

## Extraction of Lipopeptide from *Bacillus subtilis* and Histological Study on Albino Rats Tissue

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### ABSTRACT

**Introduction and Aim:** An extract of the lipopeptides from *Bacillus subtilis* has been found to be extremely useful in antimicrobial applications. Specifically, the goal of this study is to manufacture lipopeptide and assess the safety of this substance on the tissues and organs of laboratory rats.

**Materials and Methods:** *Bacillus subtilis* bacteria were isolated and identified at the postgraduate microbiology laboratory in the department of biology at Kufa University's College of Science. The bacteria became active after 24 hours of growth in the brain broth infusion broth medium. In order to determine the bacteria's capacity to produce lipopeptide. On nutrient agar medium, a quantity of lipopeptide was synthesized utilizing HCL for sediment, yielding an estimated amount of 1.5 gm during a two-month period, then the product was partially purified and lyophilized using a lyophilizer. For one week, the lipopeptide was administered on albino rat tissues such as the kidney, liver, spleen, and small intestine to determine its safety.

**Results:** The results showed no damage or any changes on tested tissues compared with control treatment and all of the previously mentioned organ tissues were completely intact and this is evidence of the safety of the lipopeptide extract for use.

**Conclusions:** It was discovered that lipopeptide had no effect on the organs that were utilized in the experiment and that it is safe for human consumption.

**Keywords-** *Bacillus subtilis*, Lipopeptide, Albino rats, tissues.

things many antibiotics are made up of lipopeptides (Hamley, 2015). In 2002, the total amount of lipopeptide produced exceeded 2.5 million metric tons. Given that the detergent business accounts for more than half of all peptide production, the rate of expansion is closely tied to worldwide demand for detergents (Deleu *et al.*, 2008). lipopeptides, there are many different chemical groups that can be found in them. The discovery of lipopeptides as a chemical compound alternative prompted a flurry of research into the use of these microbial products as a chemical compound replacement (Banat, Makkar and Cameotra, 2000). Numerous investigations on microorganisms that synthesize diverse kinds of lipopeptides using water-soluble chemicals as substrates, such as many carbohydrates: sucrose, glucose, ethanol or glycerol, have been carried out in the past few decades.

## II. MATERIALS AND METHODS

### *Activation and storage of Bacillus subtilis bacteria on the culture media*

The diagnosed bacteria that taken from the postgraduate laboratory of microbiology were identified and activated using brain broth medium, part of the bacterial colony was transported to the brain broth medium by sterile loop and incubated in an incubator for 24-48 hours for later use. The bacteria were then stored by streaking on nutrient agar slants in screw-capped tubes containing 5 ml of the medium and incubated at 30°C for 24 hours. These slants can be stored for a few months at 4°C (Maniatisietal, 1982).

### *Screening of lipopeptide production*

#### *1. Preparation of inoculums*

Using a single colony from bacterial culture broth activated on brain broth medium, inoculate 10 ml of nutritional medium in a tube and incubate at 30°C for 20 hours in a shaker incubator (180rpm). Also, at 30°C for the intermediate logarithmic phase bacteria.

#### *2. Lipopeptide extraction and production*

1ml of the aforementioned inoculum mix was used to inoculate 100 ml of nutritional medium three times in a shaker incubator at 180rpm at 30°C. (24, 48 and 72 hrs.). After centrifugation of broth medium at 4°C, 15,000 rpm for 10 minutes, the supernatant containing lipopeptide was collected.

## I. INTRODUCTION

A lipopeptide is a molecule that is made up of a lipid and a peptide combination that can self-assemble into various shapes. Many bacteria make lipopeptides as part of their metabolism, with the majority of them belonging to the genera *Bacillus*, *Streptomyces*, and *Pseudomonas* being the most common producers (Coutte *et al.*, 2017). Lipopeptides are well-known for their ability to alleviate surface stress and oxidative damage. Lipopeptides are a key component in a wide range of consumer and industrial goods, including lubricants, polymers, and solvents; they are also used in detergents, tooth paste, perfume, and oil additives, among other

### 3. Partially purified of lipopeptide production

Each culture's cell free supernatants were treated with HCl acid (6N) for 24 hours, until the pH was 2. The precipitate was then centrifuged at 15,000 rpm for 10 minutes at 4°C, re-suspended in 2 ml D.W., adjusted to pH 7 with stirring, and lyophilized and weighed.

