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RESEARCH ARTICLE

IMPROVISED METHOD FOR DETECTION OF PHOSPHATE GROUP IN GRAM NEGATIVE BACTERIAL SURFACE PROTEINS: (A MODIFIED METHOD OF FISKE SUBBARAO METHOD)

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ABSTRACT

Estimation of phosphate in *Wolbachia* Surface Protein (WSP) will explore the link between Phospholipid hydrolysis and *Wolbachia*. It is hypothesized that, the proteins with phosphate groups can actively participate in phosphate hydrolysis. In the current study, an improvised novel our approach has been adopted to quantify the presence of phosphate in WSP.

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INTRODUCTION

Outer membrane proteins (OMPs) performs a variety of functions such as: (a) They form channels in the outer membrane for uptake of nutrients or the secretion of proteins; (b) They may be as enzymes involved in, for example, in the modification of lipopolysaccharides or in proteolysis; or (c) They function as adhesins mediating the interaction of the bacterium to host cells. Despite these different functions, they appear to share a common fold, i.e. they form trans-membrane β-barrels consisting of antiparallel amphipathic β-strands. The number of β-strands in the OMPs, of which the structure has been solved, varies and ranges from 8 to 22.*Wolbachia surface protein* (WSP) is known to be a key player in host interactions. In the recent publications on WSP there was a comparison of the structure of WSP (1) in three different hosts. (Nematode, *Drosophila, and A.tabidia*) and has shown that it has 8 Beta barrel structure and has similarity with following protein structures: 2u2F, 1D4C, 1DQ6, 1FW2 (2, 3, 4, 5). The blast similarity with PDB structure sequences revealed conserved domains/motifs (unpublished data).

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It was interesting to see that there were some unusual hits derived from the analysis. These findings provide a great start for finding similar structure-function relationship. This result was exploited investigate the, evolutionary links between WSP & the non-surface proteins in the hit. Investigating the (a) Link between WSP and anaerobic respiration (b) Link between WSP and Phospholipid hydrolysis (2). This would be great interest to study the structure function relationship of *Wolbachia* proteins. In the current study simple method was adopted to detect phosphate content in WSP. Further the study will have insights to elucidate the metabolites that the *Wolbachia* may require for its growth and replication. The researchers may use this methodology to further develop assay protocols to study different functions of *Wolbachia* proteins.

MATERIALS AND METHODS

Acid Molybdate Reagent: To 9ml of water, add 3.4ml of concentrated H_2SO4. Dissolve 0.625g of ammonium molybdate in 12.5ml of water. Add this solution to the above sulphuric acid solution, and make up to 25ml with water.

Reducing Agent: Dissolve 6g of Ascorbic acid in 10ml of H_2O. Added 20µl of acetone for stability.

Table 1. Protocol for Standardization of the Phosphorus and Test protein sample of 10 and 100 µl

Standard Phosphate (ml)	D.H_2O(ml)	Molybdate Reagent(ml)	Reducing Agent(ml)	O.D. At 660nm	Conc of Phosphate(mg)
Control	1.0	1	0.4	0.00	0.00
0.2	0.8	1	0.4	0.26	0.027
0.4	0.6	1	0.4	0.34	0.054
0.6	0.4	1	0.4	0.52	0.081
0.8	0.2	1	0.4	0.71	0.108
1.0	0.0	1	0.4	0.83	0.136
Test(10µl)	0.99	1	0.4	0.003	
Test(100µl)	0.9	1	0.4	0.073	0.01

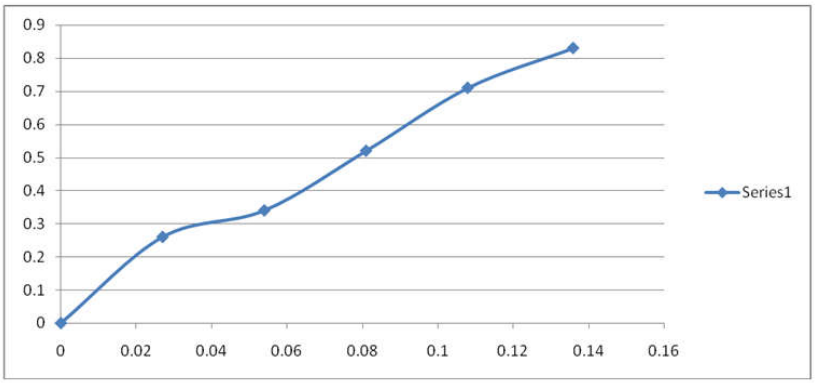

Figure 1. Graph Showing the Standard Curve

Table 2. Protocol for Standardization of the Phosphorus and Test protein sample of 390 µl

