Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(3):974-977

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Research on the contamination status of shrimp pathogenic vibrio

Jiang Hui-min

Heze University, China

ABSTRACT

To understand the contamination status of shrimp pathogenic Vibrio and provide reference for poisoning prevention and warning of pathogenic Vibrio in shrimp products, this paper adopts SN/T 2564-2010、SN/T 1022-2010 and GB/ T4789.7-2008 and gets 58 pathogenic Vibrios out of 340 shrimps of 5 varieties through separation and identification. The detection rate turns out to be 17.06%, which shows that there are pathogenic Vibrios in freshwater shrimps and reminds people to eat them with caution.

Key words: Shrimp; Pathogenic Vibrio; Separation; Identification

---

INTRODUCTION

Pathogenic Vibrio is likely to grow in waters with wide salt concentration and the surface and intestinal of maritime animals. It is worth paying attention to the existence of pathogenic Vibrio in freshwater products. Pathogenic Vibrio can easily lead to sickness of both human beings and animals, which may include the intestinal infectious diarrhea, food poisoning and bacterial diseases of cultivated fish, shellfish and shrimp. In some coastal cities, in particular, food poisoning caused by pathogenic Vibrio accounts for over 60% of bacterial food poisoning [1]. US Food and Drug Administration (FDA) and Nordic Committee on Food Analysis (NMKL, Nordisk Metodikkomité for Naringsmidler) have announced specific methods to fight against such pathogenic Vibrios, including the traditional separation and identification method and the molecular biology method through the polymerase chain reaction (PCR). FDA has published on Bacteriological Analytical Manual Online(BAM)major pathogens from high frequency to low frequency: Vibrio parahaemolyticus, non-O1Vibrio cholerae, Hollis Vibrio, Vibrio river, O1-Vibrio cholera [2]. To understand the contamination status of pathogenic Vibrio in four common freshwater shrimp products, a research on 340 shrimps of different types on market from January to December 2013 has been carried out to lay a foundation for the future risk assessment and warning of pathogenic Vibrio in shrimp products. Results are elaborated as follows.

EXPERIMENTAL SECTION

1.1 Sample source

Four kinds of freshwater shrimp Penaeusvannamei Boone, Macrobrachiumrosenbergii, Macrobrachiumnipponense and Crayfish were randomly selected in the city's major supermarket stores for a sampling test. The sampling was carried out from January to December 2013 according to the sterile sampling principle, after which samples were refrigerated at a temperature of 4℃. All these samples have received a test within 3 to 8 hours. The variety and number of samples are illustrated in Table 1.

1.2 Medium and reagent

Enrichment reagent: alkaline peptone water, halophilic bacteria and nutrient broth enrichment broth; Screening reagents: thiosulfate - citrate - bile - sucrose (TCBS) agar, CHROMagar medium; Purification Reagents: Tryptone soy (TSA) agar, nutrient broth; Identification Reagents: Blood agar, MacConkey agar and bacteria identification

biochemical reagents; VITEK2 Gram-negative bacteria and biochemical identification plate and Vibrio cholerae O1 and O139 group diagnostic serum group.

1.3 Pathogenic Vibrio composite enrichment culture

Shrimps are crustaceans. According to pretreatment methods [3,4] of SN/T 2564-2010 and GB/ T4789.7-2008, each sample should have three parallels. 25g sterile samples were weighed, then 225mL 0.5%, 1.0% 2.5%, and 4.0% alkaline peptone water (APW) were added with reference to SN / T 2564-2010 composite enrichment steps [5], and then they were altogether put into the sterile shoot click homogenizer bag, which should be slapped for 1min afterwards. Having evenly mixed them, they were enveloped and cultivated for 18 hours at 36 ℃. Finally, this enrichment broth was inoculated to TCBS flat, and cultivated at the temperature of 36 ℃ for 20 hours.

1.4 Identification of separated strains

With reference to SN / T 2564-2010, SN / T 1022-2010 and GB / T4789.7-2008, 2 to 3 suspected colonies in TCBS flat plate were chosen and crossed by CHROMagar Vibrio color tablet, then they were cultivated at 36 ℃ for 24 hours. Afterwards, 2 to 3 suspected colonies in CHROMagar color tablet were picked up and crossed by TCBS flat plate for mutual verification. Then, 5 suspected colonies were selected on both flat plates and crossed by 3.5% NaCltryptone soy agar (TSA) flat plate, and they were cultivated at 36.5℃ 24 hours. Afterwards, TSA tablet bacterial smear received Gram staining, and its morphology was observed under the microscope. The oxidase test was then carried out, and micro biochemical tube and automatic microorganism analyzer identifications were made when they were determined to be Vibrio bacteria.

