American Journal of Clinical and Experimental Medicine
2015; 3(5): 260-267
Published online November 9, 2015 (http://www.sciencepublishinggroup.com/j/ajcem)
doi: 10.11648/j.ajcem.20150305.21
ISSN: 2330-8125 (Print); ISSN: 2330-8133 (Online)

Biochemical Differentiation and Molecular Characterization of Biofield Treated *Vibrio parahaemolyticus*

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To cite this article:
Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Sambhu Charan Mondal, Snehasis Jana. Biochemical Differentiation and Molecular Characterization of Biofield Treated *Vibrio parahaemolyticus*. *American Journal of Clinical and Experimental Medicine.* Vol. 3, No. 5, 2015, pp. 260-267. doi: 10.11648/j.ajcem.20150305.21

Abstract: The recent emergence of the *Vibrio parahaemolyticus* (*V. parahaemolyticus)* is a pandemic. For the safety concern of seafood, consumer monitoring of this organism in seafood is very much essential. The current study was undertaken to evaluate the impact of Mr. Trivedi's biofield energy treatment on [ATCC-17802] strain of *V. parahaemolyticus* for its biochemical characteristics, biotype and 16S rDNA analysis. The lyophilized strain of *V. parahaemolyticus* was divided into two parts, Group (Gr.) I: control and Gr. II: treated. Gr. II was further subdivided into two parts, Gr. IIA and Gr. IIB. Gr. IIA was analyzed on day 10, whereas, Gr. IIB was stored and analyzed on day 142 (Study I). After retreatment of Gr. IIB on day 142 (Study II), the sample was divided into three separate tubes. The tubes first, second and third were analyzed on day 5, 10, and 15, respectively. The biochemical reaction and biotyping were performed using automated MicroScan Walk-Away® system. The 16S rDNA sequencing was carried out to correlate the phylogenetic relationship of *V. parahaemolyticus* with other bacterial species after the treatment. The results of biochemical reactions were altered 24.24%, out of thirty-three in the treated groups with respect to the control. Moreover, negative (-) reaction of urea was changed to positive (+) in the revived treated Gr. IIB, Study II on day 15 as compared to the control. Besides, biotype number was substantially changed in all the treated groups as compared to the control. However, change in organisms were reported in Gr. IIA on day 10 and in Gr. IIB; Study II on day 5 as *Shewanella putrefaciens* and *Moraxella/Psychrobacter* spp., respectively with respect to the control *i.e. Vibrio* sp. SF. 16S rDNA analysis showed that the identified sample in this experiment was *V. parahaemolyticus* after biofield treatment, and the nearest homolog genus-species was observed as *Vibrio natriegens* with 98% gene identity. The results envisaged that the biofield energy treatment showed an alteration in biochemical reaction pattern and biotype number on the strain of *V. parahaemolyticus.*

Keywords: *Vibrio parahaemolyticus*, Biofield Energy Treatment, Biochemical Reaction, Biotype, 16S rDNA Analysis

1. Introduction

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a Gram-negative, human-pathogenic halophilic bacterium. The species is a natural inhabitant of the marine environment as part of estuarian microflora and coastal marine waters. The organism can be present in crabs, shrimps, fish, oysters, mussels and other seafood [1-3]. The infections of *V. parahaemolyticus* occur due to the ingestion of contaminated raw or undercooked seafood and are an important cause of gastroenteritis in humans [4,5]. The organism has been recognized as the cause of sporadic cases of gastroenteritis in the coastal areas of the world, especially Japan, because they are very much habitual for the consumption of inadequately cooked seafood [6]. The pathogenicity of *Vibrio* species is due to the massive production of several virulence factors *viz.* enterotoxin, haemolysin, cytotoxin, protease, lipase, phospholipase, siderophore, adhesive factor and/or haemagglutinins [7]. Several studies have reported that the pathogenicity of *V. parahaemolyticus* is conferred by the production of two well-defined hemolysin proteins, *i.e.* thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in humans. Although the mechanism of action of these two proteins at the cellular level is not yet well characterized [6, 8]. Based on

findings of many studies, it was described that the virulent strain of *V. parahaemolyticus* carries either the gene *tdh* or *trh*, or both and transmit through plasmids or insertion elements [9]. Apart from thermostable haemolysin proteins, a thermolabile haemolysin (TLH) encoded with the *tlh* gene is considered as a species-specific marker in the strains of *V. parahaemolyticus* [10]. Based on the biochemical studies, it was reported that the *tdh* gene, marked by a *β*-type haemolysis [11], was correlated to a urease positive test [12], and served as the marker for pathogenic strains of *Vibrio* species.

