1992 and 1995) reported that the serum antibodies against the surface O-PS antigens of LPS confer protective immunity against the pathogen.

There is no commercially available vaccine to prevent infection with *P. aeruginosa*. Experimental vaccines against a variety of surface epitopes have been complicated by toxicity and/or inconsistent immunogenicity in target populations (Hemachandra *et al*., 2001; Worgall, 2005). Conjugate vaccine using O-PS coupled to recombinant exotoxin A appears particularly attractive because it presents two protective antigens. In a prior study, for example, a conjugate vaccine produced by coupling O-PS derived from *Escherichia coli* to toxin A was found to be nontoxic and capable of engendering both anti-polysaccharide and antitoxin A immune responses in animals (Cryz *et al.,* 1995).

Recent research in immunotherapy has focused on the use of the mutant, nontoxic, recombinant *Pseudomonas aeruginosa* exoprotein A (rEPA) in conjugate vaccines as a carrier to stimulate an immune response against several epitopes because it is easily expressed and purified in high concentration from *Escherichia coli*. The rEPA was also found to be effective in mice and was tested in humans, and it was found to enhance the immune response in a safe manner. It has also been successfully employed for many surface polysaccharides-based vaccines (Welch *et al*., 1996; Konadu *et al*., 1998; Pavliakov *et al*., 1999; and Ahmed *et al*., 2006).

In this study, the O-PS from *P. aeruginosa* IATS 10, one of the most prevalent strains of *P. aeruginosa* in Jordan and worldwide (Odeh, 2002), has been isolated and conjugated to rEPA. This conjugate can induce antibodies not only to neutralize the exotoxin A cytotoxicity but also to block bacterial colonization of homologous strain.

## 2. MATERIALS AND METHODS

**Bacterial Growth and LPS Preparation**.

*P. aeruginosa* IATS 10 strain (kindly provided by Professor Joseph Lam, University of Guelph, Canada) were cultivated in large scale (60 L) in Mueller-Hinton-broth medium. The cells were killed by adding phenol (90%) to a final concentration of 2%, and the cell pellets were obtained by centrifugation. The cell pellets were washed once with absolute ethanol, twice with acetone, and finally twice with petroleum ether, LPS was dried under the fume-hood until the residual ether smell disappears, then the dried cells were grounded to a fine powder (Masoud *et al.,* 1991). LPS would have then be extracted and purified from the dried cellular material by phenol-water method (Westphal *et al.,* 1952).

**O-PS Preparation**

LPS (100 mg) was hydrolyzed in 2% acetic acid (20 ml) at 100 °C with shaking for 150 min. The hydrolysate was centrifuged at 5000 rpm at 4 °C for 30 min to separate lipid A from O-PS moiety. The supernatant containing O-PS was removed and then lyophilized. Crude O-PS was purified on the Bio-Gel P2 gel filtration column chromatography (2.6 X 100 cm, 200–400 mesh, Bio-Rad, USA). The column was eluted with pyridinium-acetate buffer, and ~5 ml fractions were collected by a fraction-collector (Rikakikai, Tokyo). Collected fractions

were tested for carbohydrate by phenol-sulfuric acid method (Dubois *et al.,* 1956). The positive fractions were pooled, lyophilized, and weighed.

**Preparation of Conjugates**

The rEPA (kindly provided by Dr. Ali Fattom, Biopharmaceutical Company, USA) was derived with adipic acid dihydrazide (ADH) (Sigma) by the carbodiimide method (Cryz *et al.,* 1986). Briefly, 150 mg rEPA in 29 ml phosphate buffer saline (pH =7.2) was reacted with 300 mg ADH and mixed. After that, a 300 mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Sigma) were added dropwise. Subsequently, the reaction mixture was left at room temperature with stirring for 3 h while the pH was maintained at 4.7± 0.2 with 0.1 N HCl. Following that, the derived rEPA-ADH was dialyzed against PBS at 4°C for two days with four changes of the outer buffer. Finally, the dialysate was centrifuged at 3000 rpm for 10 min to remove insoluble material then the supernatant was concentrated by ultrafiltration (Amicon ultra tube 50 KDa, Millipore), and assayed for protein concentration by Bradford method (Bradford., 1976). The final concentration was estimated by referring to a standard curve obtained at the same time using known concentrations of bovine serum albumin (BSA).

Conjugation of O-PS to rEPA-ADH was performed as follows: 30 mg O-PS of IATS 10 strain was dissolved in 3.75 ml PBS followed by addition of 100 mg EDC and mixed at room temperature for 2 min. Subsequently, 1 ml of rEPA-ADH (20 mg/ml) was added dropwise and the reaction was carried out with continuous stirring at room temperature for 3 h. The pH was continuously adjusted to 5.6 by addition of 0.1N HCl. After the end of the reaction (when the pH become stable), the pH was neutralized using 0.1N NaOH then solution was centrifuged at 3000 rpm for 10 min to remove insoluble material.