**Dry weight of lipopeptide extraction = weight the plate with extraction after drying - weight of the empty plate**

#### Preparing of lipopeptide solutions for testing on albino rats

One mg of extract was dissolved in D.W. or PBS material, and concentrations of 250 were produced.

#### Laboratory animals

Male albino rats, aged 12-15 weeks and weighing 200-250 g, were utilized in this research to provide sufficient ventilation, feeding, and illumination. The animals were maintained at a temperature of 223°C for 1-2 weeks to acclimatize (Fetouii et al., 2007).

#### Sterility of crude extract test

To verify that the extract was devoid of germs or bacteria, the loop portion of the partially purified lipopeptide was transferred to nutrient agar and blood agar and cultivated, and then the plates were incubated at 37°C for 24 hours.

#### Safety of lipopeptide extract

To test the lipopeptide's safety, eight animals were used in the experiment, each divided into two groups of four animals. One of these two groups was intra peritoneally injected with 250 l of partially purified lipopeptide for one week, while the other group was injected with PBS for the same amount of time, while clinical features in the rats were monitored.

#### Preparation of histological sections

The histological sections were prepared in the college of science/biological department using Bancroft and Steven's method of placing models of the first experiment in formation for 24 hours and then completing the steps of dehydration, clearing, infiltration, embedding, sectioning, staining, and mounting.

## III. RESULTS DISCUSSION

#### Activation of *Bacillus subtilis*

*B. subtilis* bacteria were isolated and activated again on brain media after being kept at 4°C in nutrition agar medium.

#### Production of lipopeptide from *B. subtilis*

*B. subtilis* was cultivated three times in nutrient broth medium. After 24 hours of incubation, the lowest production of lipopeptide was observed, followed by an increase in production but at a low rate as the time was increased to 48 hours, resulting in a proportional increase in the number of bacteria producing lipopeptide

in the broth medium and thus an increase in bacterial carbon source consumption in the medium. After 72 hours of incubation in the medium, the greatest percentage of lipopeptide synthesis occurred, reaching 0.4 (g/l).

#### Partially purification and drying of lipopeptide produced by *B. subtilis*

Because such compounds tend to become insoluble at low pH due to charge neutralization and protonation of carboxylic acid side chains of glutamic acids in the peptide portions of these molecules, partial purification of lipopeptide from *B. subtilis* began with acid precipitation of lipopeptide in cell free supernatant to crystallize state (Maneerat and Phetrong, 2007). The brown precipitates were then solubilized in alkaline D.W., lyophilized using a lyophilizer (figure 1), and weighed to determine the primary yield of lipopeptide (g/L of broth), as indicated in table (1).



Figure 1: partially purified lipopeptide (dry weight)

Table 1: Weight of partially purified lipopeptide from the *B. subtilis* after cultivation in nutrient medium for the optimum period of incubation

Symbol of bacteria	Incubation Periods (hrs.)	Partially purified lipopeptide (g/l)
S1	24	0.15
S2	48	0.29
S3	72	0.4

#### Sterility test of *B. subtilis* lipopeptide

After seven days, the crude culture on blood and nutrient agar was evaluated on plates. The absence of any microbe development demonstrated the sterility of the lipopeptide extract, according to the findings.

#### Safety of Crude Extract of *B. subtilis* lipopeptide

Rats were injected intra-peritoneal with bacterial extract and monitored for seven days to verify that the extract used in the experiment was safe. When compared to a control treatment, the findings revealed no harmful indications in the organ tissue of those rats, indicating the safety of partial purified lipopeptide, as shown in figure (2), (3), (4).

