Experiment (3): Extraction and Determination of Bacterial Proteins

Aims:

- Extraction of total bacterial proteins.
- Determination of bacterial proteins using biuret method.

Introduction:

A bacterial protein is a protein which is either part of the bacterium structure or produced by bacterium as a part of its life cycle. Research on bacterial proteins has been performed with the goal of learning more about specific proteins and their function which impact human health. A bacterial protein can be toxic, causing illness or death in an organism which has been infected including humans. In addition, the information which has been gained form studding bacterial proteins can be extrapolated to gather more data about the proteins associated with larger organisms.

Furthermore, bacteria can produce foreign proteins from introduced genes, using their own gene expression machinery. Scientists routinely clone the gene that encodes 'their' protein and express large amounts of it in bacteria. Many medicines and drugs – particularly hormones – are proteins. These include insulin (for treating diabetes), erythropoietin (for treating anaemia), growth hormone (for treating growth disorders) and others. Today, bacteria (and other organisms) are used routinely as biological 'factories' to produce protein medicines in large amounts by cloning the desired genes.

Principle:

Isolation of bacterial proteins involves several steps: 1. Growth and induction of bacterial cultures, 2. Lysis of cells in a suitable buffer containing a detergent, 3. DNase and RNase treatment for the removal of the nucleic acids, 4. Determine the protein concentration using suitable method and 4. Passage of the extract through an affinity resin and finally elution of proteins.

In this lab determination of total bacterial proteins will done using biuret method. Biuret method is based on copper ions binding to peptide bonds of protein under alkaline condition to give a violet (purple) color which has maximum absorbance at 540 nm. The intensity of the color resulting from the (Cu+protein) complex is linearly proportional to the concentration of protein present in the solution.

Materials:

Chemical

LB medium, Distilled water, BSA stock solution (3 g/l), Biuret reagent, and Lysis buffer

Preparation of lysis buffer

Containing the following: 140mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4

Equipment and Glassware

Microfuge centrifuge, electronic balance, water bath, spectrophotometer, microcentrifuge tube, centrifuge tube, Pasteur pipette, micropipette, tips, pipette 5ml, test tubes, plastic cuvettes.

Protocol:

A) Extraction and isolation of bacterial proteins:

1. Centrifuge the bacterial sample (6 ml of overnight culture) for 5 minutes at 3000 rpm at 4 °C.
2. Resuspend the pellet in 1 ml lysis buffer.
3. Sonicate for 30–60 s in ice bucket until the cells are completely disrupted.
4. Transfer the resuspended sample to microcentrifuge tube, then spin 5 min at 13000 rpm at 4°C.
5. Separate soluble proteins (supernatant) from insoluble proteins (pellet). Use supernatant (soluble proteins) for next step.
6. Resuspend the pellet in another 1 ml lysis buffer and use supernatant for next step. (Insoluble proteins).

B) Determination of total bacterial proteins concentration:

1. Set up 10 test tubes as following:

Test tube	Distilled water [µl]	Stock BSA solution (….. g/l) [µl]	Sample [µl]	Protein concentration [g/l]
Blank	250	-	-	
A	200	50	-	
B	150	100	-	
C	100	150	-	
D	50	200	-	
E	-	250	-	
F (Unknown soluble proteins)	-	-	250	?
F'	125	-	125	?
G (Unknown insoluble proteins)			250	?
G'	125		125	?

2. Incubate all the tubes in water bath at 37°C for 5 minutes.
3. To each tube, add 1000 µl of Biuret reagent. Mix well and allow standing for 5-10 minutes in the 37 °C water bath.
4. Measure the absorbance of solutions at 540 nm.
5. Plot standard curve for absorbance against BSA concentration using results for solutions (A-E).
6. From the standard curve, estimate the concentration of proteins presents in your samples.

Results:

Test tube	Protein concentration [g/l]	Absorbance at 540 nm
Blank		
A		
B		
C		
D		
E		
F	_____	
F'	_____	
G	_____	
G'	_____	

References:

1. https://www.sciencelearn.org.nz/resources/1959-producing-foreign-proteins-in-bacteria
2. Todar K. "Bacterial Protein Toxins." Online Textbook of Bacteriology. University of Wisconsin, 2011.