The purified conjugate was obtained by fractionation of reaction mixture using Sephadex G-100 column (1.4 X 90 cm) base on the differences in molecular size, eluted with PBS (Masoud, 2007). The void volume of the column was determined with dextran blue 2,000 KDa (Sigma). The eluents were monitored for protein at $A_{280}$ and assayed for carbohydrate content by the phenol-sulfuric acid method (Dubois *et al.,* 1956). The collected positive fractions in void volume containing both carbohydrate and protein were pooled, concentrated by ultrafiltration (Amicon ultra tube 50 KDa, Millipore) and stored at -70 ºC. The final product is referred to as O-PS-ADH-rEPA (Scheme 1).

**Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)**

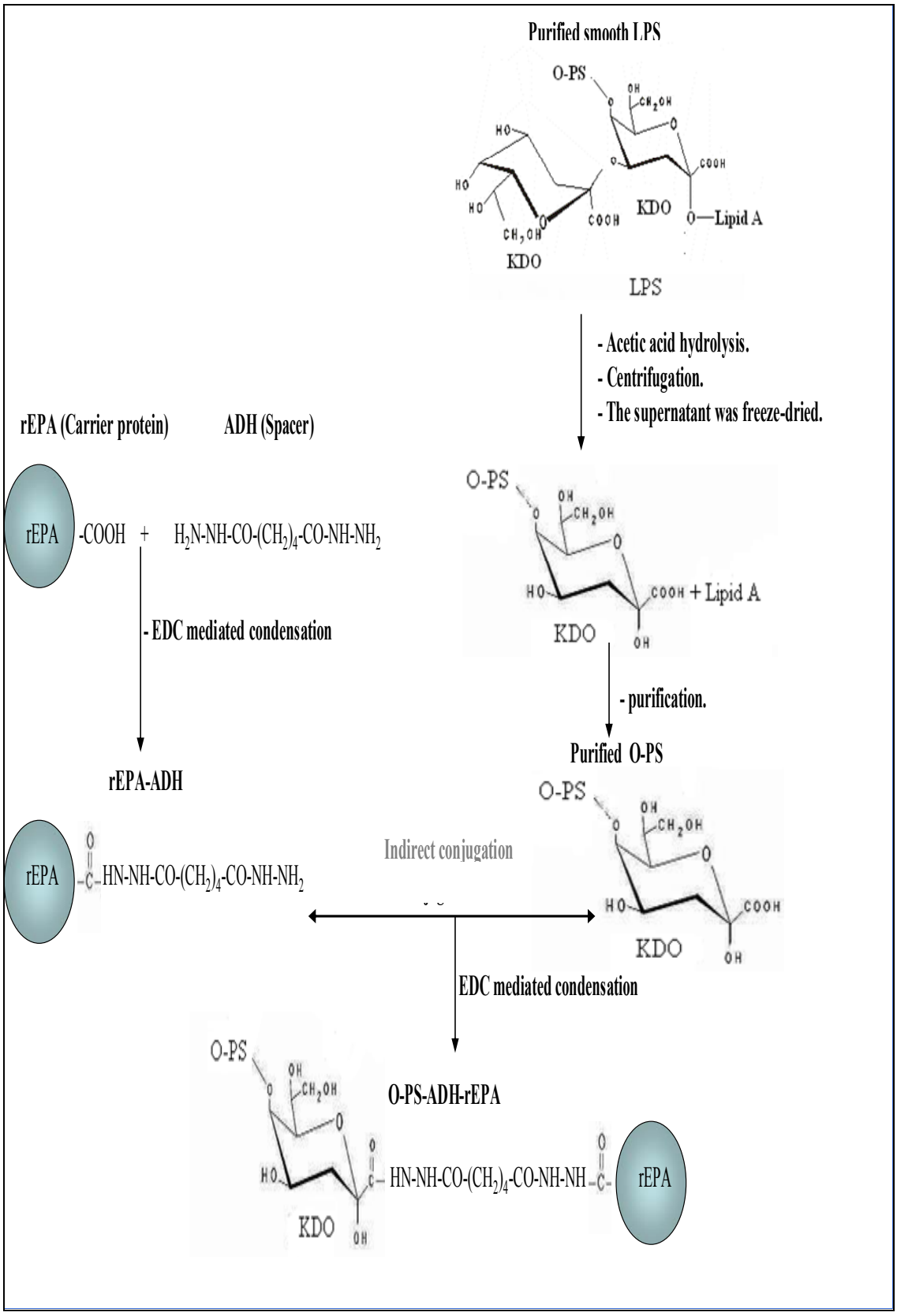
Molecular weight analysis and purity of conjugate vaccine was carried out by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (170 x 200 x 1.5 mm), by using a discontinuous buffer system (Laemmli, 1970), 8% (w/v) acrylamide resolving gel, and 4% (w/v) stacking gel. Protein molecular weight high range standards were used as marker (Sigma). Electrophoresis was carried out at 30 mA/gel constant current until the tracking dye front reached the bottom of the gel. The gel was removed and visualized by Coomassie brilliant blue R-250.