As *V. parahaemolyticus* is an important foodborne pathogen. To reduce the risk of *V. parahaemolyticus* infection and to ensure the safety aspect of seafood, an alternative strategy can be used. Biofield energy treatment has been known as an alternative approach that may be useful in that concern. Based on the bioplasma concept of consciousness in any living organism, the habitats of bioplasma are protein semiconductors, piezoelectric or organic compounds such as melanin, neuromelanin, melatonin, DNA, RNA, *etc.* [13]. Changing the information disclosed in the electrical profile in living organism and changed the biofield plasma. Bioplasma is the result of the biofield [14]. The electrical current present within the human body in the form of vibratory energy particles and they produce a magnetic field commonly known as biofield [15, 16]. Biofield (putative energy fields) or electromagnetic based energy therapies, used to promote health and healing that had been exclusively reported by National Institute of Health/National Center for Complementary and Alternative Medicine (NIH/NCCAM) [17]. Human has surround the putative energy barrier is known as biofield energy and the process is known as biofield energy treatment. Mr. Trivedi's unique biofield treatment (The Trivedi effect®) has been extensively contributes in scientific communities in several fields [18-21].

Based on the clinical importance of this organism in the field of seafood industry, the work was undertaken to assess the effect of biofield energy treatment on *V. parahaemolyticus* in relation to biochemical characteristics and biotyping followed by 16S rDNA sequencing.

2. Materials and Methods

The strain of *V. parahaemolyticus* was bearing American Type Culture Collection (ATCC) number 17802, was obtained from MicroBioLogics, Inc., USA. Biochemicals used in this study were obtained from Sigma-Aldrich, MA, USA. The MicroScan Walk-Away® (Dade Behring Inc., West Sacramento, CA, USA) with Negative Breakpoint Combo 30 (NBPC 30) panel was used for analysis of biochemical reactions and biotype number. The 16S rDNA sequencing analysis was performed using Ultrapure Genomic DNA Prep Kit; Cat KT 83 (Bangalore Genei, India).

2.1. Experimental Design

For the assessment of the effects of biofield energy treatment on *V. parahaemolyticus* the study was designed as follows -

Group I: ATCC strain in the lyophilized state was considered as control. No treatment was given to it and analyzed for biochemical reactions and biotype number as per the standard protocol.

Group II: The lyophilized state of ATCC strain was divided into two parts named as Gr. IIA and Gr. IIB. Both the groups of ATCC strain of *V. parahaemolyticus* in the lyophilized state were assigned to Mr. Trivedi's unique biofield treatment (first treatment). Gr. IIA was analyzed on day 10 while Gr. IIB sample was stored in the lyophilized state for 142 days at -70ºC. Gr. IIB was further sub-divided in two separate parts named as Gr. IIB - Study I and Gr. IIB - Study II.

Group IIB - Study I

After 142 days, biochemical reactions pattern and biotyping were performed as per the standard protocol.

Group IIB - Study II

The stored strain was revived from -70ºC and the revived culture was again provided to Mr. Trivedi's biofield treatment (re-treatment) on day 142. After biofield retreatment, the sample was sub-cultured into three separate tubes and each sample on was analyzed on day 5, 10, and 15.

2.2. Biofield Treatment Strategy

The lyophilized (Gr. IIA) sample of *V. parahaemolyticus* was subjected to Mr. Trivedi's biofield treatment followed by retreatment after storing for 142 days in revived state (Gr. IIB, Study II). The samples assigned to treatment were received Mr. Trivedi's biofield energy treatment through his unique way of energy transmission process from the short distance without touch the test item. Treated samples were assessed for biochemical reactions and biotyping as per experimental design. While handing over these cultures to Mr. Trivedi for retreatment purposes, optimum precautions were taken to avoid contamination.

