Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Extraction and purification of lipopolysaccharide from *Escherichia coli* (local isolate) and study its pyrogenic activity

A.M. Al-AAlim[1], A.A. Al-Iedani[2] and M.A. Hamad[1]

[1]Department of Microbiology, College of Veterinary Medicine, University of Mosul, Mosul, [2]Department of Microbiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

Article information

Article history:
Received November 16, 2020
Accepted February 09, 2021
Available online October 15, 2021

Keywords:
E. coli
Lipopolysaccharide
Hot-phenol extraction
SDS- PAGE
Pyrogenic activity

Correspondence:
A.M. Al-AAlim
ammarmahmmod@uomosul.edu.iq

Abstract

In this study, we tried to extract and purify the LPS from *E. coli* local isolate and determine the molecular weight, purity, and pyrogenic effect of the product and compare it with standard *E. coli* O55:B5 LPS, the *E. coli* LPS was extracted by using hot phenol method then SDS- PAGE was used with both Coomassie blue and silver nitrate stain to determine its molecular weight and protein contamination also we used HPLC to the estimation of *E. coli* LPS purity and finally the pyrogenicity of extracted *E. coli* LPS was tested by using rabbit pyrogen test. The result showed that the hot phenol method with enzymatic treatment gave highly pure LPS with a high yield reach up to 242.4 mg, staining the SDS page gel with Coomassie blue and silver nitrate uncover the high purification of the extracted LPS (ELPS) with no protein contamination, with a molecular weight range between 15-23 kDa, HPLC test reveals that purity of ELPS was 100 % compared with standard LPS. The rabbits' pyrogen test confirmed that the biological activity of ELPS. In conclusion, the LPS was extracted with high purity compare with standard LPS and without any protein or DNA contamination by using the hot phenol method also the extracted rough LPS was slightly lighter than the standard LPS used but this did not affect its biological activity which remained intact.

DOI: 10.33899/ijvs.2021.128963.1614,

Introduction

Enterobacteriaceae family is a large diverse group of Gram-negative rods considered as normal flora in the intestinal tract of both animals and humans (1). *Escherichia coli* the wide world distribute bacteria belong to this family and could be found in food and cause infection in human and animals (2-5), according to virulence, pathogenicity, and site of infection *E. coli* is classified as commensal, enteropathogenic, and extraintestinal pathotypes strains (6-10). All Gram-negative bacteria are enveloped by a cell wall consisting of the cytoplasmic inner membrane, an intermediate layer of peptidoglycan, periplasm layer, and finally, outer membrane layer (11,12) which connected covalently to peptidoglycan by lipoprotein (13). *E. coli* outer membrane composed of phospholipid and lipopolysaccharide which reflected the inner and outer layer of the membrane. The main components of lipopolysaccharide LPS are lipid A, core oligosaccharide, and O- antigen. All compartments help to protect the bacteria and play role in antigenic variation, both lipid A and core oligosaccharide involved in bacterial virulence, and O- antigen also has attachment properties as well as acts to resist many antimicrobial drugs and phagocytosis effect (12). The O- antigen alone capable to protect the bacteria from lytic action of complement and bacteriophage (14,15). There were different protocols for extraction and purification of bacterial LPS these protocols differ in their yield and purity such as Trichloroacetic acid, Aqueous butanol, Triton /Mg^{+2}, Aqueous ether, Chloroform: methanol: petroleum-ether, and EDTA method. The hot phenol extraction method was mostly used due to the short

time required with high yield production. (16,17). The LPS of Enterobacteriaceae is characterized by its high molecular weight ranging from 10-100 kDa and the most LPS of smooth *E. coli* strain reach up to 25 kDa (1,17-19).

Bacterial LPS (endotoxin) consider as a potent pyrogen which means that have the ability to induce fever with several pathophysiological effects after injected intravenously; only a very small quantity (Nanogram/kg) of LPS was able to stimulate the response of an immune system and exaggerated host response to the removal of this toxic substance from body circulation by releasing many types of mediators that mediated in pyrogenic response. (20) the liberation of LPS from the cell wall in the host relates to a wide range of bad effects such as releasing of pro-inflammatory cytokine as TNF-α, IL-1 by macrophages which were responsible for fever production and lead to hyperthermia as a result of the pyrogenic response, and lead to inflammation, capillary leak, tissue toxicity (21) coagulation (22), aseptic shock, lethality (12). The aims of this study, is to extract and purify the LPS from *E. coli* local isolate, determine the molecular weight and pyrogenic effect of the extracted LPS (ELPS), and compare it with standard LPS(SLPS) purified from *E. coli* O55: B5 as a positive control.