**MPL Preparation and Safety Test**

Monophosphoryl lipid A (MPL) adjuvant preparation was derived from lipid A of *Salmonella typhimurium* (DIFCO, USA). Lipid A was prepared as described earlier. Glycosidic phosphate of lipid A was cleaved by hydrolysis of the lipid A (50 mg) in 10 ml of 0.1 M HCl in boiling water bath with shaking for 1 h (Masoud *et al.,* 1991). Converting the MPL to monovalent cation salt was preformed by electrodialysis (Galanos and Luderitz, 1975) followed by freeze-drying. A safety test was performed to estimate the toxicity of the prepared MPL. Different concentrations of MPL (25, 50, 75, and 200 ug in 0.1 ml sterile PBS) were intraperitoneally (i.p) injected in selected five healthy BALB/c female mice, weighing between 17-20 gm. The animals were monitored for any sign of illness or weight-loss following the injection for 7 days.To prepare MPL-T mixture, 1.0 mg of MPL was combined with 1.0 mg synthetic Trehalose dicorynomycolate (S-TDCM) (Sigma, USA). The combined substances were mixed with 0.08 ml Squalene (hexamethyltetracosahexane), 0.008 ml Tween 80 and 2 ml PBS were added. Complete and incomplete Freund's adjuvant were purchased from Sigma-Aldrich Chemical Co (St. Louis, MO).

**Mice Immunization**

Groups of ten 6-8 week-old female BALB/c mice weighing 16-20 gm were immunized i.p. Three times at 3-week intervals with 2.5 ug (based on O-PS content) of conjugate vaccine and emulsified with MPL-T or Freund's adjuvant in a 1:1 ratio. Blood samples were collected 14 days after the last immunization. Sera were obtained by centrifugation at 3,000 rpm for 10 min then stored at -20 ºC.




**Scheme 1: scheme shows the indirect method for preparation of conjugation vaccine.**

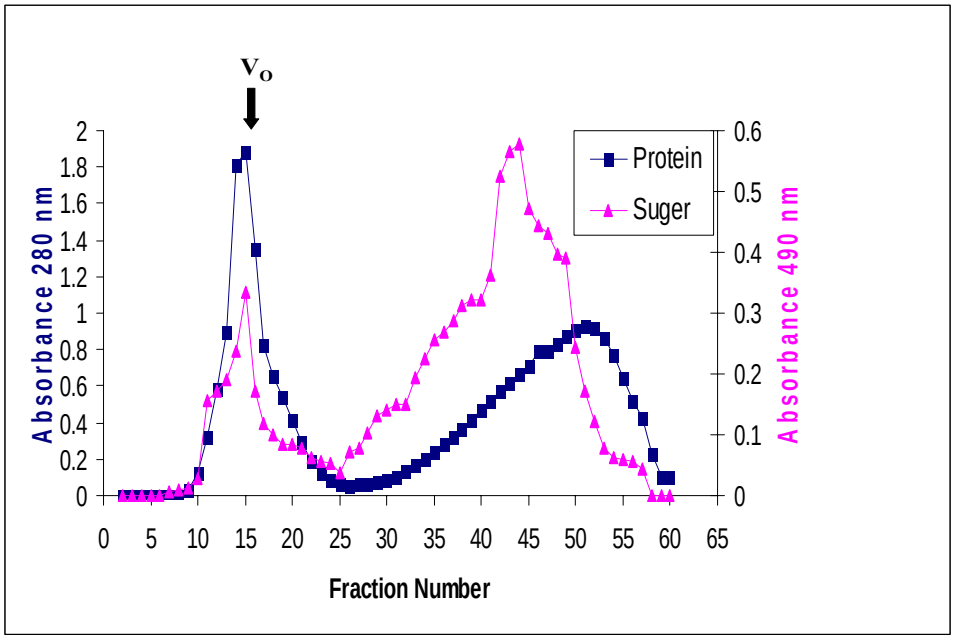



**Figure 1. Gel filtration profile of the O-PS-ADH-rEPA conjugate reaction mixture on Sephadex G-100. The protein content of the fractions was measured by the absorbance at 280 nm and polysaccharide was measured by phenol-sulfuric acid ($A_{490}$). $V_O$, Void volume of column.**

