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OUTER MEMBRANE PROTEIN PROFILE OF VIBRIO SPECIES ISOLATED FROM MARINE FISHES

Vikram S[1], Sri Renga Ramanujam G[2], Saravanan N[3], Ramesh Babu N G[4]

[1,2,3,4] *Department of Biotechnology,*
Adhiyamaan College of Engineering (Autonomous)-Hosur 635109

Abstract - *Many Vibrio species are harmful to human beings especially found in marine fishes are responsible for food poisoning. Three fish samples were examined for the presence of Vibrio species. It has been found that only one species dominated and it was determined by a series of biochemical tests according to the biochemical key. The outer membrane proteins of the Vibrio species have been isolated by series of techniques. The outer membrane of Vibrio metschnikovii containing a major protein with an apparent molecular weight of 50 kDa and one or two proteins with molecular weight ranging from 40 kDa to 70 kDa have been identified through SDS PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) profiles. Then Lowry was used to method determine the concentration of outer membrane protein. Similar protein sequence was retrieved from the protein data bank and accompanied with pairwise alignment BLAST and the ligand (CID: 54675776) interaction was identified using Molecular Docking tool SWISS DOCK. This gives a partial profiling of outer membrane protein, thus it helps in control of Vibrio associated food poisoning.*

Key Words: **Vibrio species, Marine fishes, Biochemical key, Molecular Docking, Ligand, Outer membrane protein profiling, SDS PAGE**

1. INTRODUCTION

Vibrios are among one of the surface organism in surface waters of the world. They occur in both marine and fresh water aquatic animals. Some species are pathogens of marine fishes etc., [1] .

Species such as V.cholerae, V.pharahaemolyticus, V.vulnificus, V.alginolyticus, V.mimicus, V.fluvialis, V.furnissi, V.metschnikovii, V.hollisae and V.damsela are human pathogen. Thus they account for a significant proportion of human infection such as gastroenteritis usually associated with consumption of raw or undercooked sea food [1].

Vibrio species of marine fishes usually causing food poisoning associated with consumption of sea food. It is likely that exposed surface components outer membrane proteins play an important role in interaction of Vibrio and the host during infection and may also affect the ecological distribution of the Vibrio [15]. It has been reported that cell envelope of the genus vibrio containing several major proteins with molecular weight ranging from 25,000 Da to 50,000 Da [15].

In Nigeria, V.parahaemolyticus associated gastroenteritis due to consumption of contaminated sea food has been reported as well as sporadic cases of cholera (WHO, 1991) has been reported.

In this study, Vibrio species was isolated from marine fishes and its outer membrane proteins were isolated and present data shows chemical and morphological characterization of the outer membrane protein. We also reported the biochemical assay of isolated Vibrio species of three different marine fishes. The protein sequence (outer membrane lipoprotein-sorting protein domain from Vibrio parahaemolyticus (PBID: 3BK5)) which is in accordance with obtained outer membrane protein was subjected to molecular graphics and docking. The knowledge of this helps in control of Vibrio associated food poisoning as the masses are made aware of the danger associated with consumption of raw or undercooked sea food.

2. MATERIALS AND METHODS

2.1. Enrichment of Microbial Source

Three types of fishes Acanthocybium solandri (Vanjaram) about 45.56g, Sardinella longiceps (Nei-Maththi) about 51.34g, Lutjanus campechanus (Sankara) about 47.8g represented as VJ, NM and SK respectively are taken for the isolation of the microbes. The abdomen flesh of the fishes were cut off and cleaned with distilled water. 1g of abdomen fleshes are placed in 15ml alkaline peptone water (1% NaCl + 1% Peptone) three different test tubes. They were incubated overnight at 37ºC for the nourishment of microbes.

2.2. Isolation of Vibrio species

Overnight enriched alkaline peptone water was inoculated in selective media TCBS agar (Thiosulphate citrate bile salt sucrose agar) for the isolation of the *Vibrio* species. The inoculation was done by swab technique in three sterile petri plates (25ml) which are named VJ, NM and SK. It was incubated at 37ºC for 24 hours. Development of bluish green

color denotes the growth of *Vibrio* species. It was subcultured in TCBS agar by quadrant streaking method in order to obtain pure colonies incubated at 37ºC for 24 hours. Pure colonies were sub cultured in nutrient agar slant by streaking method incubated at 37ºC for 24 hours and by using biochemical tests it has been identified as vibrio species.