Materials and methods

E. coli bacterial isolate and purity determination

Three Shiga Toxigenic *E. coli* (STEC) local isolates which isolated from cattle in Basrah governorate were gifted from the Department of Microbiology and Parasitology, University of Basrah, College of Veterinary Medicine (23). The purity of the isolates was confirmed by culturing on brain heart infusion broth, Eosin Methylene Blue agar, and MacConkey agar at 37˚C for 24 h. The isolates were confirmed using polymerase chain reaction PCR. Briefly, five single colonies from each isolate were selected for DNA extraction using DNeasy® Blood and tissue Kit (Qiagen, USA) kit and amplification of *uid A* gene. The PCR conditions were set as similar to that described by (24). The final PCR product was detected in 2% agarose using 60 V for 1.5 h and the band was visualized by using a transilluminator (23,25) the amplicon size was 623 bp according to (24).

Preparation of bacterial isolate

For extraction of lipopolysaccharide. five single colonies of STEC were transferred from eosin methylene blue agar to tubes containing 5 ml brain heart infusion broth and incubated aerobically at 37ºC for 24h, then the bacterial growth turbidity was adjusted with 0.5 McFarland standard tubes to obtain $1.5*10^8$ CFU/ml as starting bacterial inoculum. The suspension was transferred to one liter of brain heart infusion broth and incubated aerobically at 37ºC for 24h. The final bacterial turbidity was adjusted to 1.84 by spectrophotometer using OD=600 nm this approximately equal to $2*10^9$ CFU/ml. *E. coli* lipopolysaccharide was extracted by using the hot phenol-water method described by (16) with some minor modifications. in our study 4 patches (Each patch represented 1 liter of *E. coli* culture (2×10^9 CFU/ml) was used for extraction LPS from *E. coli* local isolate, bacterial suspension in each patch was collected by cold centrifuge at 4100 rpm/15 min, after washing the bacterial pellet with phosphate buffer saline (PBS) pH=7.2, contain 0.15mM $CaCl_2$ and 0.5 mM $MgCl_2$; the pellet was resuspended in 10 ml of PBS, to liberate the LPS in solution, the bacterial cells in suspension were destroyed by sonication (29 MHz / 10 min). The next steps including treatment of sonicated bacterial suspension with 100 µg/ml Proteinase K in 65˚C/1h to remove the protein contamination farther enzymatic treatment was extended to overnight at 37ºC with 40ug/ml RNase, 50 ul of working solution of DnaseI (2U/µl), 1 µL/mL 20% $MgSO_4$ and 4 µL/mL chloroform to remove any DNA or RNA contamination. The separation of LPS was done by adding an equal volume of hot (65-70ºC) 90% phenol and incubate the mixture in 70ºC/15 min with vigorous shaking, the LPS mixture was cooled in ice and centrifuged at 10000 rpm/15 min to separate aqueous phase from the phenolic phase, the phenolic phase was re extracted with 300 µl distilled water, all aqueous phase was collected in 15 ml conical centrifuge tube and the LPS was precipitate in -20ºC overnight after adding 0.5 M (final concentration) of sodium acetate and 10 volume of 95% ethanol then the LPS was collected by centrifuge in 4˚C at 4100 rpm /10 min and resuspended in 1ml distill water, for farther purification and removal of any chemical substance extensive dialysis against double distilled water occur in 4ºC /48 hours using 13 cm long Dialysis tube (3500D cutoff value,34 mm, Special laboratory, USA) (26), the final step was lyophilized The purified LPS under -55ºC in 0.001 mbar for 24 hours then stored at 4ºC for farther analysis.

Detection of impurities of protein and nucleic acids

Two µl of ELPS from *E. coli* were used for electrophoresis in 2% agarose containing ethidium bromide for detection of any DNA and/or RNA contaminants. However, the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used according to (27-29) to determine the purity of the product from protein residues and the detection of the molecular weight of LPS. The SDS-PAGE gel was stained with both instant Blue™ (expedeon, UK) for detection of protein (26,29-31) and sensitive silver stain (26,31-34) to visualized both protein contamination and the lipopolysaccharide.

Analysis of extracted LPS by High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was used to identify and quantify the components of the mixture

of ELPS and comparing with SLPS of *E. coli* O55: B5 (Chem Cruz©). Separation of both Lipopolysaccharide done by HPLC 1200 series system (Agilent Technologies, USA pressure 110 bar, and flow rate 0.8 ml/min with 210 nm UV wavelength detector, the column Supelco™ C18 of 25 cm length × 4.6 mm inner diameter, 5 µm pore size was used for separation of Lipopolysaccharide), the mobile phase consisted from acetonitrile and water in concentration 5:95. 10 µl from both standard and extracted LPS were injected into the HPLC for analysis (16,35,36). The purity of the product was calculated as follows: The purity of sample = (area under the peak of the test sample/ area under the peak of standard)×100.

When it reaches 90 -115 this indicates high purity of the sample.

Detection of the biological activity of extracted LPS

Six white albino rabbits weighing 1.5-2 Kg were used in the pyrogen test, animals were divided into three groups. The first group was the negative control group and the rabbits were given normal saline, the rabbits in the second and third groups were given an intravenous injection of 10 µg/kg of both standard *E. coli* O55: B5 LPS and extracted *E. coli* LPS respectively. Rectal temperature before and after injection was recorded in all groups in the period interval of 30 mint up to 4 hours by using a medical rectal thermometer. The positive result was recorded as elevated in body temperature by 1-2 ° C (20,26).