2.3. Study of Biochemical Reactions

Biochemical reactions of *V. parahaemolyticus* were determined using MicroScan Walk-Away®, system with NBPC 30 panel. The system contains a photometric or fluorogenic reader. On the basis of nature of bacilli (*i.e.* Gram-negative), computerized reports were generated using the conventional panel, which utilizes the photometric reader. The panel was allowed to equilibrate to room temperature before rehydration. All opened panels were used on the day. The tests were carried out with miniaturized of the broth dilution that has been dehydrated. Concisely, 0.1 mL of the standardized suspension of *V. parahaemolyticus* cultured cells were taken into 25 mL of inoculum water using pluronic and inverted 8 to 10 times and inoculated, rehydrated, and then subjected to incubation for 16 hours at 35°C. After that, rehydration and followed by inoculation were performed using the RENOK® system with inoculators-D (B1013-4). Approximately 25 mL of standardized inoculum suspension was poured into the inoculum tray. Before commencing the experiment, the NBPC 30 panel was first incubated and read on the system. After evaluating the experimental reading on

the system, the NBPC 30 panel was removed from the system and recorded on the Biomic system within 1 hour. The instrument consists of a database associated with collective information, which was required to identify the microbes with respect to group, genera, or species of the family. The detailed experimental procedure was followed as per manufacturer-recommended instructions [22].

2.4. Biotyping for Identification of Organism

The biotype number of *V. parahaemolyticus* was determined on MicroScan Walk-Away® processed panel data report with the help of biochemical reactions data. The similar experimental procedure was followed for identification of biotype number as described in biochemical reaction study, and as per manufacturer-recommended instructions [22].

2.5. Amplification and Gene Sequencing of 16S rDNA

DNA was made from *V. parahaemolyticus* cells (Gr. IIA, sample coded as 1A) using genomic DNA purification kit (Cat KT 83, Bangalore Genei, India), as per manufacturer information. The DNA was resuspended in tris-ethylene diamine tetra acetic acid (TE), (10 mM Tris-HCl, 100 mM EDTA, pH 7.8) buffer and stored at −20°C. The quantity of genomic DNA was then measured at 260 nm with the help of a spectrophotometer. The concentration of DNA was adjusted approximately to 25 ng/μL using distilled deionized water for polymerase chain reaction (PCR). 16S rDNA gene (~1.5 kb) fragment was amplified with the help of high-fidelity PCR using universal primers; forward-5′-AGAGTTTGATCCTGGCTCAG-3′ and reverse-3′-ACGGTCATACCTTGTTACGACTT-5′. The PCR assay was done at 94°C for 5 min followed by cycles of 94°C for 1 min, 63°C for 1 min and 72°C for 2 min in a DNA thermal cycler [23]. The PCR fragments of amplified DNA were subjected to gel electrophoresis on a 1.0% agarose gel, stained with ethidium bromide and visualized under UV light in a gel doc apparatus (BioRad, USA). The PCR amplified fragment was then purified from the agarose gel using DNA gel extraction kit. Sequencing of amplified product was performed on a commercial basis from Bangalore Genei, India. The obtained 16S rDNA sequences were aligned and compared with the stored sequences in GenBank database available from National Center for Biotechnology Information (NCBI) with the help of algorithm BLASTn program. Based on the multiple sequence of alignment a phylogenetic tree was established using MEGA3.1 molecular software [24].

