# Isolation and Partial Purification of Cell wall Lipopolysaccharides of *Pseudomonas Aeruginosa* and Using It as Vaccine

**Taghreed A. Mohammad**
**Sawsan Abdul Hussain Mahdi**
Dept. of Chemistry/ College of Education for Pure Science(Ibn AL-Haitham)/ University of Baghdad

**Received in:21/February/2016,Accepted in:17/April/2016**

## Abstract

This study ,the samples were collected from "118 patients " suffering from burn wound contaminated with *Pseudomonas aeruginosa* and 100 health individuals (male and female ) as a control group ,the samples were wound swap and blood sample .

Chromatography technique was employed to extract and purify cell wall containing lipopolysaccharide by using *P. aeruginosa* isolate ATCC 15692,the purification done by addition of ammonuium sulfate, sodium dodecyl sulfat (SDS) anddialysis, gel filtration chromatography by using sepharose-4B.

Immunogenicity of LPS component was determined by mice injection under the skin ,then Ab concentration against LPS component formed and determined by ELISA .

These findings indicated ability of LPS to make immune system stimulated to release specific Ab towards *P. aeruginosa* isolate ATCC 15692 , therefore, this result may be a promising procedure to produce vaccine against *P. aeruginosa* isolate ATCC 15692.

**Key words**: *Pseudomonas aeruginosa* and Lipopolysaccharides

# Introduction

*P. aeruginosa* is a rod-shaped bacterium almost all types of strains are motile by means of a polar flagellum and includes bacteria slightly curved rods or straight. [1].

Lipopolysaccharide (LPS) form outer leaflet projecting outside and the inner leaflet containing phospholipids and lipoproteins [2]. The LPS consists of three different sectors: Lipid, core polysaccharide comprising the inner and outer cores and O-specific polysaccharide chains projecting outward [3].

Active acquired immunity to a particular disease is enhanced by a biological preparation which is called vaccine contains is often made from weakened or killed forms of the microbe or bacteria derived toxins or a surface proteins [4].The agent the body's immune system stimulated by vaccine as foreign material, destroy it, and keep a memory, so that the immune system can destroy a specific microbe with short onset of reaction [5].

If a bacterium composed of an outer coating of sugar component called polysaccharides, as many harmful bacteria do, researchers may try making a conjugate vaccine for it[6].Polysaccharide hides (Ag) of bacterium so that the immature immune systems in younger children & infants cannot recognize or respond to them. Conjugate vaccines, a subunit vaccine, can solve this problem [7].

Conjugate vaccine composing of (polysaccharide – protein) proves to be effective primarily due to the effect of the bonded carrier protein which is combined to the polysaccharide[8], as this ensures that T cells (or T- helper lymphocytes) are involved in the activation of β-cells which then go on to produce the antibody proteins that are required for immune defense again these extracellular pathogenic bacterial types[9].

# Materials and Methods

## Subjects

Samples were collected from patients who attended Al-KadimiyaTeaching hospital and from Feb 2014 to Nov 2014 and were investigated for *Pseudomonas aeruginosa* by many tests including [Culture, Vitek ,API and ELISA KIT-Immunolab ].The results of investigation revealed that most of the *P. aeruginosa* isolated in this study to the isolate ATCC 15692.

# Methods

## 1-Isolation and partial purification of LPS-Antigen from *P. aeruginosa* (isolate ATCC 15692).

1. *P. aeruginosa* (isolate ATCC 15692) was cultivated blood agarto yield 50 mlfrom colony concentrations for *P. aeruginosa* (isolate ATCC 15692), the cells were harvested by centrifugation at 4000 rpm for 30 minut.
2. *P. aeruginosa* suspension was treated with lysis buffer (0.1 M acetate buffer, PH=6 ,containing 2% sodium dodacylsulphate and 5% mercaptoethanol treated with water. The resultant suspnsion was vortexes strongly, incubated in boiling water for 5 minutes and refrigerated in ice bath for two minutes.
3. Fifty ml of the digested mixture was centrifuged at 14000xg for 10 minutes ,the supernatant was precipitated using ammonium sulphate $(NH_4)_2SO_4$ at saturation ratio 80%. The precipitate was collected by centrifugation at 20,000xg for 10 minutes, suspend in 5 ml of 0.1 M acetatebuffer, pH= 6 also dialyzed again.
4. Purifications of LPS-Antigen by-gel filtration chromatography preparation of sepharose-4B was preparedas suggested by pharmacia fine chemicals company aamount of