Results

The *E. coli* isolates were confirmed by amplification of *uid A* gene using conventional PCR and separated in agarose gel, and the results confirmed the presence of *uid A* gene with 623 bp product size (Figure 1).

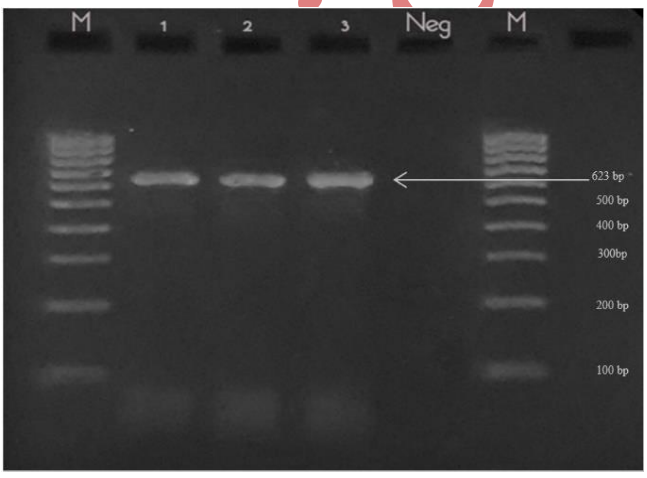

Figure 1: *Escherichia coli uid* A gene (M: ladder 100 bp, 1, 2 and 3: Shiga toxigenic *E. coli*)

Extraction of *E. coli* lipopolysaccharide

The results of the hot phenol extraction method used to isolate the LPS from - *E. coli* cultures (2×10^9 CFU/ml), showed that the bacteria gave about 242 mg of purified LPS after dialysis. The difference in the percentage of ELPS purification before and after dialysis was about 29.76% (Table 1).

Table 1: Percentage of *E. coli* LPS lost in the extraction and purification procedure

Patch No.*	Weight of crude LPS (mg)			
	before dialysis	after purification by dialysis	lost during purification	Lost %
1	10	6	4	40%
2	148	124.1	23.9	16.14%
3	147	88.2	58.8	40%
4	40	24	16	40%
Total	345	242.3	102.7	29.76%

* Each patch represented 1 liter of *E. coli* culture 2×10^9 CFU/ml.

Detection of DNA and RNA contaminants

The ELPS was separated in agarose gel to detect the presence of any traces of nucleic acids (DNA and RNA). All patches of extracted LPS were 100% free from nucleic acid contaminants due to the effect of DNase and RNase added during the extraction procedure (Figure 2).

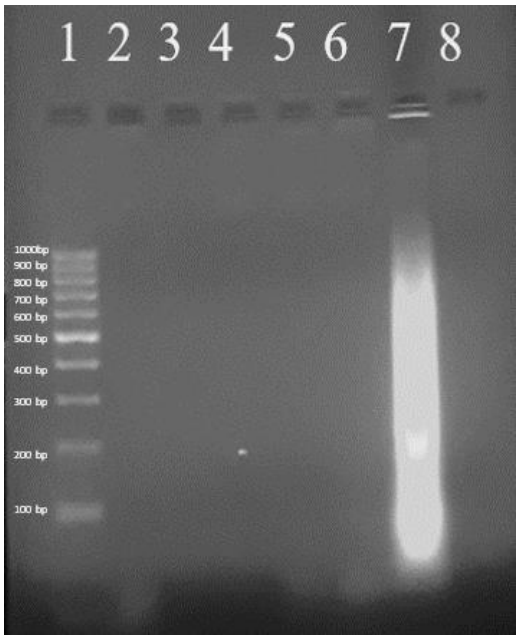

Figure 2: Extracted LPS of STEC in agarose gel (1: molecular weight marker, 2-6: ELPS, 7: bacterial

suspension after sonicating and before DNase and RNase enzymes digestion, 8: negative control).

Detection of protein contaminants

The SDS-PAGE electrophoresis followed by staining with instant Blue (Coomassie blue-based) stain specific for protein was used to detect any residues of protein in LPS extract. The extracted LPS was 100% free from protein contaminants (Figure 3).