1.5 Identification of Vibrio parahaemolyticus virulence genes through the PCR method

With reference to the identification method of Vibrio parahaemolyticus in BAM, which has been announced by FDA, specific tlh genes in suspected Vibrio parahaemolyticus strains were amplified through PCR. After they were confirmed to be Vibrio parahaemolyticus, the PCR method was used again to detect Dead parahaemolyticus pathogenicity genes tdh and trh genes. Afterwards, UNIQ-10 column bacterial genomic DNA extraction kit was used to extract Vibrio parahaemolyticus genomic DNA. Primers and their sequence are shown in Table 1; PCR amplification conditions are illustrated in Table 2.

Table 1 Primers and sequence

gene	primer	sequence	amplification length
tlh	tlh-L	5' aaagcg gat tat gcagaagcactg 3'	450
	tlh-R	5' gct act ttc tag cat tttctctgc 3'	
tdh	tdh-L	5' gtaaaggtctctgacttttgg ac 3'	270
	tdh-R	5' tggaatagaaccttcatcttcacc 3'	
trh	trh-L	5' ttggcttcgatattttcagtatct 3'	500
	trh-R	5' cat aacaaa cat atg ccc atttcc g 3'	

Table 2 Amplification conditions

gene	denaturation	annealing	extension	Cycle number
tlh	94℃ 4 min	60℃ 1 min	72℃ 5 min	25 cycles
tdh	94℃ 11min	60℃ 1 min	72℃ 11min	25 cycles
trh	94℃ 4 min	60℃ 1 min	72℃ 5 min	25 cycles

1.6 Statistics method

SPSS 11.5 software was adopted to compare detection rates of pathogenic Vibrios in four freshwater shrimps.

RESULTS AND ANALYSIS

2.1 Detection of pathogenic Vibrio

Pathogenic Vibrios were separated from 340 shrimp samples, and results were demonstrated in Table 3. The Vibrio parahaemolyticus virulence genes distribution was identified through the PCR method, and the Vibrio cholerae serotype was identified through the serological experiment. As the research result illustrates, the detection rate of 340 seawater pathogenic Vibrio samples is 27.06%, and 58 pathogenic Vibriosare separated. Among pathogenic Vibrios in shrimps, there are 37 Vibrio parahaemolyticuses (which account for 10.88%), among which there are 3 tdh genes and none trh gene; following Vibrio parahaemolyticus, there are 14 Vibrio vulnificuses (4.12%); in the third place are 4 non-O1Vibrio choleraes, which account for 1.18%; in the fourth place were 2 Vibrio alginolyticuses, which account for 0.59%. The detection rate of pathogenic Vibrio in Penaeusvannamei Boone, Macrobrachiumrosenbergii, Macrobrachiumnipponense and Crayfish are respectively 27.06%, 15.31%, 14.13%, and 10.77%.

2.2 The statistical significance of pathogenic Vibrio

A pathogenic Vibrio investigation on these four kinds of shrimp products that are commonly seen in the market was conducted and the average detection rate was 17.06%. From table 3, we can see that the detection rate of pathogenic Vibrio varies significantly among different kinds of shrimps($\chi2$= 8.60, $0.01<p<0.05$). The detection rate of Penaeusvannamei is 10 percent higher than the average detection rate of pathogenic Vibrio and has significant difference with that of Macrobrachiumrosenbergii and Procambarus clarkia respectively and insignificant difference with that of Macrobrachiumnipponense($\chi2$ Penaeusvannamei / Macrobrachiumrosenbergii =4.56 $0.01<p<0.05$,$\chi2$ Penaeusvannamei / Procambarus clarkia =6.11 $0.01<p<0.05$,$\chi2$ Penaeusvannamei / Macrobrachiumnipponense =3.82 $p >0.05$);the detection rate of Macrobrachiumrosenbergii has insignificant difference with that of Macrobrachiumnipponense or Procambarus clarkia($\chi2$ Macrobrachiumrosenbergii / Macrobrachiumnipponense =0.00 $p >0.05$,$\chi2$ Macrobrachiumrosenbergii / Procambarus clarkia =0.65 $p >0.05$); and the detection rate of Macrobrachiumnipponense has insignificant difference with that of Procambarus clarkia($\chi2$ Macrobrachiumnipponense / Procambarus clarkia =0.69 $p >0.05$).