sepharose-4B dissolved in 0.1M acetate buffer pH=6 degassed and packet in column (2.5 x 78) cm, then equilibrated the same buffer. Added 5 ml from sample that concentrated by ammonium sulphate was set onto sepharose-4B colum.

Elution achieved at a flow rate(F.R) of 5 mL fraction(12 tube through one hour) by the similar buffer of equilibration then the absorbance of each fraction was assessed at 280 nm. LPS-Antigen collected (tube No. 15, 16….., and 26 fraction) and concentration by dialysis tube against sucrose.

### 2-Computation of LPS concentration in stock and determination of LPS-Antigen immunogenicity

Detection of LPS (stock) concentration by used ELISA technique. principle of ELISA technique was sandwich method and constrictions of antibody against LPS-antigen was measured in serum samples mice using ELISA Kit .

### 3-Estimationof LD50 to LPS-Antigen

Micewere separated into 4 groups: 4 mice for each group. All mice were injected subcutaneously with diverse concentrations of LPS-Antigen (25,50,100, and 200 µg/dl) and waited for 48 hours, LD50 for mice was 119.05 µg/kg.

### 4-Preliminary trial of vaccination *to P.* aeruginosa using local preparation of LPS – antigen

Eighteen mice were divided 3 groups ,6 mice in each groups. Group A and C survived as control ,group B was test group.

## Results and Discussion

In Table (1)and Figure(1), it was found that appear "Isolation and Purification for LPS" dissolved with lyses buffer [containing 2% sodium dodacylsulphate and 5% mercaptoethanol treated with water] and vortexes for breakingthe cell wall of *P. aeruginosa* [isolate ATCC 15692] and prepared in addition to chromatography column to" isolation and purification" the LPS for otherproteins which were consistent with the previous study of Phil [10]. Portion turbidity was measured using spectrophotometer at 280 nm and assessed the LPS concentration by sandwichELISA methods [11].

Figure (2) shows that detection of LPS (stock)concentration by using ELISA technique. Principle of ELISA technique was sandwich method ,detection the LPS as antigen and reaction with antibody , the concentration of LPS was 200 µg/dl, the ELISA method was high sensitive and specific technique which were consistent with the study [12].

Table (2) showed that in the step of vaccine preparation, LD50 and immunogenicity to LPS must know, LD50 for mice was 119.05 µg/kg [13].

The findings in table (3) and figure (3), regardingpreface trial vaccine to *P.seudomonas* barbicans mice were separated into three groups (six mice for each group). Group A (positive control group), group B (sample group), and group C (negative control group). Group A and B were injected subcutaneously with 11.905 µg/dl of LPS [1/10 dilution of 11.905µg/dl]. This concentration was selected cause it was the most excellent concentration to domain the level of immune system and selected as a vaccine. These results agreed with the study of Al-Lammi [14], and waited for 4 weeks, group B was treated with the immunosuppressive drug (25 µg/dL prednisolone) and after 24 hours group (b andc) were infected with *P. aeruginosa* isolate ATCC 15692 by creation wounds in the skin, then the wounds were contaminated with the colony of *P. aeruginosa* isolate ATCC 15692 and waited for 48 hour. And showed that 6 mice from group C were infected with *P. aeruginosa* and all the mice from

group B were not infected. A result which meant that the mice in group B had protection against *P. aeruginosa* infection as a result of vaccination by 11.905 microgram/dl LPS. These findings were in agreement with the study of Sandini et al [15].