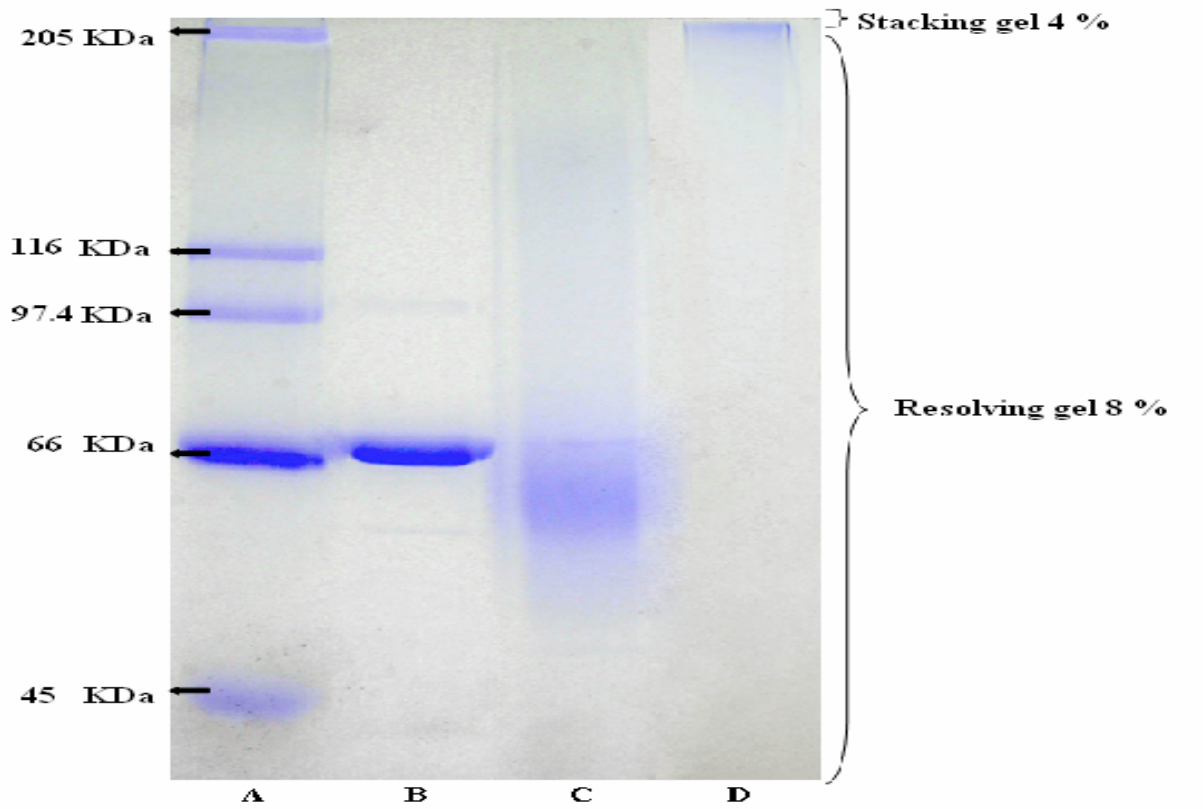



**Figure 2.6. SDS-gel electrophoresis of the conjugate vaccine was stained with Coomassie Blue R-250. Lane A: high range molecular weight standard proteins, lane B: The recombinant exotoxin A (3.2 µg protein), lane C: The ADH-rEPA (3.2 µg protein), lane D: The conjugate vaccine made from O-PS of *P. aeruginosa* IATS 10 (32 µg protein).**