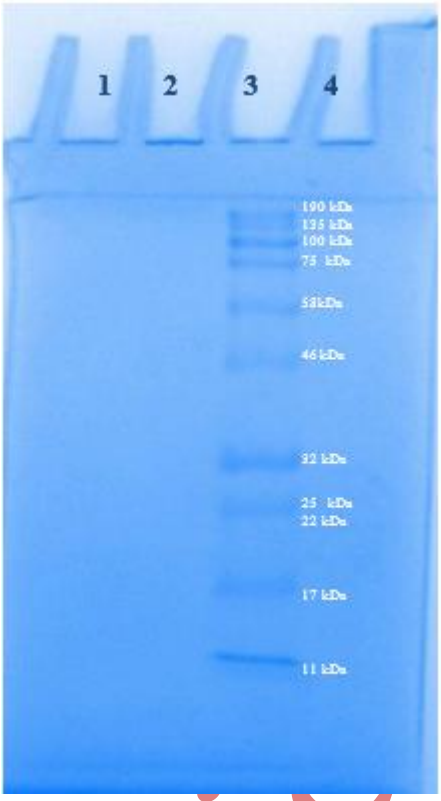

Figure 3: Electrophoresis of ELPS on SDS -PAGE (1: ELPS, 2: Standard LPS of *E. coli* O55: B5, 3: Protein weight marker [11- 190] kDa, 4: negative control).

Detection of the molecular weight and structure of extracted LPS

The result of SDS-PAGE electrophoresis which was stained with sensitive sliver stain for LPS shows that the ELPS has three-bands with molecular weights ranged (15,20 and 32) kDa, however, the standard LPS molecular weights ranging between (15-50) kDa. The upper bands represent rough O-polysaccharide containing LPS (30-50) kDa, the ELPS lighter than that of standard *E. coli* O55: B5 LPS, while the lower bands of both extracted and standard LPS represent the core lipopolysaccharide with lipid A and have molecular weights (15- 20) kDa. The intensity of the band was different when using different concentrations of LPS injected in the SDS-PAGE. The intensity of the bands was the best with 20 ng/µl followed by 100 ug/µl then 500ng/ml as in (Figure 4). The addition of 4M Urea in resolving gel was not effective in-band separation but it gives more clear background gel.

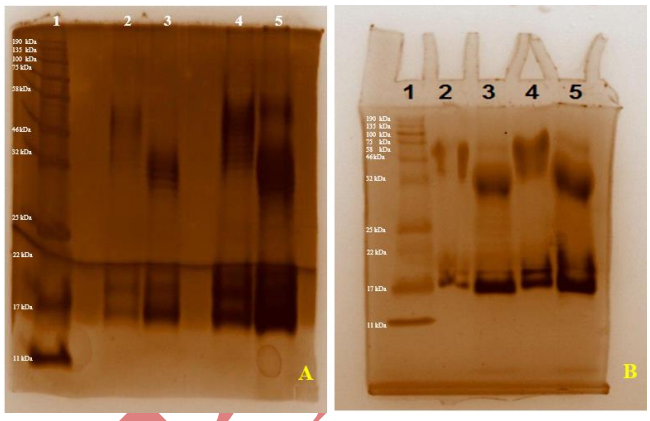

Figure 4: SDS-PAGE of the standard and extracted lipopolysaccharide from *E. coli* stained with the silver stain. A: (1: protein marker, 2: 20ng/µl standard *E. coli* O55:B5 LPS,3: 20ng/µl Extracted *E. coli* LPS,4:100ng/µl standard *E. coli* O55:B5 LPS,5: 100ng/µl Extracted *E. coli* LPS). B: (1: protein marker, 2: 1.7ug/µl standard *E. coli* O55:B5,3 LPS: 1.7ug/µl Extracted *E. coli* LPS,4:500ng/µl standard *E. coli* O55:B5 LPS, 5: 500ng/µl Extracted *E. coli* LPS,4) upper band show rough O-polysaccharide containing LPS and lower band show core lipopolysaccharide with lipid A

Detection of purity of extracted *E. coli* LPS using HPLC analysis

The purity of extracted *E. coli* LPS was analyzed by HPLC method using both extracted *E. coli* LPS and standard *E. coli* O55: B5 LPS, the result shows the appearance of one single peak with different retention time which reaches 1.650 in extracted *E. coli* LPS and 1.829 for standard *E. coli* O55: B5 LPS (Figure 5). The purity of ELPS was 100 % in comparison with a standard.

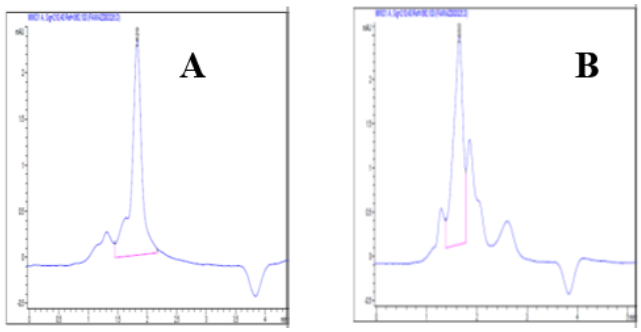

Figure 5: HPLC electropherograms of standard *E. coli* O55:B5 LPS (A) and extracted *E. coli* LPS (B).

Detection of pyrogenic activity of extracted *E. coli* LPS

The rabbit pyrogen test was used to detect the pyrogenicity of ELPS and SLPS by intravenous injection into the rabbits. The body temperatures reached 39.9ºC and 40.6ºC of the ELPS and SLPS respectively in comparison with the temperature of the control rabbit 38.5ºC (Figure 6).