## Conclusions

Lipopolysaccharide was found to have immunogenicity therefore ,it was used as a vaccine , it was hoped that it will become the first antibiotics vaccine approved for human use.

## Reference

1. Keith ,H.; Turner,B.; Aimee, K.; Wessel, M. , Gregory, C.; Palmer G. , Justine, L. ;Murray F. ; and Marvin,W.; (2015). Genome of Pseudomonas aeruginosa in cystic fibrosis sputum, Proceedings of the National Academy of Sciences of the United States of AmericaPNAS,112,13,4110–4115.
2. Chiang, W.; Nilsson,P. ;and PJensen, O.;(2014).“Extracellular DNA shields against aminoglycosides in Pseudomonas aeruginosa biofilms,” Antimicrobial Agents and Chemotherapy, 57, 5, 2352–2361 .
3. Taha, A. ; and Essien, J.;(2005)."Growth Profile and Hydrocarbonoclastic Potential of Microorganisms Isolated from Tarballs in the Bight of Bonny, Nigeria", World Journal of Microbiology and Biotechnology, 21, 6, 1317-1322.
4. Englund, J.; Walter, E.; andGbadebo, A.;(2006).Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. Pediatrics,118,3,79-85.
5. José, M.;(2006).Live attenuated and inactivated vaccines.Vaccines,Chapter 8, 2nd editin, brooks / cole , cengage . Learning ,p. 98.
6. Manisha, N.;(2012). Protein Conjugate Polysaccharide Vaccines: Challenges in Development and Global Implementation, Indian J Community Med,37,279–82.
7. Brien, K.; Hochman, M.;and Goldblatt,T.; (2014).Combined schedules of pneumococcal conjugate and polysaccharide vaccines: journal name plos one, 7,9 ,597-606.
8. Sara,H. ; and Nancy, M.; (2014) . Use of 13-Valent Pneumococcal Conjugate Vaccine and 23-Valent Pneumococcal Polysaccharide Vaccine Among Adults Aged ≥65 Years: Recommendations of the Advisory Committee on Immunization Practices (ACIP), Centers for Disease Control and Prevention 1600 Clifton Road Atlanta,37 ,37 ,822-825.
9. Kathleen, S.; Andrew, F.; Erin, R.; and Ellen,W.; (2011) . Effects of Vaccines, the. National Academy of Sciences , 31 ,10 , 892.
10. Phil, C.; (2014). Gel-Filtration Chromatography , School of Biotechnology and National Centre for Sensor Research, Dublin City University, Dublin, Ireland, Clifton, N.J,1, 12,25-33.
11. .Aung,T.; and Fatimah Ibrahim.; (2015). A Colorimetric Enzyme-LinkedImmunosorbent Assay (ELISA)Detection Platform for a Point-of-Care Dengue Detection System on a Lab-on-Compact-Disc, Centre For Innovation in Medical Engineering, Faculty of Engineering, University of Malaya,3, 7, 11431-11441.
12. .Kragstrup, T.; Vorup-Jensen, T.; Deleuran, and B.; Hvid, M.; (2013) . "A simple set of validation steps identifies and removes fals results in a sandwich enzyme-linked

immunosorbent assay caused by anti-animal IgG antibodies in plasm,2 and MårtenSegelmark a from arthritis patients". Springer Plu ,149 ,7, 263.

13. .Al-Ali, A .; Alkhawajah, A.; Randhawa, M.; and Shaikh, N.; (2008). Oral and intraperitoneal LD50 of thymoquinone, an active principle of Nigella sativa, in mice and rats.JAyub Med CollAbbotabad, National Center for Biotechnology Information, U.S. National Library of Medicine,20, 4,25.
14. Al-Lammi, T. J.; (2009). Study the effect of Neem and the cell wall of mannan of Candida albicans as immunomodulators on vaccination of mice with Brucella vaccine-Rev-1vaccine.Ph.D. Thesis, aUniversity of Al-Muthana , Iraq, international journal of advanced biological research ,2, 3, 742-746.
15. Sandini, S.; Lavalle, R.; Debernardis, F.; Macri, C. ; and Cassone, A.; (2007) .The 65 KDamanno proteins gene of Candida albicans encodes a putative Bglucanaseadhesine required for hyphal morphogenesis and experimental pathogenicity. J. Immunol ,178 ,35,2171-2181.