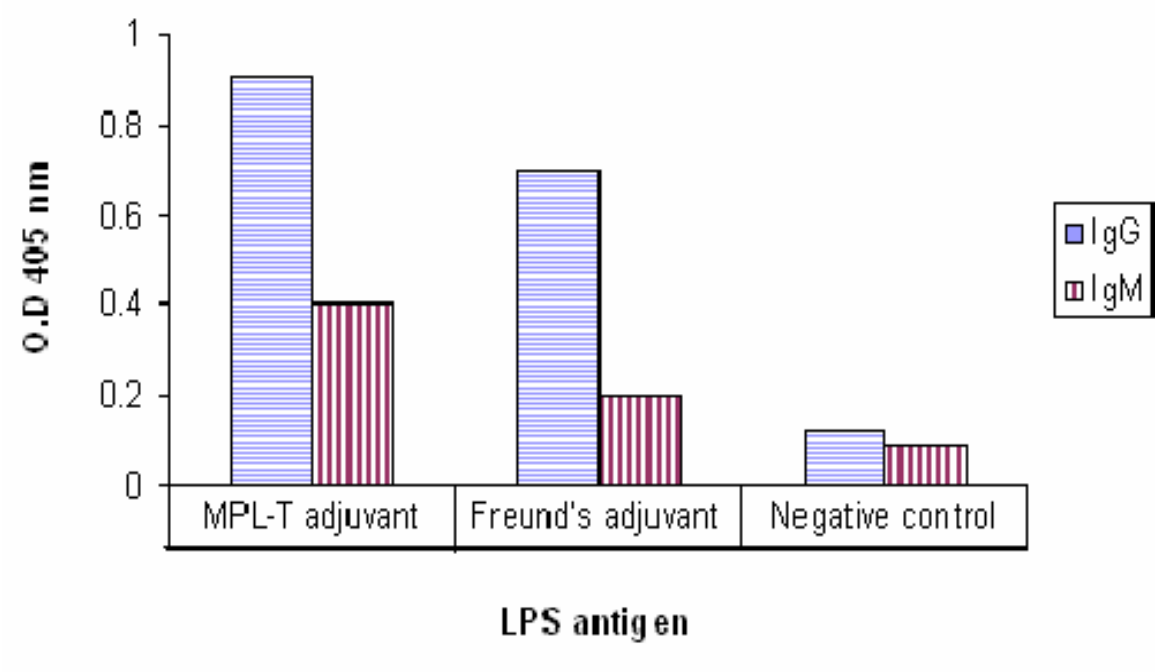



**Figure 3. ELISA IgG and IgM titers elicited against the conjugate vaccine emulsified with MPL or Freund's adjuvants compared with the negative control. The serum dilution is 1/3200, and the titers level is depicted as $OD_{405}$.**

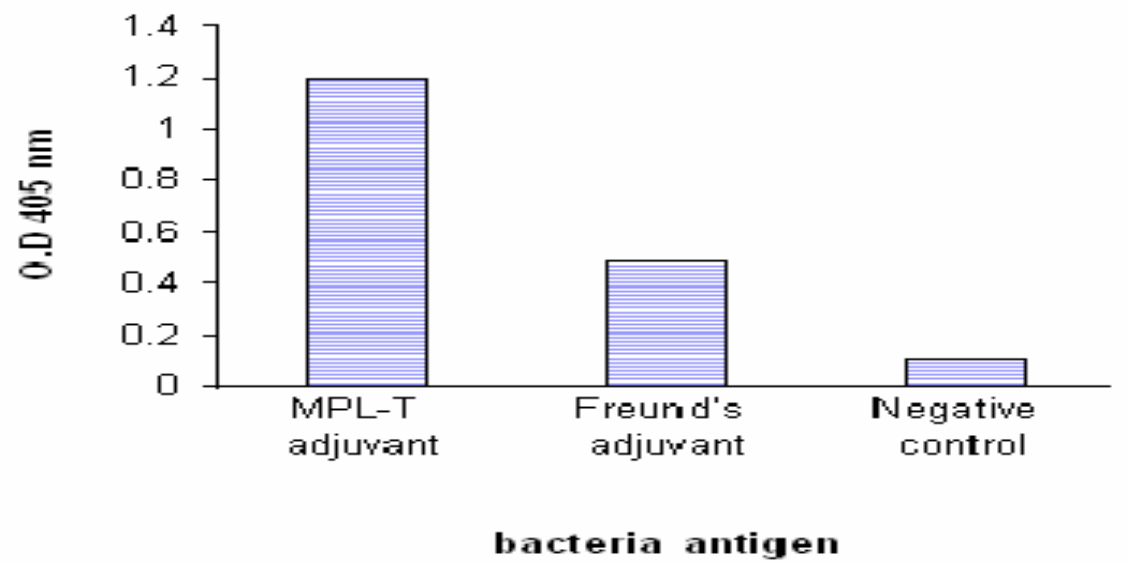



**Figure 4. IgG titers elicited against conjugate vaccine measured using whole bacterial cell ELISA. The serum dilution is 1/3200, and the IgG level is depicted as $OD_{405}$.**