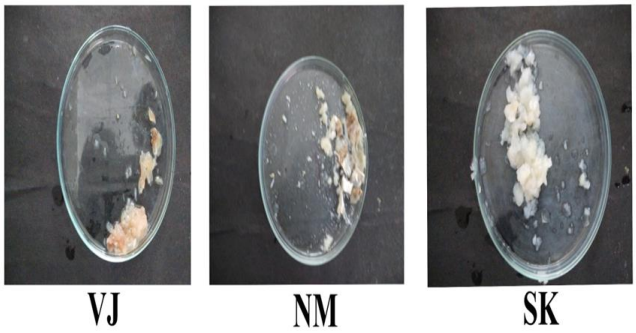

Fig.1. Abdomen flesh of marines fishes

2.3. Biochemical test

The biochemical key was designed by using Bergey's manual and FDA manual was used in biochemical characteristics of *Vibrio* species. Eight biochemical tests were included in identifying the species namely urease test, catalase test, oxidase test, motility test, staining, citrate test, indole test, and gas production test.

2.4. Bacterial cultivation

All three strains of *Vibrio* species from streaked TCBS agar plate was incubated in nutrient broth of 25 ml containing 3% NaCl and incubated at 37ºC for 24 hours.

2.5. Preparation of cell envelopes and outer membrane

Cells were harvested by centrifuging the overnight cultures at 5000 rpm for 10 minutes. The supernatant was removed and the pellet was mixed with 1x PBS (phosphate buffer saline). It was then subjected to heat shock at 100ºC for 24 hours and then immediately kept at 4ºC for 15 minutes and it was repeated thrice. It was again centrifuged at 5000 rpm for 10 minutes and the pellet was mixed with 1× PBS buffer and the samples are stored at 4ºC.

2.6. SDS-PAGE

SDS-PAGE was performed in order to determine molecular weight of outer membrane protein. Then the sample (NM, VJ and SK) was mixed with loading buffer. Then they were loaded to their respective wells. Protein markers also loaded which play a vital role in the determination of molecular weight. Then the whole experimental setup is made to run for 1 hour at 50 volts. Then gel was subjected for determination of molecular weight.

2.7. Estimation of protein concentration

Protein concentration was determined by Lowry's method [9] with Bovine serum albumin used as a standard. Protein concentration was determined by observing OD at 620 nm in a UV spectrometer (spectronic 200-Thermo scientific).

2.3. Bioinformatics analysis

All the similarity searches of outer membrane protein sequences with Outer membrane protein A (Vibrio metschnikovii CIP 69.14) were analyzed by BLAST [10]. Pairwise sequence alignments were conducted between respective sequences using the Clustal Omega software website for confirmation of sequence. Molecular graphics visualization of the protein molecule was performed using RASMOL. The predicting and discriminating OMP were performed with Hidden Markov Models PRED-TMBB. Molecular Docking between outer membrane lipoprotein-sorting protein domain from *Vibrio parahaemolyticus* (PBID: 3BK5) and zinc ligand tetracycline (usually used for treating food poisoning) (CID: 54675776) were performed using the online Docking Tool SwissDock [3] and molecular graphics of the interaction between the protein and the ligand were visualized using USCF Chimera package: Chimera was developed by the Resource for Biocomputing , Visualization and Informatics at the University of California, San Francisco (Supported by NIGMS P41-GM103311).

3. RESULTS

3.1. Biochemical assays: identification of vibrio species

The results of biochemical tests based on the biochemical key was designed by using Bergey's manual and FDA manual and *Vibrio metschnikovii* strains was identified in all the samples.

3.2. Composition of outer membrane protein of Vibrio Species isolated from three different marine fish samples

SDS PAGE profiles of the outer membrane protein obtained from *Vibrio metschnikovii* strain grown in 3% NaCl nutrient broth are shown in the fig.2. All three marine fishes contained a major outer membrane protein with an apparent molecular weight of 50 kDa and one or two other major membrane protein with molecular weight ranging from 60 kDa to 40 kDa.