**Table (1) Estimation of LPS concentration by absorbance at 280 nm at step gel-filtration chromatography**

| No. of fraction | Absorbance At 280 nm | Concentration LPS mg/dl |
|---|---|---|
| 15 | 1.1 | 0.04 |
| 16 | 1.3 | 0.03 |
| 17 | 1.6 | 0.09 |
| 18 | 2 | 0.2 |
| 19 | 2.3 | 0.9 |
| 20 | 2.5 | 1.2 |
| 21 | 2.2 | 1.4 |
| 22 | 2 | 1.7 |
| 23 | 1.7 | 2 |
| 24 | 1.4 | 1.6 |
| 25 | 1.1 | 1.1 |
| 26 | 1 | 1 |

**Table (2) Determination of LD50 of LPS purified from *P. aeruginosa* ATCC 15692 and its mortality rate**

| Groups | Type | LPS injected subcutaneously | Prednisolone | Infections |
|---|---|---|---|---|
| **A** | **Control** | **11.905 µg/ mouse** | | **0** |
| **B** | **Vaccination** | **11.905 µg/mouse** | **25µg/dl** | **6** |
| **C** | **Control** | | | **6** |

**Table (3) Immunization of LPS as a vaccine in mice body**

| | Dose/moue | Number of Animals | Mortality Rate [%] |
|---|---|---|---|
| LPS | 25µg | 4 | 0 |
| | 50µg | 4 | 0 |
| | 100 µg | 4 | 50 % |
| | 200µg | 4 | 75% |

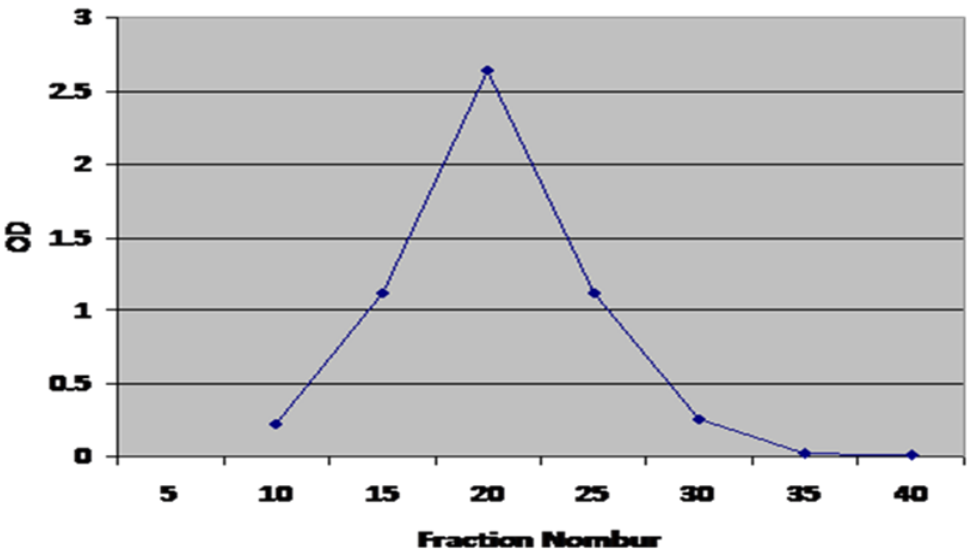
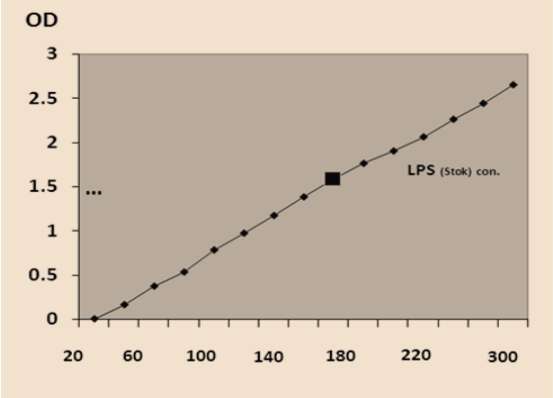
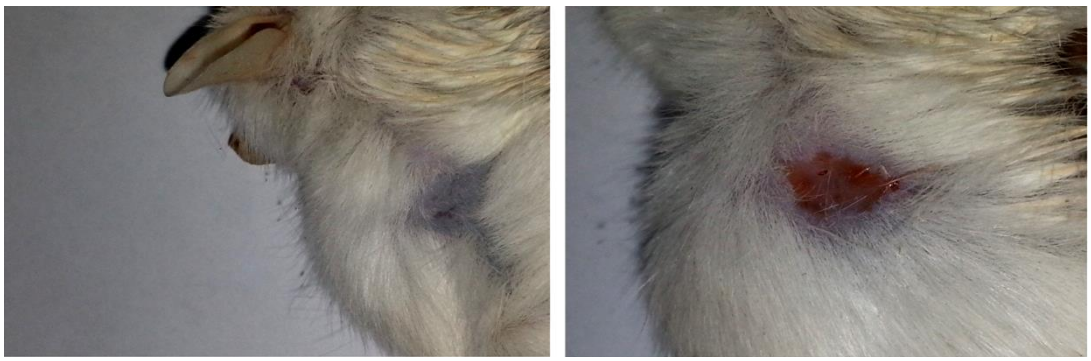