**Enzyme Linked Immunosorbent Assay (ELISA)**

Specific IgG and IgM anti-*P. aeruginosa* LPS antibodies were detected in sera collected from mice raised against the conjugates and then were evaluated by using an ELISA as previously described (Cryz *et al.*, 1986) with some modifications. Briefly, LPS sample solution (100 µl/well) was loaded into ELISA plate at a concentration of 10 µg LPS/ml in 0.1 M carbonate buffer (pH 9.6). Plates were kept overnight at 4 °C. After the plates were washed three times (PBS, pH 7.2, 0.05% $NaN_3$, and 0.05% Tween 20), nonspecific sites were blocked with 200 ul of blocking buffer (1% BSA in PBS,

pH 7.2,and 0.05% $NaN_3$) for 1 h at 25 °C. After washing, serial dilutions of mice sera (1/100 then 2X diluted in 1% BSA–PBS) were added (100 ul/well) and incubated at 25 °C for 3 h. Alkaline phosphatase-conjugated goat-specific anti-mouse IgM and IgG (Sigma, USA) were added (100 µl/well, diluted 1/3000 in 1% BSA–PBS) and incubated for 1 h at 25 °C. Washing using the buffer system described above was performed between the steps. A solution of P-nitrophenylphosphate-disodium (Sigma, USA) in concentration of 1 mg/ml in carbonate buffer (pH 9.6) was prepared, and 100 µl/well were added. Optical density (O.D) was determined after 1 h using ELISA reader (das, Italy) set to 405 nm.

**Whole Bacterial Cell ELISA**

Single colony was inoculated into 20 ml Trypticase soy broth (TSB) medium and grown overnight at 37°C with shaking. On the next day, all the bacterial cells were killed by formaldehyde and incubated at 4ºC overnight then spun at 5000 rpm at 4°C for 10 min and washed once in sterile PBS (pH 7.2). The pellet was resuspended in 20 ml of sterile carbonate buffer (pH 9.6) and diluted to an $O.D_{600}$ 0.45, which is approximately equal to 6 x $10^8$ CFU/ml. Bacterial suspension (100 µl/well) were loaded into ELISA plates, followed by overnight incubation at 4°C. The blocking and addition of other reagents were performed as mentioned above for ELISA (Robert *et al.,* 1984).

**$LD_{50}$ Determination**

*P. aeruginosa* were grown in TSB medium overnight at 37 °C with agitation. Cells were harvested and washed twice with PBS by spinning at 5000 rpm for 10 min. The cell suspension was measured by spread plate technique after a serial dilution in PBS. BALB/c female mice weighing 22-24 gm (14–16 weeks old) were divided into 3 groups, each of which consisted of 5 animals. Each mouse received 0.1 ml of the bacterial suspension dilution ($10^6$-$10^8$ CFU/ 0.1 ml per mouse) using the i.p. route. Negative control group received sterile PBS. Mortality was recorded 7 days after challenge. $LD_{50}$ values were calculated using Reed-Muench equation (1938).

**In vivo *Protection of Mice***

For evaluating biological activity *in vivo*, BALB/c female mice weighing 16-20 gm (6–8 weeks old) were divided into 3 groups, each of which consisted of five animals that were immunized with the conjugates emulsified with various adjuvants and negative control group received PBS only as described above. Fourteen days after the last injection, mice were challenged i.p. with ten times the determined $LD_{50}$ of the homologous strain as described earlier (Cryz *et al*., 1983). The mice were daily monitored for mortality for 7 days (Holder *et al.,* 1982; Specht *et al*., 1995).

## 3. RESULTS

**MPL toxicity**

The safety test showed that up to 200 ug MPL dose did not kill any of the tested mice (Table 1). None of the tested animals showed any abnormal reaction during the observation period, demonstrating that the prepared MPL is safe to be used as an adjuvant *in vivo* studies.

**Characterization of the Conjugate**

The O-PS of IATS 10 was coupled indirectly through to rEPA catalyzed by EDC reagent. The conjugate was fractionated on Sephadex G-100 column chromatography and eluted as a single peak in the void volume (Figure 1). SDS-gel electrophoresis showed that the conjugate vaccine did not enter into 8% resolving gel (Figure 2, lane D), accordingly, it is clear that this vaccine has a molecular weight more than 205 KDa, greater than the molecular weight of either unconjugated O-PS or ADH-rEPA. No bands corresponding to free rEPA or ADH-rEPA were observed (Figure 2, lane D). Based on the SDS-gel electrophoresis, high degree of conjugation was obtained between O-PS and rEPA, indicating that the conjugation strategy was successful.

**Table 1. Lethal toxicity of the MPL prepared from *S. entritidis* LPS on female BALB/c mice.**

| Concentration µg/ mouse | Lethality Dead/Total animals |
|---|---|
| 25 | 0/5 |
| 50 | 0/5 |
| 75 | 0/5 |
| 200 | 0/5 |

**Immunogenicity of the Conjugate Vaccine**

The immunogenicity of the conjugate was evaluated by immunization of mice with the prepared conjugate vaccine emulsified with either MPL-T or Freund's adjuvant compared to the negative control. Elicited IgG

and IgM were assayed against the homologous LPS using ELISA method (Figure 3). The conjugate emulsified with MPL-T adjuvant elicited the highest level of IgM and IgG followed by Freund's adjuvant whereas very weak titers were observed in the negative control groups. Elicited IgG reported higher titers than IgM titers after the second post of immunization, which provide a good evidence for the possible protection against subsequent infection with the pathogen. Similar results were obtained using the whole bacterial cell as a coating antigen in ELISA, where the highest titer was observed with MPL-T adjuvant (Figure 4).