3. Results

3.1. Study of Biochemical Reactions

The biochemical reactions data of biofield treated *V. parahaemolyticus* obtained from MicroScan Walk-Away® system are presented in Table 1. Identification of the enzymatic and metabolic behaviors of microbes can be obtained through a series of biochemical reactions. The microbes can be categorically differentiated based on the utilization of specific biochemicals as nutrients during the process of enzymatic or metabolic reactions. The conventional biochemical tests are necessary for the differentiation of *V. parahaemolyticus*. The biochemical reactions study of *V. parahaemolyticus* was performed using thirty-three biochemicals. The negative (-) reactions of arabinose (ARA), inositol (INO), and raffinose (RAF) in control sample were changed to positive (+) reactions in revived treated group (Gr. IIB; Study II) on day 10, while unchanged *i.e.* negative (-) in rest of the treated groups. In similarly way, the negative (-) reactions of untreated *V. parahaemolyticus* in glucose (GLU), penicillin (P4), and urea (URE) in control sample were converted into positive (+) reactions in revived treated group (Gr. IIB; Study II) on day 15, after retreatment on day 142, while unchanged *i.e.* negative (-) reactions in rest of the treated groups. The biochemical data of indole (IND) was changed from positive (+) to negative (-) reaction in all the biofield treated groups, except in Gr. IIB; Study II on day 10 after retreatment on day 142, with respect to the control (Gr. I). Moreover, the biochemical reaction pattern of nitrate (NIT) showed positive (+) reaction in the lyophilized treated group (Gr. IIA) on day 10, while again converted into negative (-) in rest of the treated groups as compared to the control (Table 1). Overall, eight out of thirty-three *i.e.* 24.24% biochemical reactions were changed in biofield energy treated *V. parahaemolyticus* sample as compared to the untreated strain. The rest of the biochemicals did not report any type of alteration in treated group at any time-point with respect to the control group (Table 1).

Table 1. *Biochemical and morphological characterization of biofield treated Vibrio parahaemolyticus (ATCC 17802).*

S. No.	Code	Biochemical	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 142)	Gr. IIB (Study II; 142)		
						Day 5	Day 10	Day 15
1.	ACE	Acetamide	-	-	-	-	-	-
2.	ADO	Adonitol	-	-	-	-	-	-
3.	ARA	Arabinose	-	-	-	-	+	-
4.	ARG	Arginine	-	-	-	-	-	-
5.	CET	Cetrimide	-	-	-	-	-	-
6.	CF8	Cephalothin	-	-	-	-	-	-
7.	CIT	Citrate	-	-	-	-	-	-
8.	CL4	Colistin	-	-	-	-	-	-
9.	ESC	Esculin hydrolysis	-	-	-	-	-	-
10.	FD64	Nitrofurantoin	-	-	-	-	-	-
11.	GLU	Glucose	-	-	-	-	-	+
12.	H_2S	Hydrogen sulfide	-	-	-	-	-	-

S. No.	Code	Biochemical	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 142)	Gr. IIB (Study II; 142)		
						Day 5	Day 10	Day 15
13.	IND	Indole	+	-	-	-	+	-
14.	INO	Inositol	-	-	-	-	+	-
15.	K4	Kanamycin	-	-	-	-	-	-
16.	LYS	Lysine	-	-	-	-	-	-
17.	MAL	Malonate	-	-	-	-	-	-
18.	MEL	Melibiose	-	-	-	-	-	-
19.	NIT	Nitrate	-	+	-	-	-	-
20.	OF/G	Oxidation-fermentation/glucose	-	-	-	-	-	-
21.	ONPG	Galactosidase	-	-	-	-	-	-
22.	ORN	Ornithine	-	-	-	-	-	-
23.	OXI	Oxidase	+	+	+	+	+	+
24.	P4	Penicillin	-	-	-	-	-	+
25.	RAF	Raffinose	-	-	-	-	+	-
26.	RHA	Rhamnose	-	-	-	-	-	-
27.	SOR	Sorbitol	-	-	-	-	-	-
28.	SUC	Sucrose	-	-	-	-	-	-
29.	TAR	Tartrate	-	-	-	-	-	-
30.	TDA	Tryptophan deaminase	-	-	-	-	-	-
31.	TO4	Tobramycin	-	-	-	-	-	-
32.	URE	Urea	-	-	-	-	-	+
33.	VP	Voges-Proskauer	-	-	-	-	-	-

-, (negative); +, (positive); Gr.: Group

3.2. Biotyping for Identification of Organism

Based on the utilization of specific nutrients (*i.e.* biochemicals) for catabolic or anabolic activities, *V. parahaemolyticus* showed different types of reactions. Biotyping was obtained based on the interpretation of biochemical reaction pattern of biofield energy treated *V. parahaemolyticus* on specific biochemicals. Then, the biotype number can be used for the identification of the specific organism. In this experiment, biotyping was performed using automated systems, and the processed panel data are shown in Table 2.