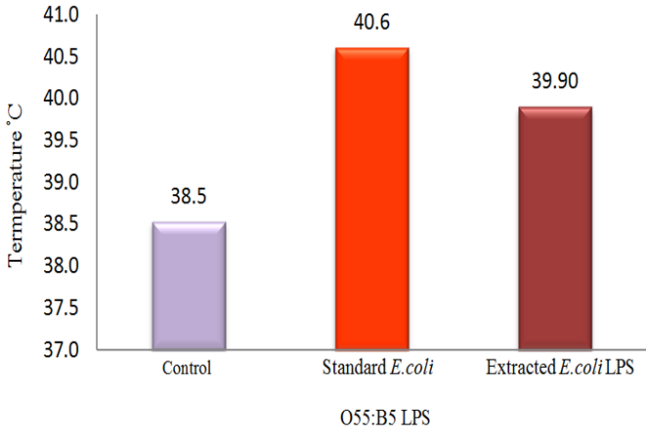

Figure 6: The mean body temperature of rabbits after intravenous injection with 10 µg/kg of *E. coli* LPS.

Discussion

The LPS also called endotoxin, is an essential structural part of the *E. coli* cell wall, as control permeability, antibiotic resistance, and further of its role as a structural component also it can modulate the immune response so play an important role in parasite-host interaction (15).

Many methods were used to extract the LPS with keeping high purity and biological activity as Trichloroacetic acid, Aqueous butanol, Triton /Mg+2, Aqueous ether, Chloroform: methanol: petroleum-ether, and EDTA method (37) and hot-phenol extraction method. The hot-phenol extraction method still the best method used although it has many dangerous and poisoning materials as phenol (26). The result of this study shows a high yield of 242.3 mg of purified LPS from 4-liter of *E. coli* cultures with a mean 60 mg/liter. These results were different from other related studies (17,26,37-39) and this difference in LPS yield maybe explain by differences in extraction methods and differences of Gram-negative bacterial species (40,41).

Most methods used to extract bacterial LPS were not given a high purity LPS and many proteins and nucleic acid contaminants were found in ELPS, the hot phenol method was not excluded from this disadvantage and may contain up to 24% of RNA (36). The results of this study show that the LPS product was 100% free from protein in Coomassie blue staining and nucleic acid contaminants. these results were in agreement with that recorded by (16,26) who used the proteinase k and DNase and RNase enzymes during the hot phenol extraction method.

The results of SDS-PAGE electrophoresis stained with silver stain showed that a more clearly detectable band was found in LPS concentration 20ng/µl and this agrees with (34), the sliver stain was highly sensitive to detection of LPS. The effect of the addition of urea on the SDS-page does not affect the separation of the band but is believed to give more clear ground of gel as its increased oxygen permeability and water vapor transmission rate (42). The ELPS product has both rough and smooth parts of LPS, molecular weight ranged from 15-32 kD in comparison with standard LPS used which have 15-50 kD which lie in the standard molecular weight of LPS ranged between 2-100 kD (1,17,18).

The difference in molecular weight between standard and ELPS may be due to differences between bacterial strains which have different LPS structures to give *E. coli* five core types K1, R1- R4 (19,43). The ladder formation appeared in rough LPS and this can explain by increasing of polysaccharide unit in the rough LPS core (44) as a modification of core LPS by adding more galactose, rhamnose, PEtN, or Kdo group as a response to stress or to increase bacterial resistance to the antimicrobial peptide (15,45).

The HPLC result confirms the high purity of ELPS by showing only one sharp peak in comparison with the standard LPS this result agrees with (16) as a result of using enzymes to remove all contamination from ELPS. The most important in any extraction method is to preserve the biological activity of LPS, and its purity at a high level by removing chemical materials (used in the extraction process), protein, DNA, or RNA that affect and interfere with the signal downstream of biological and immunological responses (26).

The rabbit pyrogenic test was performed to evaluate the biological effect of the product, results showed that the mean rise of rabbit temperature was 1.4 ˚C which give the test a positive result and indicate the biological activity of the extra pure *E. coli* LPS and this agrees with (20,46).

This elevation in animal's body temperature related to bacterial LPS which considered as a potent trigger for the immune system and capable to produce pro-inflammatory cytokine such as TNFα, IL1 as well as Prostaglandin E2 that mediated in the production of pyrogen reaction and appear its biological effect (16,20,21).

Conclusion

In conclusion, the LPS was extracted with high purity compare with standard LPS and without any protein or DNA contamination by using the hot phenol method also the extracted rough LPS was slightly lighter than standard LPS used but this did not affect its biological activity which remained intact.

Acknowledgment

The Authors are thankful for the efforts and support of the head of the Department of Microbiology and Deanship of the College of Veterinary Medicine, University of Mosul.