**Table 2. Determination of the $LD_{50}$ for *P. aeruginosa* IATS 10.**

| | **Dose (CFU of *P. aeruginosa* IATS 10/ mouse)** | | |
|---|---|---|---|
| | **$5.5 \times 10^5$** | **$5.5 \times 10^6$** | **$5.5 \times 10^7$** |
| **Total number of animals per group** | 5 | 5 | 5 |
| **Number of animals died** | 0 | 0 | 4 |
| **Number of animals survived** | 5 | 5 | 1 |
| **Sg*** | 11 | 6 | 1 |
| **$Ds^{\Delta}$** | 0 | 0 | 4 |
| **Sg + Ds** | 11 | 6 | 5 |
| **% Mortality = $Ds^{\Delta}$ / (Sg+ Ds)* 100** | 0 | 0 | 80 |

Sg* : Number of animals survived at this dose and greater doses.

$Ds^{\Delta}$ : Number of animals died at this dose and smaller doses.

$$\text{Log } LD_{50} = \log 5.5 \times 10^6 + \frac{0.5 - 0}{0.8 - 0} \times \log \frac{5.5 \times 10^7}{5.5 \times 10^6} = 2.3 \times 10^7 \text{ CFU/ mouse}$$

**Table 3. Protective capacity of the prepared conjugate vaccine of the *P. aeruginosa* IATS 10 against 10-fold $LD_{50}$ of homologus strain.**

| **Group of mice** | **Dead/ Total Animals** | **Survival %** |
|---|---|---|
| MPL-T adjuvant | 0/5 | 100% |
| Freund's adjuvant | 0/5 | 100% |
| Negative control (PBS alone ) | 5/5 | 0% |

**$LD_{50}$ of IATS 10**

The $LD_{50}$ for *P. aeruginosa* IATS 10 was determined according to Reed and Muench method and was found to be $2.3 \times 10^7$ CFU/mouse (Table 2). All the mice treated with PBS survived and appeared healthy throughout the experiment.

***In vivo* Protection of Mice**

*In vivo* protective capacity of the conjugates was performed by challenging the immunized mice with ten times the determined $LD_{50}$. The prepared conjugate provided high level of protection (up to 100%) when the mice were immunized with the conjugate emulsified with MPL-T or Freund's adjuvants (Table 3). No protection was observed in the negative control group.

## 4. DISCUSSION

*P. aeruginosa* is a major cause of morbidity and mortality in many hospital-acquired infections, especially in immunocompromised patients such as cancer- or burns-victims and children with cystic fibrosis (Bodey *et al*., 1983; and Lyczak *et al*., 2000). The emergence of antibiotic resistant strains of *P. aeruginosa*, in recent years, increased the demand for the development of a protective vaccine against this pathogen (Stanislavesky *et al*., 1997; and Holder**,** 2004).

The LPS is the major surface antigen of *P. aeruginosa*. Elicited antibodies directed against the O-PS antigen have shown high efficiency to protect against *P. aeruginosa* infection. The level of protection is correlated

with anti-LPS antibodies (Cryz *et al*., 1984). The O-PS antigen is attractive target to prepare vaccine because it is non-toxic and highly immunogenic when conjugated with immunogenic protein. Several protein carriers were evaluated to stimulate the immune system against *P. aeruginosa* such as tetanus toxoid (Cryz *et al*., 1986), native exotoxin A (Cryz *et al*., 1989; Zuercher *et al*., 2006), and BSA (Tsay *et al*., 1984; Al-Zeer and Masoud, 2007).

Because exotoxin A cross-reacts antigenically in most serotype strains, it plays an important role in the infectious process, and it is highly immunogenic (Ohman *et al*., 1980; and Vidal *et al*., 1993). Modified exotoxin A (rEPA) was selected in this study as a carrier protein against *P. aeruginosa*. The indirect conjugation of the O-PS from *P. aeruginosa* IATS 10 to rEPA protein appeared to be promising for the production of LPS-based vaccine because it is simple, easy, and an efficient conjugation method.

