



Alexandria University Faculty of Veterinary Medicine

Molecular studies on Vibrio species isolate from fish.

A Thesis

Presented to the Graduate School Faculty of Veterinary Medicine, Alexandria University In Partial Fulfillment of the Requirement for the Degree

of

Ph. D. of Veterinary Science

Specialization

(Bacteriology & Mycology)

By

Eslam Abd El-Latif Asran Hamada

(B. V. Sc. Fac. Of Vet. Kafr El-Sheikh Univ., 2009) (M.V.Sc. Fac. Of Vet. Alexandria Univ., 2015)

(2020)

CONTENTS

CONTENTS	Peg
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. History of Vibrio species	4
2.2.Vibrios in human and aquaculture	7
2.2.1. vibriosis in human	7
2.2.2.Vibriosis in aquaculture	9
2.3. Bacteriological characters of vibrio species	16
2.3.1. Morphology	17
2.3.2. Biochemical characterization of vibrio species	22
2.4. Molecular identification of vibrio species and virulence	28
factors detection	20
3. MATERIAL AND METHODS	44
3.1.1. Material	44
3.1.1. Samples	44
3.1.2. Media used for bacteriological examination	44
3.1.3. Solutions and reagents	48
3.1.4. Staining	49
3.1.5. String test material and solutions	49
3.1.6. Vibriostatic agent(0/129) disks	50
3.1.7. Reagents and chemicals used for polymerase chain reaction	50
3.2.Methods	52
3.2.1.Clinical and macroscopic examination	52
3.2.2. Sampling of fish	53
3.2.3. Isolation of Vibrio species	53

3.2.4. Identification of the isolates	53
3.2.4.1. Morphological characterization	53
3.2.4.2. Cultural characterization	54
3.2.4.3. Detection of motility	54
3.2.5. Biochemical characterization	54
3.2.6. Sensitivity to vibriostatic agent O/129	57
3.2.7. Procedure of String Test	57
3.2.8. Detection of Vibrio species and virulence factors by PCR	58
3.2.8.1.Bacterial template DNA preparation	58
3.2.8.2.Oligonucleotide primers	58
3.2.8.3.The protocol of the PCR	59
3.2.8.4. Detection of PCR products	61
4. RESULT	63
4.1. Results of bacteriological isolation	63
4.2. Results of biochemical identification	64
4.3. Results of polymerase chain reaction (PCR)assay	66
4.3.1. Results of molecular characterization of genus vibrio	66
4.3.2. Results of species specific detection of different Vibrio spp	68
4.3.3. Detection of virulence genes of isolated vibrio spp	73
5. DISCUSSION	82
6- SUMMARY	91
7- REFERENCES	93
8. ARABIC SUMMARY	

LIST OF TABLE

Table NO.	Titled	Page
1	The isolated Vibrio species from diseased shrimp and their identification according to Jayasree et al. (2006).	24
2	The biochemical characteristics of several of family Vibrionacea according to Jayasinghe et al., (2008).	25
3	Morphological and biochemical characteristics of Vibrio sp. isolated from fish samples according to Ravikumar and Vijayakumar (2017).	27
4	List of V. anguillarum genes associated with virulence	39
5	The Oligonucleotide primer sequences used in this study	51
6	Vibrio species on TCBS.	54
7	Thermo cycling program for all gene primers used in this study.	60
8	Colonies morphology of isolated samples (suspected to be vibrio spp.).	64
9	Biochemical results of suspected isolates	65
10	Results of biochemical examination of suspected isolates	66
11	The lighted bands produced by Vibrio species specific primers	68
12	Results of detection and identification of species specific of different Vibrio spp.	68
13	The expected size of DNA bands that regarded as positive results for the existence of the virulence factors	73

LIST OF FIGURES

No.	Title	Page
1	Yellow and green colonies on TCBS.	63
2	Microscopic examination of Vibrio species bacteria	64
3a	Agarose gel electrophoresis analysis (1.5 %) (stained with ethidium bromide) of 16SrRNA(700 bp) for identification and characterization of vibrio species .	66
3b	Agarose gel electrophoresis analysis (1.5 % and stained with ethidium bromide) of 16SrRNA(700 bp) for identification and characterization of vibrio species.	67
4	Agarose gel electrophoresis analysis of PCR of toxR (366 bp) for identification and characterization of vibrio parahaemolyticus.	69
5	Agarose gel electrophoresis analysis of PCR of Van gene (429 bp) for identification and characterization of vibrio anguillarum.	70
6	Agarose gel electrophoresis analysis of PCR of Vh_toxR gene (390 bp) for identification and characterization of V.harveyi.	71
7	Agarose gel electrophoresis of PCR of VP gene (320 bp) for identification and characterization of vibrio alginolyticus.	72
8a	Agarose gel electrophoresis of PCR of V. Parahaemolyticus virulence factors (tdh at 251 bp).	74
8b	Agarose gel electrophoresis of PCR of V. Parahaemolyticus virulence factors (trh at 373 bp).	75
9a	Agarose gel electrophoresis of PCR of V. Anguillarum virulence factors (angM 453 at 453 bp).	76

9b	Agarose gel electrophoresis of PCR of V. Anguillarum virulence factors (virA 314 at 314 bp).	77
10a	Agarose gel electrophoresis of PCR of V. harveyii virulence factors (Partial hly at 647 bp).	78
10b	Agarose gel electrophoresis of PCR of V. harveyii virulence factors (Vhh at 234 bp).	79
11a	Agarose gel electrophoresis of PCR of V. alginolyticus virulence factors (trh at 250 bp).	80
11b	Agarose gel electrophoresis of PCR of V. alginolyticus virulence factors (tdh at 373 bp).	81

6-Summary

Fish is a very important food of high protein value in Egypt. Vibriosis is a very serious disease affecting fish production, that caused by *Vibrio* species, affecting both fresh and marine water fishes causing pathogenic symptoms including dermal ulceration, septicemia and ascites. Leading to highly economical losses.

In our study, we examined 100 fish samples collected from fish markets at Kafr ElSheikh governorate and transported immediately to our Microbiology departement at (AHRI) Animal Health Research Institute, Kafr ElSheikh branch for bacteriological examination as 25 apparent healthy and 75 naturally infected (diseased) fish. Liver, kidney, spleen, gills, external skin lesions and heart were collected and cultivated for bacteriological examination.

The collected samples cultivated on TCBS agar media and the green and yellow colonies stained by Gram stain.

About 90 samples showed green and yellow colonies, that examined biochemically and about 83 samples suspected to be positive biochemically to vibrio.

Biochemical tests not produced accurate identification results, so PCR assay seems to be a sensitive, rapid and effective method for bacteriological characterization. 10 random samples examined by PCR for 16S rRNA that act as a specific primer for vibrio species that show 8 positive samples to vibrio.

The8positivesamplesexaminedforV.harveyi,V.parahaemolyticus, V.anguillarum and V. alginolyticusspeciesspeciesspecificprimers.

Vibrio alginolyticus detected by VP32 and VP33 gene, *Vibrio anguillarum* detected by Van-ami8 and Van-ami417 gene, Vh_toxR used to identify *Vibrio harveyi* and *Vibrio Parahaemolyticus* identified by toxR gene .

As in our thesis, PCR amplification show 8 vibrio strains show 1 *V.alginolyticus*, 1*V. anguillarum*, 2 *V.harveyi* and 4 *V.parahaemolyticus* as 12.5%,12.5%,25% and 50% respectively.

Also PCR technique used to identify some of virulence factors which found in each species.