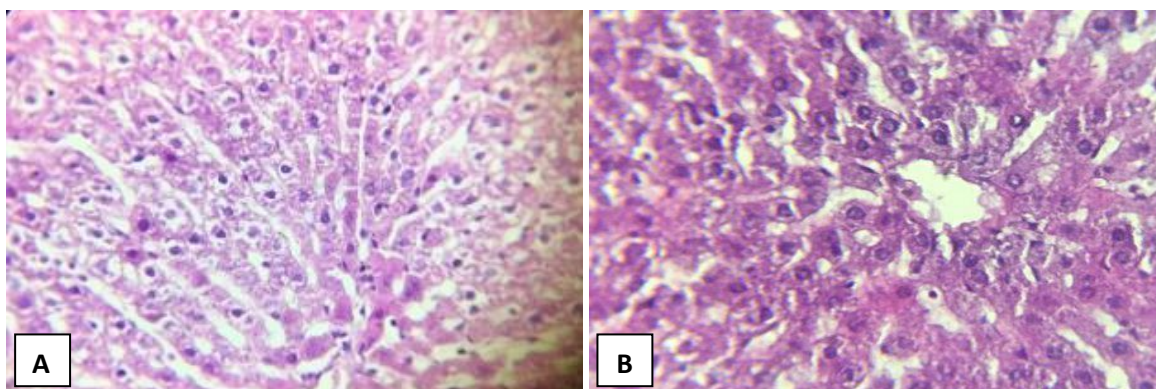


Figure 2: The histological section of liver tissue in different concentration after 7 days, 100x magnification, A- control treatment, B- 250 µl

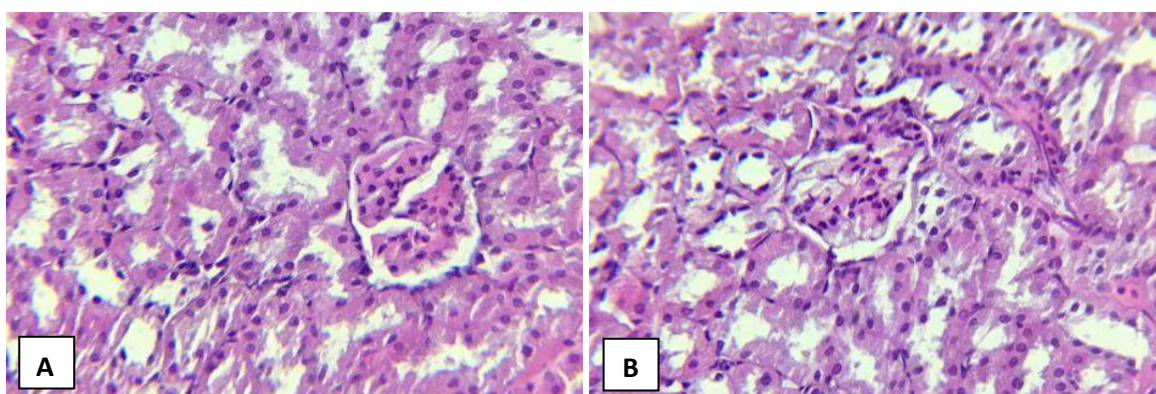


Figure 3: The histological section of kidney cortex tissue in different concentration after 7 days, 100x magnification, A- control treatment, B-250 µl