In the present study, the O-PS from *P. aeruginosa* IATS 10 was conjugated indirectly through ADH to rEPA by carbodiimide mediated condensation reaction. The immunogenicity of the prepared conjugate was evaluated using MPL-T and Freund's adjuvants in mice model. High IgG titers were obtained against O-PS when the conjugate was emulsified with MPL-T adjuvant. These results were not surprising since MPL-T adjuvant is already known for being more effective than the Freund's adjuvant in mice (Schneerson *et al*., 1991; Al-Zeer and Masoud, 2007). The elicited IgM antibodies showed higher titers when the conjugate was emulsified with MPL-T than with Freund's adjuvant. Similar observation was reported in the previous studies using the conjugate vaccine of *P. aeruginosa* with the same adjuvants (Schneerson *et al*., 1991; Al-Zeer and Masoud, 2007).

Previous studies have used whole bacterial cell ELISA technique to measure the antibody levels (Robert *et al.,* 1984). Similarly, in this study the whole bacterial cell ELISA was used to ensure that the LPS orientation did not change during the extraction procedure. Although we used two different coating antigens (formalin-killed bacteria and LPS), we obtained similar results with respect to the antibody titer. This indicates that LPS isolation procedure did not change the original state of the molecules.

*In vivo* protections are utilized widely for measurement of immune responses to vaccines because the circulated antibodies play an important role in the protection of the host against many infectious diseases (Cryz *et al*., 1986). In this study, mice immunized with conjugate vaccine emulsified with MPL-T or Freund's adjuvant were provided high level of protection (100%) against 10-fold $LD_{50}$ of the homologous strain because it presents two protective antigens (rEPA and O-PS) which were found to be nontoxic. These findings offer additional evidence that conjugate vaccine may have potential usefulness as vaccine to prevent the infection with *P. aeruginosa*. Moreover, the results showed a good correlation between the anti-LPS titer and survived mice.

Because of the variation in O-PS antigenic structures of different strains of *P. aeruginosa*, further studies are required on a polyvalent conjugate vaccine of O-PSs from several strains of *P. aeruginosa* conjugated to rEPA protein.

## Abbreviations

ADH: Adipic acid dihydrazide, BSA: Bovine serum albumin, CFU: Colony forming unit, EDC: 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide, ELISA: Enzyme linked immunosorbant assay, IATS: International antigenic typing scheme, i.p.: Intraperitoneal, KDO: 3-deoxy-D-manno-octulosonic acid, LD50: Half lethal dose, LPS: Lipopolysaccharide, MPL: Monophosphoryl lipid A, MPL-T: Monophosphoryl lipid A-trehalose dicorynomycolate, O-PS: O-polysaccharide, rEPA: Recombinant Pseudomonas aeruginosa exoprotein A, SDS-PAGA: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, S-TDCM: Synthetic trehalose dicorynomycolate, TSB: Trypticase soy broth.

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# تركيب ودراسة الخواص والصفات المناعية للمطعوم المقترن المكون من O-متعدد السكريات المعزول من *سيدوموناس ايروجينوزا* السلالة 10(حسب التصنيف العالمي) المربوط الى البروتين الخارجى " أ " المعدل جينيا

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## ملخص

سيدوموناس ايروجينوزا هي بكتيريا ممرضة انتهازية وخاصة عند المرضى الذين يعانون من نقص في المناعة. متعدد السكريات الدهني الموجود على سطحها هو من اهم المركبات في عملية تحفيز انتاج الاجسام المضادة في الحيوانات المخبرية. إن متعدد السكريات هو الجزء الأهم في تحديد خاصية الاجسام المضادة، ولقد تم ربطه مع البروتين المعدل جينيا "أ" بطريقة تفاعل التكثيف باستعمال مادة الكاربوديمايد. ولقد تم تطعيم فئران المختبر بالمطعوم المقترن الممزوج مع محفزات جهاز المناعة سواء الدهن"أ" أحادي الفوسفات او فروند. ولقد كشفت النتائج ان المطعوم المقترن الممزوج مع الدهن"أ" أحادي الفوسفات قد اعطى اعلى مستوى من الاجسام المضادة سواء من النوعين "ج" و"م"يليه المحفز فروند. ولقد وجد ان مستوى الاجسام المضادة من النوع "ج" اعلى فى جميع المعاملات من النوع "م". وكذلك اظهرت نتائج الحقن بعشرة اضعاف الجرعة القاتلة 50٪ في فئران المختبر اكتساب تلك الحيوانات حماية كاملة تصل الى 100٪. إن النتائج المنبثقة عن هذه الدراسة تظهر بشكل واضح بأن المطعوم المقترن الناتج يكسب مستوى جيداً من الحماية ضد الإصابات بهذه البكتيريا الخطرة وبالتالي من الممكن ان يكون المطعوم المحضر مرشحاً قوياً لاستعماله كمطعوم للوقاية من هذه البكتيريا.

**الكلمات الدالة:** متعدد السكريات الدهني، المطعوم، سيدوموناس ايروجينوزا.

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