Table 2. *Effect of biofield treatment on biotype number of Vibrio parahaemolyticus.*

Feature	Gr. I (Control)	Gr. IIA (Day 10)	Gr. IIB (Study I; Day 142)	Gr. IIB (Study II; 142)		
				Day 5	Day 10	Day 15
Biotype	1000 0002	0000 0006	0000 0002	0000 0002	1000 0402	4004 0001
Organism Identification	*Vibrio* sp. SF	*Shewanella putrefaciens*	*Vibrio* sp. SF	*Moraxella/Psychrobacter* spp.	*Vibrio* sp. SF	*Vibrio parahaemolyticus*

Gr.: Group

The biotype number of biofield treated *V. parahaemolyticus* was changed in all the treated groups as compared to the control. The biotype numbers with new organisms were observed in Gr. IIA, on day 10 (0000 0006 → *Shewanella putrefaciens*) and in Gr. IIB; Study II, on day 5 (0000 0002 → *Moraxella/Psychrobacter* spp.) with respect to the control (1000 0002 → *Vibrio* sp. SF). The rest of the treated groups of *V. parahaemolyticus* did not exhibit any change, either genus or species as compared to the control (Table 2).

3.3. 16S rDNA Genotyping

The microorganisms that are poorly differentiated by conventional methods and to contribute better characterization, need molecular analysis method such as 16S rDNA sequence [25]. Thus, the molecular method was used in this experiment that may be useful in the monitoring of ATCC strain of *V. parahaemolyticus*. This molecular-based technique is a suitable tool for identification of most of the bacteria on their genus and/or species level by comparison with databases in the public domain. As because of, most of the bacteria have small ribosomal subunit with their species-specific variability [26]. The 16S rDNA sequence was determined in *V. parahaemolyticus* on Gr. IIA sample. The alignment and comparison of the gene sequences were performed with the help of stored sequences in GenBank database available from NCBI using the algorithm BLASTn program. Based on nucleotide homology and phylogenetic analysis the microbe (Sample 1A) was detected as *V. parahaemolyticus* (GenBank Accession Number: BA000032). The nearest homolog genus-species of *V. parahaemolyticus* was found as *Vibrio natriegens* (Accession No. AJ874352) with 98% similarity. Some other closely homologs of *V. parahaemolyticus* were to be found from the alignment as shown in Table 3. The distance matrix based on nucleotide sequence homology data are presented in Table 4. The phylogenetic tree was established using BLAST-Webpage (NCBI). According to Fig. 1, ten different related bacterial species of *V. parahaemolyticus* were selected as

Operational Taxonomic Units (OTUs) to establish the phylogenetic relationship of *V. parahaemolyticus*. About 1511 base nucleotides of 16S rDNA gene sequences analyzed, and multiple alignments were constructed using ClustalW in MEGA3.1. The numbers of each base substitutions per site from pairwise distance analysis between sequences are illustrate in Table 3. All results were based on the pairwise analysis of 11 sequences. According to the data in Table 4, the lowest value of genetic distance from *P. vulgaris* was 0.005 base substitutions per site. This value is due to the distance between *V. parahaemolyticus* and *Vibrio natriegens*. P-distance method was used to correlate the pairwise distance analysis using MEGA3.1 software. The value presented in the Table 3 are programmed into Fig. 1 with the help of optimal bootstrap consensus tree. In the phylogram, there were eleven OTUs. The results suggested that *Vibrio haemolyticus* was found to be closely related to the *Vibrio natriegens* with genetic distance 0.005 base substitutions per site (Fig. 1).