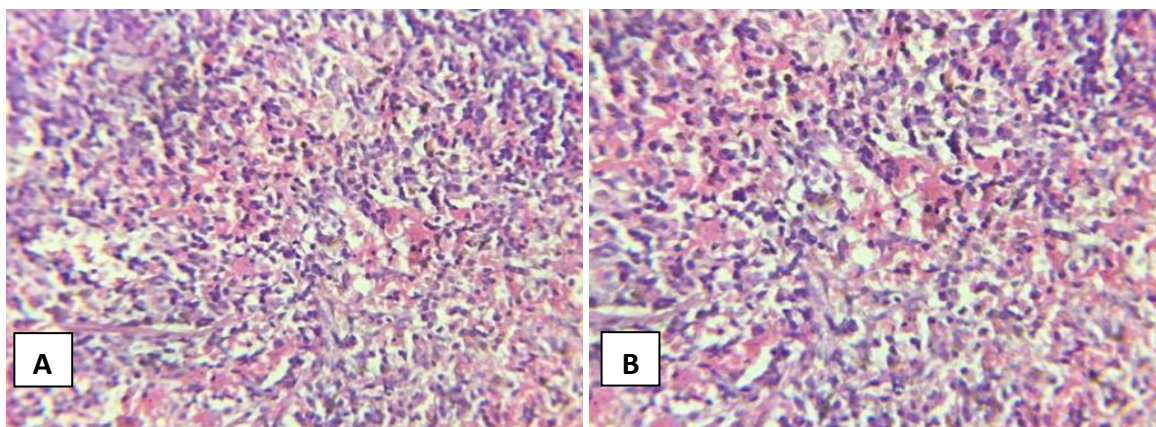


Figure 3: The histological section of spleen tissue in different concentration after 7 days, 100x magnification, A- control treatment, B-250 µl

#### IV. DISCUSSION

Lipopeptidella is a cyclic antibiotic generated by *B. subtilis* strains and categorized based to amino acid changes (Singh and Cameotra, 2004). Lipopeptides have a high level of preoperative effectiveness against bacteria, fungus, and yeasts, as well as a high level of stability due to their structure. Because of their unusual structure including D amino acid residues, lipopeptides are seldom digested by ordinary proteases, and therefore

have an impact exclusively on microbes, according to all investigations (Grangemard *et al.*,1999), No previous or current research has indicated the existence of lipopeptide impact on laboratory animal tissues and organs, confirming its appropriateness for human usage and its safe use on people since it is regarded antibacterial on many pathogens. Lipopeptide was a stable compound in the presence of heat, pH, and proteolysis enzymes, and it was not absorbed from the intestine. The pharmacokinetic natures of lipopeptide

revealed a short half-life, rapid clearance, and poor bioavailability, limiting its biomedical use, but it was favorable from a toxicity standpoint. This research should also offer basic data for future dosing studies and application form design.

## V. CONCLUSION

It was found through the study that lipopeptide had no effect on the organs that were used in the experiment and it is suitable for use.

## CONFLICT OF INTEREST

Author declares that there is no conflict of interest for this study.

## REFERENCES

- [1] Banat IM, MakkardRS and Cameotra SS (2000) Potential commercial applications of microbial surfactants. *Appl Microbiol Biotechnol.*q53:495–508.
- [2] Coutte F, Lecouturier D, Dimitrov K, Guez JS, Delvigne F, Dhulster P, Jacques P (July 2017). "Microbial lipopeptide production and purification bioprocesses, current progress and future challenges" *Biotechnology Journal.* 12 (7): 1600566. doi:10.1002/biot.201600566.
- [3] Deleu M, Paquot M and Nylander T (2008) Effect of fengycin, ga lipopeptide produced by *Bacillus subtilis*, gon model biomembranes. *Biophys J.* 94:2667–2679f.
- [4] Fetoui H, Mahjoubi-Samet A, Jamoussi K, Ayadi F, Ellouze F, Zeghal N (2007) *Nutr Res* 27:788–793.
- [5] Grangemard., Bonmatin, J.M., Bernillon, J. Das. B.C. and Peyponx, F. (1999): Lichenysins G, a novel family of lipopeptide biosurfactants from *Bacillus licheniformis*. I M.1307: Production, Isolation and structural evaluation by MNR and mass spectrometry. *J. Antibiot.*, 52, 363-373.
- [6] Hamley IW (May 2015). "Lipopeptides: from self-assembly to bioactivity" (PDF). *Chemical Communications (Cambridge, England).* 51 (41): 8574–83. doi:10.1039/c5cc01535a.
- [7] Maneerat, S. and Phetrong, K. (2007). Isolation of biosurfactant-producing marine bacteria and characteristics of selected biosurfactant. *Songklanakarin J. Sci. Technol.*, 29(3): 781-791.
- [8] Maniatis, T.; Fritsch, E. F. and Sambrook, J. (1982). *Molecular cloning: A laboratory manual*. Gold Spring Harbor Laboratory, New York.
- [9] Singh p. and Cameotra, s.s (2004): Potential applications of microbial surfactants in biomedical sciences. *Trends Biotechnol.*,22,142-146.