Conflict of interest

The authors declare no conflict of interest with any official institute and this research was achieved without any financial support from any third party. We announce that the manuscript has been read and approved by all named authors and the order of authors listed in the manuscript has been approved by all of us.

References

1. Brooks GF, Carroll CK, Butel SJ, Morse AS, Mietzner AT. Medical microbiology. 26th ed. USA: McGraw-Hill eBooks Publisher; 2013. 229-245. [available here]
2. Neamah AJ, Al-Yassari AS, Hamed M, AlRammahi MA. Inhibition of *Escherichia coli* biofilm formation by Streptomyces sdLi crude extract. Iraqi J Vet Sci. 2020;34(2):305-310. DOI: 10.33899/ijvs.2019.125965.1202
3. Naji HA, Saleh WMM, Hanoon M, Imad I, Salim Y. Serotyping, virulence gene expression and phenotypic characterization of *E. coli* O157:H7 in colibacillosis affecting buffalo calves in Basra governorate. Iraqi J Vet Sci. 2019;33(2):447-451. DOI: 10.33899/ijvs.2019.163198
4. Klaif S F, Saleh ZF, Hussein MT, Jawad AA, Jawad MS. Molecular characterization of enterohemorrhagic *E. coli* O157 and O153isolated from tissue camel and human stool samples in Al-Diwaniyah, Iraq. Iraqi J Vet Sci. 2019;33(1):81-86. DOI: 10.33899/ijvs.2019.125530.1052
5. Al-Sharook MN, Hassan AA. Isolation and identification of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from broiler in Erbil, Iraq. Iraqi J Vet Sci. 2017:31(1):31-38. DOI: 10.33899/ijvs.2017.126717
6. Ibrahim HM. Constructed of phylogenetic tree for *Escherichia coli* causing mastitis in Basrah province [master's thesis]. Basrah: College of Veterinary Medicine University of Basrah. 2018.
7. Gupta SK and Sharma A. Dynamic properties of *Escherichia coli*. World J Pharm Pharm Sci. 2015;4(7):296-307. [available at]
8. Hussain T. An introduction to the serotypes, pathotypes and phylotypes of *Escherichia coli*. Inter J Microbiol Allied. 2015:2:9. [available at]
9. Scott MD, Kennedy M, Chengappa MM. Veterinary microbiology. 3rd ed. UK: John Wiley & Sons; 2013. 62-74. [available here]
10. Abdul-Razzaq MS, Abdul-Lateef LA. Molecular phylogeny of *Escherichia coli* isolated from clinical samples in Hilla, Iraq. African J Biotechnol. 2011;10(70):15783-15787. DOI: 10.5897/AJB11.1273
11. Morè N, Martorana AM, Biboy J, Otten C, Winkle M, Serrano CKG, Silva AM, Atkinson L, Yau H, Breukink E, Den Blaauwen T. Peptidoglycan remodeling enables *Escherichia coli* to survive severe outer membrane assembly defect. Mol Biol. 2019;10(1):1-18. DOI: 10.1128/mBio.02729-18
12. Sampath V. Bacterial endotoxin-lipopolysaccharide; structure, function and its role in immunity in vertebrates and invertebrates. Agricult Nat Resources.2018:52(2):115-120. DOI: 10.1016/j.anres.2018.08.002
13. Mayer C, Kluj RM, Muhleck M, Walter A, Unsleber S, Hottmann I and Borisova M, Bacteria's different ways to recycle their own cell wall. Inter J Med Microbiol. 2019;309(7):151326. DOI: 10.1016/j.ijmm.2019.06.006
14. Putker F, Bos MP, Tommassen J. Transport of lipopolysaccharide to the gram-negative bacterial cell surface. FEMS Microbiol Rev. 2015;39(6):985-1002. DOI: 10.1093/femsre/fuv026
15. Bertani B, Ruiz N. Function and biogenesis of lipopolysaccharides. EcoSal Plus. 2018;8(1):1-33. DOI: 10.1128/ecosalplus.ESP-0001-2018
16. Rezania S, Amirmozaffari N, Tabarraei B, Jeddi-Tehrani M, Zarei O, Alizadeh R, Masjedian F, Zarnani AH. Extraction, purification and characterization of lipopolysaccharide from *Escherichia coli* and *Salmonella typhi*. Avicenna J Med Biotechnol. 2011;3(1):3-9. [available at]
17. Al-Musleh RM, Jawad Th A. Extraction and purification of lipopolysaccharide from *Citrobacter fruendii* and determination molecular weight with its carbohydrates, protein contain. J Karbala Uni. 2009;7(4):246-253. [available here]
18. Hulko M, Dietrich V, Koch I, Gekeler A, Gebert M, Beck W, Krause B. Pyrogen retention: Comparison of the novel medium cut-off (MCO) membrane with other dialyser membranes. Sci Rep. 2019;9(1):1-7. DOI: 10.1038/s41598-019-43161-2
19. Stenutz R, Weintraub A, Widmalm G. The structures of *Escherichia coli* O-polysaccharide antigens. FEMS Microbiol Rev. 2006;30(3):382-403. DOI: 10.1111/j.1574-6976.2006.00016.x
20. Elkhateeb MA, Elkhatib W, Aboulwafa M. An expert review on current approaches for endotoxin detection in various biological products. Arch Pharmaceut Sci Ain Shams Uni. 2019;3(2):142-153. DOI: 10.21608/APS.2019.13058.1000
21. Hung YL, Suzuki K. The pattern recognition receptors and lipopolysaccharides (LPS)-induced systemic inflammation. Int J Res Stud Med Health Sci. 2017;2:1-7. [available here]
22. Stief TW. Coagulation activation by lipopolysaccharides. Clin Appl Thromb .2009;15(2):209-219. DOI: 10.1177/1076029607309256
23. Farhan Z, AL-Iedani A. Molecular detection of shiga toxin (stx1 and stx2) and intimin (eae A) genes in *Escherichia coli* isolated from fecal samples of cattle, sheep, and human in Basrah governorate. Basrah J Vet Res. 2019;18(2):288-305. [available at]
24. Moyo SJ, Maselle SY, Matee MI, Langeland N, Mylvaganam H. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. BMC Infect Dis. 2007;7(1):92-99. DOI: 10.1186/1471-2334-7-92
25. Sambrook HC. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor; 1989. 1-1441.
26. Kalambhe DG, Zade NN, Chaudhari SP. Evaluation of two different lipopolysaccharide extraction methods for purity and functionality of LPS. Inter J Curr Microbiol App Sci. 2017;6(3):1296-1302. DOI: 10.20546/ijcmas.2017.603.150
27. Mcilwaine C, Strachan A, Harrington Z, Jerreat M, Belfield LA, Sandor V, Foey A, Zaric S. Comparative analysis of total salivary lipopolysaccharide chemical and biological properties with periodontal status. Arch Oral Biol. 2020; 110:104633-104639. Doi:10.1016/j.archoralbio.2019.104633
28. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat. 1970;227(5259):680-685. DOI: 10.1038/227680a0
29. Arqam A. Biophysical and kinetic analysis of *Escherichia coli* DNA ligase activity and inhibition [PhD dissertation]. Portsmouth: University of Portsmouth, UK; 2018.
30. Beer LA, Speicher DW. Protein detection in gels using fixation unit 10.5. Curr Protoc Protein Sci. 2019;21(91):10-51. DOI: 10.5.20.10.1002/cpps.48
31. Chowdhury FA, Islam MN, Saha A, Mahboob S, Mosaddek ASM, Faruque MO, Begum MF, Bhattacharjee R. Purification, extraction and visualization of lipopolysaccharide of *Escherichia coli* from urine samples of patients with urinary tract infection. Avicenna J Clin Microbiol Infect. 2015;2(4):31990-31990. DOI: 10.17795/ajcmi-31990
32. Li H, Yang T, Liao T, Debowski AW, Nilsson HO, Fulurija A, Haslam SM, Mulloy B, Dell A, Stubbs KA, Marshall BJ. The

