

Identification the Presence of *Vibrio* Species by TCBS Media in Different Water Samples Collected from Different Locations

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Abstract: The purpose of this study was to identify the presence of *Vibrio* species in the water samples collected from different locations of Himachal Pradesh, Gujarat and Uttar Pradesh. About 30 water samples were collected from different locations. *Vibrio* species were identified by using TCBS agar medium. Sucrose included as a fermentable carbohydrate, with the help pH indicator (Bromothymol blue and Thymol blue indicators) allows in distinguishing bacteria with yellow and green colour colonies on plates. Out of 30 samples, 22 samples showing positive results for *Vibrio* species. Identification of *Vibrio* species in different types of aquatic habitats (Sea water, River water, tap water, pond water and Ground water) was done.

Keywords: *Vibrio*, Isolation, TCBS, Water samples

1. Introduction

Vibrio is a genus of Gram-negative bacteria that possess a curved rod shape and naturally inhabits aquatic environments worldwide [1-4]. Within the genus *Vibrio*, several species are known to be important human pathogens [5]. Among these, *Vibrio cholerae*, *Vibrio vulnificus*, and *Vibrio parahaemolyticus* are the major pathogenic *Vibrio* species. *V. cholerae* and *V. parahaemolyticus*, contracted through consumption of contaminated seafood and seawater, can cause gastroenteritis whereas *V. vulnificus* can cause septicemia by exposure of an open wound to seawater or consumption of contaminated seafood [6].

The genus *Vibrio*, within the family Vibrionaceae, is a diverse group of Gram-negative bacteria found exclusively in the aquatic environment. Important pathogenic members include *Vibrio cholerae*, the causative agent of cholera, and *Vibrio parahaemolyticus* and *Vibrio vulnificus*, which have been implicated in diarrhea, septicemia, and wound infections [7]. There is several water borne diseases which are highly pathogenic to humans. Among all, *Vibrio* plays an important role. The most common disease (cholera) caused by *Vibrio*

TCBS agar is commercially available and easy to prepare, requires no autoclaving, and is highly differential and selective. However, it has a relatively short shelf life once prepared (3 to 5 days) unless plates are carefully protected against drying. TCBS is subject to lot-to-lot and brand-to-brand variations in selectivity, and growth on this medium is not suitable for direct testing with *V. cholerae* O1 antisera. TCBS agar is green when prepared. Overnight growth (18 to 24 hours) of *V. cholerae* will produce large (2 to 4 mm in diameter), slightly flattened, yellow colonies with opaque centers and translucent peripheries. The

yellow color is caused by the fermentation of sucrose in the medium. Sucrose non-fermenting organisms, such as *V. parahaemolyticus*, produce green to blue-green colonies. Suspicious colonies for further testing should be sub-cultured to a non-inhibitory medium, such as gelatin agar, heart infusion agar (HIA), Kligler's iron agar (KIA), or triple sugar iron agar (TSI). [8]

2. Material and Methods

2.1 Collection of Water Sample

30 samples of water (Salt and Sweet) were collected from different water bodies like Pond Water, River water, Tap Water, etc. from Himachal, Gujarat, and Uttar Pradesh (India).

2.2 Isolation on TCBS agar medium

Isolation of 30 strains of *Vibrio* spp. were done from water samples collected from different environment. It was isolated by using TCBS agar medium. Sucrose included as a fermentable carbohydrate, with the help pH indicator (Bromothymol blue and Thymol blue indicators) allows in distinguishing bacteria with yellow and green colored colonies on plates. Yellow colonies indicate those *Vibrio* spp. (*Vibrio cholerae*, *Vibrio alginolyticus*, *Vibrio furnisci*, *Vibrio mimicus*) which ferment sucrose and green color colony (*Vibrio parahaemolyticus*, *Vibrio vulnificus*) indicated as a non-sucrose fermenting bacteria. For isolation of *Vibrio* species 200 µl of different water sample was taken for spreading onto TCBS (selective media) agar plates using L-shape glass rod and left for incubation for 48 hours incubation period at 37°C for development of the *Vibrio* colonies.