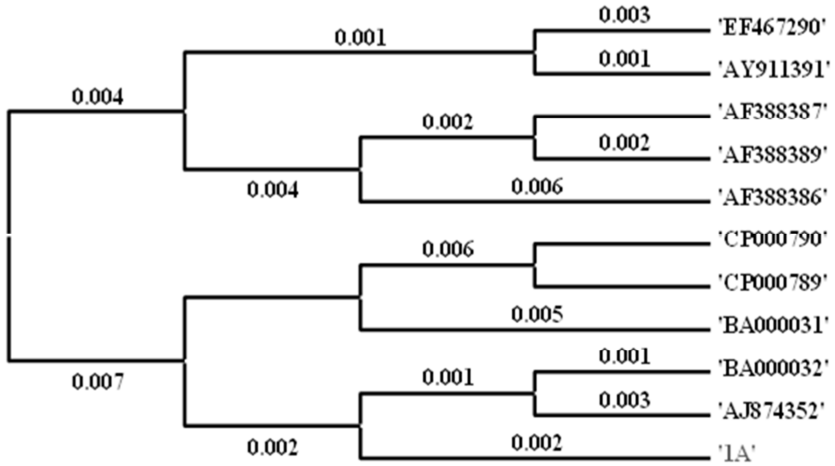

Figure 1. *Phylogenetic tree of the partial 16S rDNA gene sequencing of Vibrio parahaemolyticus using MEGA 3.1 software using neighbor joining method. Numbers represent GenBank accession number.*

Table 3. *The closest sequences of Vibrio parahaemolyticus from sequence alignment using NCBI GenBank and ribosomal database project (RDP).*

Alignment View	AN	Alignment Results	Sequence Description
	1A	0.96	Sample studied
	BA000031	0.97	*Vibrio parahaemolyticus* RIMD 2210633
	BA000032	0.97	*Vibrio parahaemolyticus* RIMD 2210633
	CP000790	0.99	*Vibrio harveyi*
	CP000789	0.99	*Vibrio harveyi*
	AY911391	0.97	*Vibrio parahaemolyticus* strain MP-2
	EF467290	0.97	*Vibrio parahaemolyticus*
	AJ874352	0.97	*Vibrio natriegens* strain 01/097
	AF388387	0.96	*Vibrio parahaemolyticus*
	AF388389	0.95	*Vibrio parahaemolyticus*
	AF388386	0.96	*Vibrio parahaemolyticus*

AN: GenBank Accession Number

Table 4. *Distance matrix of Vibrio parahaemolyticus sample based on nucleotide sequence homology (using kimura-2 parameter).*

Distance Matrix												
AN		1	2	3	4	5	6	7	8	9	10	11
CP000790	1	—	0.978	0.990	0.989	0.999	0.976	0.990	0.974	0.972	0.981	0.990
EF467290	2	0.022	—	0.983	0.983	0.979	0.990	0.980	0.988	0.986	0.997	0.982
BA000032	3	0.010	0.018	—	0.997	0.990	0.979	0.992	0.977	0.975	0.984	0.997
AJ874352	4	0.011	0.018	0.003	—	0.990	0.978	0.990	0.975	0.973	0.982	0.995
CP000789	5	0.001	0.021	0.010	0.010	—	0.975	0.989	0.973	0.971	0.980	0.989
AF388387	6	0.024	0.010	0.021	0.022	0.025	—	0.978	0.998	0.992	0.992	0.980
BA000031	7	0.010	0.020	0.008	0.010	0.011	0.022	—	0.976	0.974	0.983	0.992
AF388389	8	0.026	0.012	0.023	0.025	0.027	0.002	0.024	—	0.990	0.990	0.978
AF388386	9	0.028	0.014	0.025	0.027	0.029	0.008	0.026	0.010	—	0.988	0.975
AY911391	10	0.019	0.003	0.016	0.018	0.020	0.008	0.017	0.010	0.012	—	0.985
1A	11	0.010	0.018	0.003	0.005	0.011	0.020	0.008	0.022	0.025	0.015	—