استخلاص وتنقية متعدد السكريد الشحمي من الإشريكيا القولونية (العزلة المحلية) ودراسة نشاطه البيولوجي

عمار محمود العالم[1]، علي عبود العيدان[2] و محمد علي حمد[1]

فرع الأحياء المجهرية، كلية الطب البيطري [1]جامعة الموصل، الموصل، [2]جامعة البصرة، البصرة، العراق

الخلاصة

تهدف الدراسة الحالية إلى استخلاص وتنقية متعدد السكريد الشحمي من جراثيم الاشريكيا القولونية (العزلة المحلية) مع تحديد الوزن الجزئي، النقاوة والفعالية البيولوجية له ومقارنته مع متعدد السكريد الشحمي القياسي المستخلص من جراثيم الاشريكيا النمط المصلي O55:B5. تم استخدام طريقة الفينول الحار في استخلاص متعدد السكريد الشحمي لجرثومة الاشريكيا القولونية وتم تحديد الوزن الجزئي ومدى تلوث البروتيني لمتعدد السكريد الشحمي المستخلص باستخدام الفصل الكهربائي لهلام كبريتات دوديكل الصوديوم متعدد الأكريلامايد مع التصبيغ بصبغة البروتين وصبغة نترات الفضة الحساسة لصبغ متعدد السكريد الشحمي، واستخدم الاستشراب السائل عالي الأداء لتحديد مدى النقاوة مستخلص متعدد السكريد الشحمي كما تم قياس مدى إنتاجه للحمى في الأرانب باستخدام اختبار إنتاج الحمى في الأرانب، بينت الدراسة أن استخدام طريقة الفينول الساخن مع المعاملة الانزيمية أعطت نسبة استخلاص عالية ٢٤٢ ملغرام مع نقاوة مرتفعة لمتعدد السكريد الشحمي وبدون تواجد الملوثات البروتينية والنووية. تراوح الوزن الجزيئي لمتعدد السكريد الشحمي بين ١٥-٢٣ كيلو دالتون باستخدام طريقة الترحيل الكهربائي في هلامة متعدد الاكريلامايد، واظهر متعدد السكريد الشحمي المستخلص نقاوة وصلت إلى ١٠٠% عند مقارنته مع متعدد السكريد القياسي بواسطة طريقة الاستشراب السائل فائق الأداء، كما تم إثبات وجود الفعالية الإحيائية لمستخلص متعدد السكريد الشحمي باستخدام اختبار إنتاج الحمى في الأرانب. ختاما أظهرت الدراسة إمكانية استخلاص متعدد السكريد الشحمي بنقاوة عالية مقارنا مع متعدد السكريد القياسي باستخدام طريقة الفينول الحار مع عدم وجود تلوث بالأحماض النووية أو بالبروتين، كما بينت الدراسة أن الجزء الخشن من متعدد السكريد الشحمي المستخلص كان أخف وزنا عند مقارنته مع متعدد السكريد الشحمي القياسي مع عدم وجود تأثير مثبط لفعاليته البيولوجية التي بقيت سليمة.