Table 1: List of water samples

S. No	Sample	Location /Address	Location Id
1	Sea water	Dahej sea port, Disst-Bharuch, Gujarat	SW
2	Pond water	Disst-Bharuch, Gujarat	PW
3	River water	Disst-Bharuch, Gujarat	RW
4	River water	Pung River, Vill-Saud, P.O-Jol Lambri, Tehsil-Sujanpur, Distt.-Hamirpur(H.P)	PR-1
5	River water		SKTC
6	River Water	Tehsil-Sujanpur, Distt.-Hamirpur, H.P	VR
7	Ground Water (Bawadi)		BNVR
8	Ground Water (Naun)		NNFS
9	Ground Water (Naun)	Vill-Deenge de gran, P.O-Patlander, Tehsil-Sujanpur, Distt-Hamirpur, H.P.	DDGN
10	River Water (Pung River)	Vill-Bhugol, P.O-Patlander, Tehsil-Sujanpur, Distt-Hamirpur, H.P.	PR-2
11	River Water		PRWT
12	Ground Water (Naun)	V.P.O-jol Lambri, Tehsil-Sujanpur, Distt-Hamirpur, H.P.	JLN
13	River Water (Pung River)	Village-Chamiyola,P.O-Chauri, Tehsil-Sujanpur, Distt-Hamirpur, H.P.	KMPR
14	River Water (Pung River)	Village-Chamiyola,P.O-Chauri, Tehsil-Sujanpur, Distt-Hamirpur, H.P.	KMPH
15	Ground water	Gram panchyat – Kyarwan, Distt-Kangra	BJT
16	Water Reservoir	Water Reservoir Sample, Ankleshwar, Gujarat	WR
17	Tap Water	Tap Water, Axar Nagar, Bharuch, Gujarat	TW
18	River Water	Narmada River sample near neelkanth mahadev temple, Bharuch Gujarat	NR
19	River Water	Narmada River sample near Kabir Badd, Bharuch , Gujarat	KB
20	Pond Water	Pond water sample collected from Village-Bhensali, BHARUCH, GUJARAT	BV
21	Tap water	Ajamgarh, Lucknow	ATW
22	Tap water	Jankipuram, Lucknow	JTW
23	River Water	Allahabad	GR
24	River Water	Lucknow	GO
25	Tap water	Lucknow	SN
26	Tap water	Lucknow	GN
27	River Water	Lucknow	GHR
28	Pond water	Lucknow	MP
29	Pond water	Lucknow	MOL
30	Pond water	Lucknow	TP

3. Result and Discussion

3.1 Isolation of *Vibrio* spp. from water sample on TCBS media-

Green colonies and yellow colonies of *Vibrio* organism were observed. On TCBS, yellow colonies were presumptively identified as *V. alginolyticus*, *V. cholerae*, and *V. mimicus*. Green or blue-green colonies were assumed to be *V. parahaemolyticus*, and *V. vulnificus*. A total of 22 water samples were found positive for *Vibrio* species out of 30 water sample.