AN: GenBank Accession Number

4. Discussion

Identification of *Vibrios* species on the basis of biochemical reactions are pose difficult due to their atypical type of reactions pattern [27]. Ben Kahla-Nakbi *et al.* in 2007 reported that most of the *Vibrios* species are indole (IND) negative. Although in this experiment, the biochemical data of IND showed positive (+) which was corroborated with another literature mentioned that the production of IND is variable. Apart from few biochemicals most of the biochemical reactions were well matched with the literature data [7]. Based on several reports that not all the coastal strains are pathogenic to humans due to the production of various haemolysin proteins, only 1-2% strains are TDH positive [28,29]. The negative (-) reaction of urea (URE) in the control strain of *V. parahaemolyticus* was corroborated with literature, while some researchers were reported that the positive reaction of URE biochemical reaction directly correlated with the specific gene *tdh* innervated with the TDH protein molecule [7,11-12]. If we look the biochemical reaction of URE it was positive (+) after long-term assessment point after second-time biofield treatment on day 15 as compared to the control. Consequently, it is assumed that the positive biochemical reaction of URE may be due to the act on the genetic level after biofield energy treatment on *V. parahaemolyticus*. Moreover, the positive reaction of galactosidases (ONPG) indicated the identifiable characteristics feature of *V. parahaemolyticus* rather than sugar fermentation process *i.e.* sucrose [30]. Identification of *V. parahaemolyticus* with the help of PCR-based 16S rDNA technique is more efficient, reliable and faster in comparison with the conventional biochemical reaction pattern analysis [23]. Based on literature *toxR* gene is specific for identification of *V. parahaemolyticus* genome profile. However, it does not produce any virulence property but it regulates the expression of virulence factor genes [31]. Here, based on nucleotides homology and phylogenetic analysis the microbe (Sample: 1A) was detected as *Vibrio parahaemolyticus* (GenBank Accession Number: BA000032) and the nearest homolog species was found to be *Vibrio natriegens* (Accession No. AJ874352) with 97% similarity. Some researcher had found the nearest homologous species of the *toxR* gene was *V. cholerae* with 52% identity [5].

Based on the phenotypic and genotypic differences the strains can be identified. The process of differentiating is known as 'typing'. The same species that have very small differences between them can be differentiated by additional methods so called biotyping. These species are then subdivided based on their special characteristic features, percent probability, and rare typing into subspecies, subgroups, serotypes, variants, *etc*. Most of the seafood from the coastal region of Southeast Asia have a high risk for the presence of *V. parahaemolyticus* approximately 20 - 70% [32, 33]. The hot and salty marine water is the good contributor for the growth and burden of *V. parahaemolyticus*. Biofield treatment could be responsible for the change in biochemical reaction followed by biotyping due to the alteration of enzymes activities or genetic make-up, which may act on particular receptor domain. The manifestation of different phenotypic characteristics may be due to the alteration of receptor-ligand conformation or their signaling cascade [34].

Even though, *V. parahaemolyticus* is widely abundant in the environment of the coastal region seafood all over the world. The peoples those who are depending on seafood have to take precaution that this seafood should cook properly. Although, the most of the microbes did not show their pathogenicity to humans. Based on the findings of a biochemical reaction, biotyping followed by molecular analysis, it was observed that there was an impact of Mr. Trivedi's biofield treatment on the ATCC strain of *V. parahaemolyticus*. So far our group had been documented several scientific evidences regarding effects of biofield treatment on ATCC strain [19]. Based on these results, it is expected that biofield treatment has the scope to be an alternative approach than the existing antimicrobial therapy in near future.

5. Conclusions

Based on study outcome, the biofield energy treated *V. parahaemolyticus* showed 24.24% alteration in biochemical reaction pattern with respect to the control. Moreover, the biotype number of *V. parahaemolyticus* was changed in all the treated groups analyzed at different time-points as compared with the control. Apart from biotype number, the new organisms were observed as *Shewanella putrefaciens* and *Moraxella/Psychrobacter* spp., in the treated Gr. IIA, on day 10 and in Gr. IIB; Study II, on day 5, respectively as compared to the control *i.e. Vibrio* sp. SF. Molecular technique based on 16S rDNA analysis showed that the treated sample in this experiment was detected as *V. parahaemolyticus*. While, the nearest homolog genus-species was reported as *Vibrio natriegens* with 98% similarity. Based on these results, it seems that Mr. Trivedi's unique biofield treatment could be utilized as an alternate therapeutic window concurrent with other existing drug therapy in the field of seafood industry in the near future.

Abbreviations

ATCC: American Type Culture Collection; NBPC 30: Negative Breakpoint Combo 30; MIC: Minimum Inhibitory Concentration; OTUs: Operational Taxonomic Units; NCBI: National Center for Biotechnology Information; MEGA: Molecular Evolutionary Genetics Analysis

Acknowledgements

Financial assistance from Trivedi science, Trivedi testimonials and Trivedi master wellness is gratefully

acknowledged. We also are grateful to the whole team from PD Hinduja National Hospital and MRC, Mumbai, Microbiology Lab for their support.

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