redefinition of *Helicobacter pylori* lipopolysaccharide O-antigen and core-oligosaccharide domains. PLoS Pathogens. 2017;13(3):1-21. DOI: 10.1371/journal.ppat.1006280

33. Li H, Benghezal M. Crude preparation of lipopolysaccharide from *Helicobacter pylori* for silver staining and Western blot. Bio Protoc. 2017;7(20):1-8. DOI: 10.21769/BioProtoc.2585
34. Tsai CM, Frasch CE. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Analyt Biochemist. 1982;119(1):115-119. DOI: 10.1016/0003-2697(82)90673-x
35. Fadel MA, Hassan HM. Validated method for extraction and purification of Lipopolysaccharide from *Escherichia coli* and Salmonella by HPLC. Anim Healt Res J. 2019;7(3):1-10. [available here]
36. Zankharia US, Desai PV. Chemical analysis of lipopolysaccharide extracted from *Salmonella enterica* serovar *typhi*. IJRSI. 2015;II(VI):75-78. [available here]
37. Al-Masalmawi TH, Al-Khafji MSH. Study of some chemical and biological properties of Lipopolysaccharide extracted from *Pseudomonas aeruginosa*: First scientific conference of pure science educational college. Karbala Uni J. 2012;1:242-252. [available here]
38. Nile RS, Darweesh MF, Al-Rufaie MM. Liposomal-lipopolysaccharide vaccine extracted from *Proteus mirabilis* induces moderate TLR4 and CD14 production. Curr Iss Pharm Med Sci. 2019;32(2):81-86. DOI: 10.2478/cipms-2019-0016
39. Luchi M, Morrison DC. Comparable endotoxic properties of lipopolysaccharides are manifest in diverse clinical isolates of gram-negative bacteria. Infect Immunit. 2000;68(4):1899-1904. Doi: 10.1128/IAI.68.4.1899-1904.2000
40. Ebrahimi Z W, Mousavi G S, Rajabi b M, Mohamadi M, Vaise M Z. Comparison of different LPS extraction methods for isolation of smooth and rough LPS. First International and 12th Iranian congress of Microbiology. Kermanshah. Iran; 2011 23-26. [available here]
41. Uchida K, Mizushima S. A simple method for isolation of lipopolysaccharides from *Pseudomonas aeruginosa* and some other bacterial strains. Agricul Biol Chem. 1987;51(11):3107-3114. DOI: 10.1080/00021369.1987.10868532
42. Schmid M, Prinz TK, Stabler A, Sangerlaub S. Effect of sodium sulfite, sodium dodecyl sulfate, and urea on the molecular interactions and properties of whey protein isolate-based films. Frontiers Chemist. 2017;4:1-10. DOI: 10.3389/fchem.2016.00049
43. Ebbensgaard A, Mordhorst H, Aarestrup FM, Hansen EB. The role of outer membrane proteins and lipopolysaccharides for the sensitivity of *Escherichia coli* to antimicrobial peptides. Frontiers Microbiol. 2018;9:.2153-2172. DOI: 10.3389/fmicb.2018.02153
44. Ismail HA. Study on virulence factors of salmonella isolated from patients in Kirkuk city and compared with some standard isolate [PhD dissertation]. Mosul: College of Science, Mosul University, Iraq; 2005.
45. Mazgaeen L, Gurung P. Recent advances in lipopolysaccharide recognition systems. Inter J Molecul Sci. 2020;21(2):379-397. DOI: 10.3390/ijms21020379
46. Al-Sultany SJ, Jassim YA. Physiological and immunological effect of lipopolysaccharide of *Escherichia coli* was extracted by hot phenol-water in rabbits. Res J Pharmaceut Biol Chem Sci. 2016;7(3):1530-1535. [available here]