DISCUSSION

The separation of Vibrio is associated with sampling technique, culture medium selection, culture condition (time, temperature) and identification technique [6]. Therefore, this experiment combines SN/T 1022-2010and GB/ T4789.7-2008 with the methods released by the U.S. FDA and Nordic Committee on Food Analysis and employs highly targeted alkaline peptone water of sodium chloride with different concentrations to separate and recombine the enrichment of pathogenic Vibrio. For initial screening, TCBS culture medium is used for mutual authentication with CHROMagar. For identification, the biochemical reaction tube and strain identification instrument are used for the identification of mutual authentication. Ultimately, the PCR method is used to identify the distribution of virulence gene in Vibrio parahaemolyticus and the serological test used to identify the serotype of Vibrio cholera, which is traditional, accurate, simple and convenient. The collected samples in the investigation are four kinds of common shrimps in the market, among which the Penaeusvannamei has been under desalting culture in China and can grow normally in 0-2 ‰ salinity. Thus, the collected samples are all freshwater shrimps and have representative significance. The investigation shows that the detection rate of pathogenic Vibrio of these four kinds of freshwater shrimps is 27.06% and that O1 Vibrio cholera and Vibrio metchnikovi are not detected. Five kinds of pathogenic Vibrio are detected and in descending order, the detection rate is Vibrio parahaemolyticus, Vibrio vulnificus, non O1 Vibrio cholera and Vibrio alginolyticus. In the light of the statistical result of SPSS software, this research prompts that it is necessary to be wary of the occurrence of Vibrio pathogenicity with regard to freshwater shrimps. As the national standard of the Vibrio cholerae test has not been introduced, this research refers to the industry standard of Vibrio cholera and BAM in the process of separating and identifying Vibrio cholera. Based on the serotype analysis of Vibrio cholera, this research identifies that Vibrio cholera is detected in Penaeusvannamei and prompts consumers that eating such raw shrimps has the risk of infecting Vibrio cholera and that Vibrio parahaemolyticus has greater risks and entails high vigilance. The research of Davis B D et al demonstrates that pathogenic Vibrio can cause human intestinal infection, wound infection and sepsis [7]. Since the occurrence of pathogenic Vibrio is not confined to the marine environment and marine products, the control and monitoring of prompting the pathogenic Vibrio of freshwater aquaculture environment needs to be strengthened. Freshwater aquaculture environment and potential security risks of freshwater products merit attention. This research provides a scientific basis for the risk assessment and effective prevention of pathogenic Vibrio in freshwater products and the control of pathogenic Vibrio. Besides, further virulence gene identification of other pathogenic Vibrio isolates is also in urgent need.

Table 3 Distribution and detection of pathogenic Vibrio of four kinds of shrimps

Sample source	The number of samples	pathogenic Vibrio							total	
		Vibrio parahaemolyticus	non 01 Vibrio cholerae	Vibrio hollisae	Vibrio vulnificus	01 Vibrio cholerae	Vibrio metchnikovi	Vibrio alginolyticus	The number of positive	Positive rate (%)
Penaeusvannamei	85	11	2	0	9	0	0	1	23	27.06
Macrobrachiumrosenbergii	92	12	0	0	1	0	0	0	13	14.13
Macrobrachiumnipponense	98	9	1	1	3	0	0	1	15	15.31
Procambarusclarkia	65	5	1	0	1	0	0	0	7	10.77
Total	340	37	4	1	14	0	0	2	58	17.06

Table 4 Physiological and biochemical responses of five kinds of pathogenic Vibrio

		Vibrio cholerae	Vibrio parahaemolyticus	Vibrio vulnificus	Vibrio hollisae	Vibrio alginolyticus
	Gram stain	-	-	-	-	-
	Driving force	+	+	+	+	+
	Cytochrome oxidase	+	+	+	+	+
	Growth at 42 ℃	+	+	+	nd	+
	The reduction of nitrate to nitrite	+	+	+		+
	Arginine dehydrogenase	-	-	-	-	-
	Lysine decarboxylase	+	+	+	+	+
	ONPG	+	-	+	-	-
Acid production	Glucose	+	d	-	-	d
	Sucrose	+	-	-	-	+
	Cellobiose	-	-	+	-	-
	0%Nacl	+	-	-	-	-
Halophilism	6%Nacl	-	+	+	+	+
	8%Nacl	-	+	-	-	+
	10%Nacl	-	-	-	-	+

Acknowledgment

Research projects in shandong province department of education：Heavy metal toxic effect on the Yellow River carp accumulation rule and research.Issue number：J11LC53

REFERENCES

[1] Chen Ruiying, Lu Jianzhang. *Food Science*. **2007**, 28 :341-347.
[2] US FDA Bacteriological Analytical Manual Online (BAM online), Chapter 3,**2004**
[3] National standard of the People's Republic of China GB/T4789.7-**2008** Microbiological examination of food hygiene--Examination of Vibrio parahaemolyticus P2-5.
[4] Industry standard of the People's Republic of China SN/T 1022-**2010** Detection of Vibrio cholerae in food for import and export.P2-6.
[5]Industry standard of the People's Republic of China SN/T 2564-**2010** Detection of Vibrio cholerae in seafood--MPCR-DHPLC.P2-4.
[6]Han Shanqiao, Yu Jiyao,JiangTao."An analysis of antimicrobial susceptibility and isolation of Pathogenic Vibrio in brine" **2008**,33(4):239-241.
[7]Davis B D,Dulbecco R,Eisen H N,et al.Microbiology[M].4thed.Philadelphia:J.B.Lippincott Company,**1990**:582-587