Standard Phosphate (ml)	D.H_2O(ml)	Molybdate Reagent(ml)	Reducing Agent(ml)	O.D.	Conc of Phosphate(mg)
Control	1.0	1	0.4	0.00	0.00
0.1	0.9	1	0.4	0.122	0.013
0.2	0.8	1	0.4	0.231	0.027
0.3	0.7	1	0.4	0.33	0.040
0.4	0.6	1	0.4	0.43	0.054
0.5	0.5	1	0.4	0.52	0.068
Test(390µl)	0.61	1	0.4	0.33	0.04

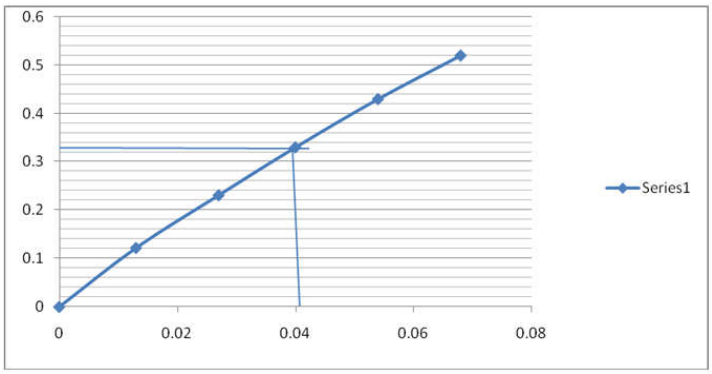

Figure 2. Graph Showing the Standard Curve and estimation of protein

Standard Phosphate Solution

The stock of 13.6mg in 10ml was prepared, from the stock the Working Solution was diluted to 10times with H_2O. i.e, after diluting the stock to 10 times, concentration of phosphate will be 0.136 mg/ml.

Recombinant *Wolbachia surface protein*

DNA was extracted from *Wmel* Strain maintained in the laboratory and Cloned into PET 19b which was over expressed and purified thorough nickel affinity chromatography to produce rWSP.

Procedure

- Pipette out 0.2 to 1.0 ml aliquots of the standard phosphate solution into different test tubes and make up the volume to 1ml with distilled water.
- Add 1.0ml of Acid Molybdate followed by 0.4ml of reducing agent to all tubes.
- Mix them well, stand for 30min at room temperature.
- Make up the volume in each case to 10.0ml with distilled H_2O and read the absorbance at 660nm against a suitable blank.

Again we tried with decreasing standard phosphate concentration and increasing protein concentration.

RESULTS AND DISCUSSION

Although the test was failed to detect phosphate in the 10 and 100 µl, we got good results when total protein was used i.e 390 µl. The results indicate that 0.04mg of phosphate is present in 390 µl of protein. i.e., 1 mg protein sample there was about 5.1ug phosphate present. The evolutionary relationship of *Wolbachia* revealed by blast analysis of WSP showed structural and functional similarity with Outer membrane proteins (OMPs) .This led us to hypothesize that there is a link between WSP and anaerobic respiration and phosphate hydrolysis. The presence of phosphate indicates that WSP is undergoing Phosphate hydrolysis, In comparison to other Outer membrane protein phospholipase A (OMPLA) is an integral membrane enzyme that catalyses the hydrolysis of phospholipids. Enzymatic activity is regulated by reversible dimerisation and calcium-binding (5). In conclusion we provide a clue that *Wolbachia surface protein* undergoes hydrolysis by using a simple protocol of estimation of phosphate by modifying protocol given by Fiske Subbarao Method (6).

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