introduction

A bacterial protein is a protein which is either part of the structure of the bacterium or produced by bacterium as a part of its cycle.

Proteins are an important part of all living organisms , and bacteria are no exception.

Research on bacterial proteins has been performed with the goal of learning more about specific proteins is important *because bacteria has a crucial role in human health status , and also the information we get by studding bacterial proteins can be extrapolated to gather more data about the proteins associated with larger organisms, including human.*

Bacterial proteins are of interests to human for a number of reasons.

1-Understanding which proteins are involved in structure of particular bacteria

2- can help researches develop medications which identify and target a particular bacterial proteins.

3-Understanding individual proteins can also allow monitoring mutations and to keep track of the ways in which these mutations occurred , and how they can be addressed .

Bacterial proteins has the ability to bind with other protein.

Protein binding involves the formation of very strong links between tow different proteins . Once proteins bind , they can trigger a reaction which may vary from an immune system response to an infection to the onset of a disease.

Some bacteria produce proteins which have a deleterious effect on the human body.

A bacterial protein can be toxic, causing illness or death in an organism which has been infected by bacteria, and bacterial proteins can also bind with specific proteins in the body to cause a variety of symptoms.

Objectives:

To extract and purify bacterial protein

Principle

Isolation of bacterial protein involved the following:

1- growth and induction of bacterial culture.

2-Lysis of cells in a suitable buffer containing a detergent

3-Dnase and RNase treatment for removal of the nucleic acid fraction

4- passage of the extract through an affinity resin (Ni+ -NTA agarose for His-tagged proteins)

5- elution of bound protein

Biuret method is one of the simple method for protein estimation.

This method can be used to monitor the concentration of protein during its purification.

This method is based on copper ions binding to peptide bonds of the protein under alkaline conditions to give a violet to purple color.

The intensity of the color resulting from the Cu-protein complex is linearly proportional to the mass of protein present in the solution.

Material

Chemicals:

1-Lysis Buffer (PBS) :

140 mM NaCl + 2.7 mM KCL + 10 mM Na_2HPO_4 +1.8 mMKH_2PO_4 (PH=7.3)

2- BSA stock solution (50mg/ml)

3-Biuret Reagent $CuSO_4$ dissolved in NaOH

4- Distilled Water

Equipments and Glassware:

1-Test tubes

2-10 ml pipette

3- Spectrophotometer

4-water bath at 37˚C

Method

A- Extraction and Isolation of bacterial Proteins:

1- centrifugation 7 min 1300 rpm 4˚C

2-Re suspend pellet in 1 ml PBS lysis solution

2- place the tubes in ice bucket for 10 seconds or more if the cells are not completely disrupted *(lysis is complete when the cloudy cell suspension become translucent)*

3- Spin 5 min 1300 rpm 4˚C .

4- Separate soluble proteins (supernatant) from insoluble proteins (pellet).

Use supernatant for the next step .

Keep sample of 40 µl of supernatant for PAGE – SDS and Western blot(insoluble proteins)

Method

b- Determination of Total Bacterial Proteins Concentration:

1- Set up 8 test tube A-H, G is soluble sample and H is the insoluble sample.

(Concentration of the Stock solution = 140 m(µg / (µl).

Test tube	Dis. H2O(µl)	BSA stock solution(µl)	Sample(µL)	Protein conc. (mg/ml)
A(blank)	250	-	-	0
B	200	50		28
C	150	100		56
D	100	150		84
E	50	200		112
F	-	250		140
G	-	-	250	?
H	-	-	250	?

2- Incubate all the tubes in water bath at 37° C for ⑤ min.

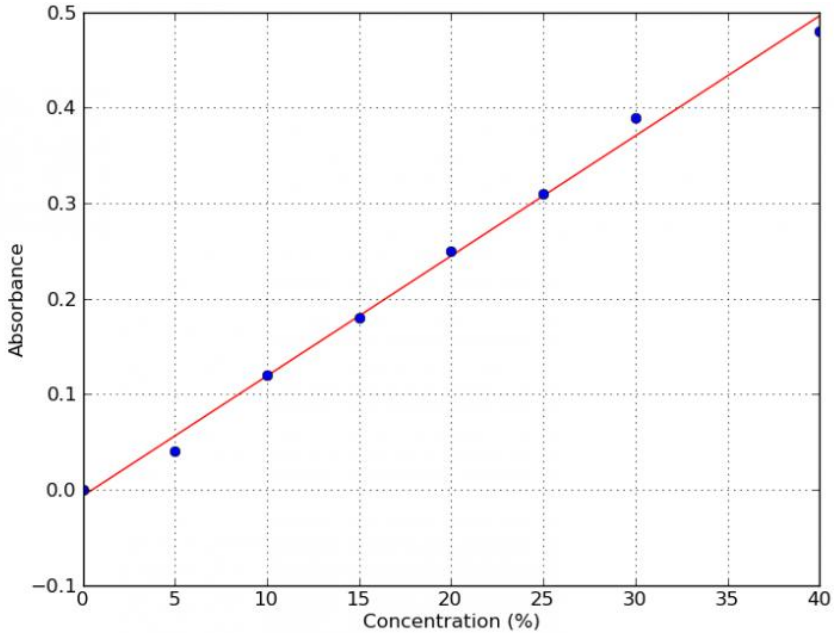
3- To each tube add 1000 µl of Biuret reaget, mix well and allow standing for ⑳ min in the water bath

Method

4- Measure the absorbance of solutions at 540 nm (B-H) using A as blank.

5- Plot the standard curve for absorbance against BSA concentration using results for solutions (B-H).

6- From your standard curve , estimate the



Result:

Concentration of protein (x) =

volume of fraction / Total volume in each tube x Concentration of standard **mg / dl.**

tube	A	B	C	D	E	F	G Soluble protein	H Insoluble protein
Concentration of protein (x)							?	?
Absorbance (y)							?	?

Thank You