Table 1: Results of biochemical test

Biochemical Test	VJ	NM	SK
Growth in TCBS agar	Yellow	Yellow	Yellow
Citrate	Positive	Positive	Positive
Urease	Positive	Positive	Positive
Motility	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative
Catalase	Positive	Positive	Positive
Indole	Positive	Positive	Positive
Gram Staining	Gram Negative	Gram Negative	Gram Negative

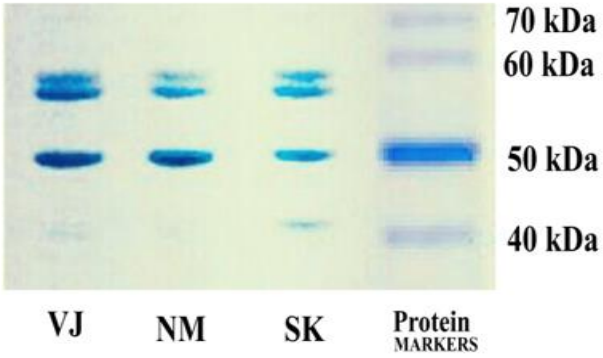

Fig.2. SDS-PAGE profile of outer membrane protein of vibrio isolates

3.3. Profiling of outer membrane protein concentration of three different *vibrio* isolates

Outer membrane protein concentration was determined by co relating OD values of three different samples with standard graphs. Concentration of outer membrane protein it was given in the Table 2.

3.4. Bioinformatics analysis

3.4.1 Identity of outer membrane protein A (Vibrio metschnikovii CIP 69.14) with related protein sequences

BLAST result of outer membrane protein A (*Vibrio metschnikovii* CIP 69.14) and pairwise sequence alignments were conducted between respective sequences (outer membrane lipoprotein-sorting protein domain from *Vibrio parahaemolyticus* (PBID: 3BK5)) using the Clustal Omega software website for confirmation of the sequence.