**Figure (1) Gel filtration chromatography of LPS produced from *P. aeruginosa* ATCC 15692**




.

**Figure (2) Curve of LPS concentration in stock by ELISA kit**



**Group B [not infected]** **Group C[infected]**

**Figure (3) Comparing group C (control) with group B (vaccinated)**

# فصل وتنقية جزئية للايبوبولي سكرايد من جدار بكتريا سيدوموناس ارجينوزا واستعماله كلقاح

**تغريد علوم محمد**
**سوسن عبدالحسين مهدي**
قسم الكيمياء/ كلية التربية للعلوم الصرفة ( ابن الهيثم )/ جامعة بغداد

**استلم في:21/شباط/2016،قبل في:17/نيسان/2016**

## الخلاصة

هدف الدراسة هو فصل وتنقية جزئيه للايبوبوليسكرايد واستعماله كلقاح. اد تضمنت الدراسة 118 شخصا (رجال ونساء) مصابين بحروق ملوثه ببكتريا السيدوموناس و100 شخص سليم بوصفهم كمجموعه مقارنه ، اد اخدت العينات على شكل نماذج دم ومسحه للزرع .

تم فصل وتنقية اللايبوبولي سكرايد من غشاء الخلية لبكتريا السيدوموناسدات السلالة(اي تي سي سي 15692) من خلال الترسيب بكبريتات الامونيوم ،كبريتات الدودوسيل الصوديوم والديلزة وباستعمالالكروموتوغرافي الترشيح الهلامي بواسطه السيفروز 4بي .

تم اختبار القدرة المناعية للايبوبوليسكرايد عن طريق حقنه بجسم الفئران وقياس تركيز الأجسام المضادة بتقنية الإليزا .

اذ أشارت النتائج إلى قدرة اللايبوبوليسكرايد على تحفيز نظام المناعة وتكوين الأجسام المضادة ضد بكتريا السيدوموناس ذات السلاله (اي تي سي سي 15692) لذا فان هذه النتيجة قد تكون إجراءلإنتاج لقاح ضد هذه البكتريا.

**الكلمات المفتاحية :**-بكتريا السيدوموناس ارجينوزا(الزائفة الزنجارية) ، الدهون للبوليمرات السكرية( اللايبوبولي سكرايد)