Table 2. Results of Lowry's method

Samples	OD at 620nm	Concentration (µg/ml)
VJ 1	0.608	260
VJ 2	0.667	
SK 1	0.428	100
SK 2	0.448	
NM 1	0.438	117
NM 2	0.592	

```
# Aligned_sequences: 2
# 1: C9P350_VIBME
# 2: Q87Q63_VIBPA
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 361
# Identity:      68/361 (18.8%)
# Similarity:   113/361 (31.3%)
# Gaps:         132/361 (36.6%)
# Score: 40.0
#
#
#=======================================

C9P350_VIBME       1 MKKL-----VAMISASLMAASSSAMADVYLGGKLGQTWLNDSCRAGEVCE     45
                     |||:     |.|:||||:|:..              |:.|:. :..|:.:
Q87Q63_VIBPA       1 MKKMNHVLQVLMLSASLVASPF--------------TYANEE-KGLEIAQ     35

C9P350_VIBME      46 DRDTSMGAFLGYNLNEWLAVEAGYDMLGKFTGIGLADDRVKAITLAPKFT     95
                     :|.              |.:.|:             .|.:.:.:.:.|
Q87Q63_VIBPA      36 ERK---------------ARDEGW------------GDSIATMEMILK--     56

C9P350_VIBME      96 LSLSPSVGAYAKLGGAYVDYGDESDYSFLAAAGLEFNVSPNVSLRTEYQV    145
                                 :...||...:....||.....:..| |.:
Q87Q63_VIBPA      57 ------------------NAQGESSTRLMRLKSLEVEGDGDKGL-TIF--     85

C9P350_VIBME     146 LTDINNDQTRAEANTVSLGVSYRFGAAAQPTQVVEIRPVTVIEEVIVVEE    195
                        ||.|....|..|..|:..||..|...      :..::.|.:.:.
Q87Q63_VIBPA      86 ------DQPRDVTGTAFLNHSHTIGADDQWLY------LPALKRVKRISS    123

C9P350_VIBME     196 IVETGPVFVNKTYDFQRLDSSSFAFDSVSLNQESKQQLT-KLVRFLNQYP    244
                     ..::|| |:...:.:: |.|||..:....|....::.. :.|..|.|.|
Q87Q63_VIBPA     124 RNKSGP-FMGSEFAYE--DLSSFEIEKYRFNHLKDEKFNGQDVFVLEQIP    170

C9P350_VIBME     245 QAKVVITGHT------------------DATGPAAYNQTLSEKRAKAVA    275
                     ..|  .:|:|                  |..|  |..:||:....|...
Q87Q63_VIBPA     171 TDK--NSGYTKQVVWLDKAHYRPLKVEFYDRKG--ALLKTLTFANYKQYL    216

C9P350_VIBME     276 DALVEQGIQRSRITWRGEGETNPIASNSTAEGRELNR---RVDITIPEFD    322
                     |..           ||  ..|..:.::.|.:..|||.   |....:.|.|
Q87Q63_VIBPA     217 DKY-----------WR--AHTMAMTNHQTGKSTELNTSDLRFQTGLEEND    253

C9P350_VIBME     323 Y------QVKQ    327
                     :      :||
Q87Q63_VIBPA     254 FNKNVLKRVK-    263
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Fig.3. Pairwise sequence alignments between respective sequences (outer membrane lipoprotein-sorting protein domain from Vibrio parahaemolyticus (PBID: 3BK5)) and outer membrane protein A (Vibrio metschnikovii CIP 69.14)

3.4.2 The predicting and discriminating outer membrane protein

Prediction in Pred-TMBB for the protein localization at the membrane of Outer membrane protein A (*Vibrio metschnikovii* CIP 69.14)[10].

is responsible for food poisoning. It was found that the isolated bacteria is hemophilic bacterium *Vibrio* species requires at least 3% NaCl for growth. Sea water and sea food is an important vehicle of transmission of this species and other *Vibrio* and the possible spread of *Vibrio* to marine invertebrates.

In this study 1x PBS was used to lead to alter outer membrane and early release of some outer membrane protein and SDS-PAGE was used to determine molecular weight of such proteins .In the present study, SDS-PAGE revealed that the outer membrane *Vibrio metschnikovii* consisted of four to six major proteins with molecular weights ranging from 40,000 to 60,000, depending on the strain. Protein A (molecular weight, 44,000) occurred commonly and predominantly in the vibrio together with a close-neighboring protein (molecular weight, 50,000). Using cell envelopes from three strains of *V. metschnikovii*, from three different marine fishes, a major protein was detected with a molecular weight ranging from 43,000 to 50,000. The presence of peptidoglycan-associated proteins in the outer membrane has been well documented in various Enterobacteriaceae [8]. One or two peptidoglycan-associated proteins with molecular weights ranging from 27,000 to 40,000 remained bound to peptidoglycan after treatment with 2% (w/v) SDS at 60ºC, which is in accordance with our results of the present study.

The Lowry method was used in the determination of outer membrane protein concentration, it was shows that protein presence was similar in the three samples, but concentration varied based on the concentration of NaCl [4].

Bioinformatics analysis was done for partial profiling of outer membrane protein. Based on BLAST result, it is found that series of protein are in accordance with outer membrane protein A (*Vibrio metschnikovii* CIP 69.14). Pairwise sequence alignment was done between outer membrane protein A (*Vibrio metschnikovii* CIP 69.14) and outer membrane lipoprotein-sorting protein domain from *Vibrio parahaemolyticus* (PBID: 3BK5), score was found to be 40.0. Prediction in Pred-TMBB for the protein localization at the membrane of Outer membrane protein A (*Vibrio metschnikovii* CIP 69.14) showed discrimination score as 2.989 and it also showed the localization of outer membrane protein. Molecular visualization is done by using UCSF chimera which gives structural information about the outer membrane protein and tetracycline ligand. Molecular docking between respective receptor and ligand showed that interaction occurs by 179 hydrogen bond and major interaction site is found to be GLY 77 HN-#1.94 LIG1 03 2.224Å. Thus it evident that outer membrane protein in *vibrio* species are causes some *Vibrio* associated food poisoning.

5. CONCLUSION

In this study, isolation of *Vibrio* species from marine fishes and partially profiling the outer membrane protein based on its chemical and morphological characteristic was done. This knowledge will help in controlling of *Vibrio* associated food poisoning due to the awareness of danger associated with consumption of raw or undercooked sea food. However, it remains to be determined whether the proteins are responsible for the formation of permeability channels in the outer membrane.

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