Figure 2: Location: KMPH



Figure 1: Location: BJT

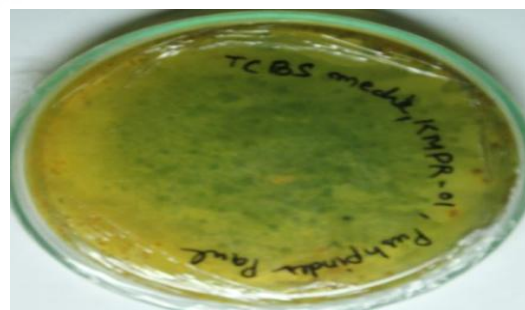


Figure 3: Location: KMPR

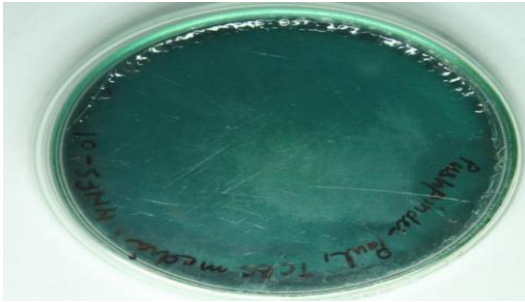


Figure 4: Location: NNFS

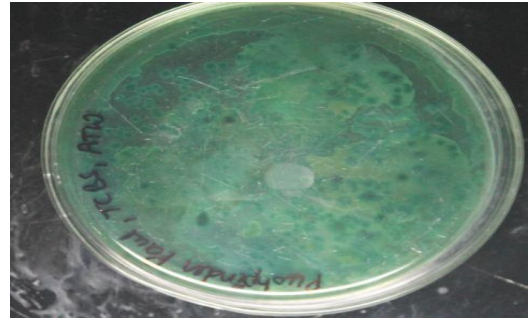


Figure 9: Location: ATW



Figure 5: Location: PR1

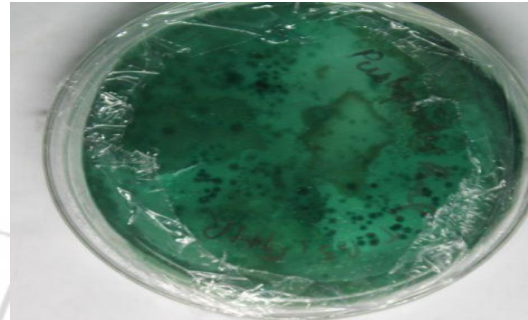


Figure 10: Location: GHR

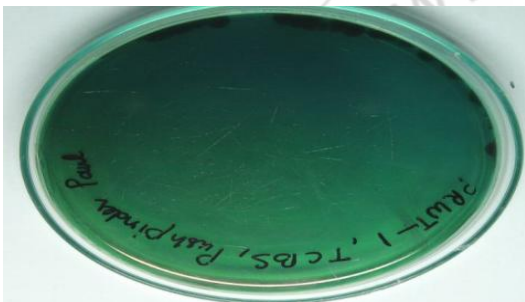


Figure 6: Location: PRWT

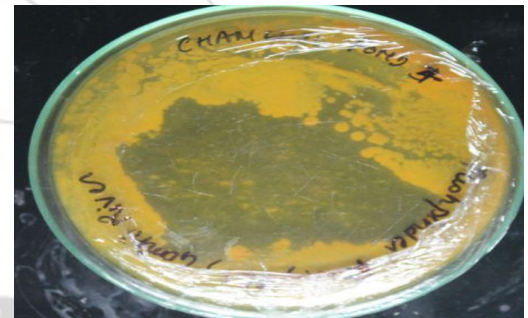


Figure 11: Location: GO

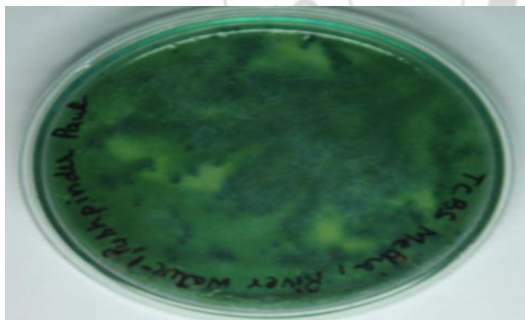


Figure 7: Location: R

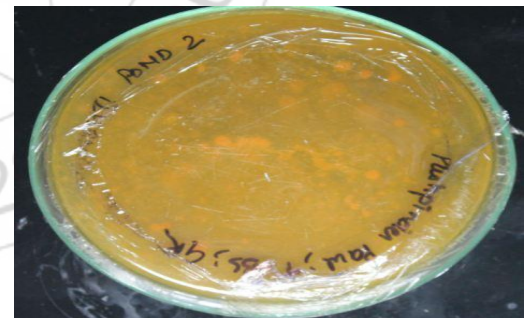


Figure 12: Location: GR



Figure 8: Location: SKTC



Figure 13: Location: GTW

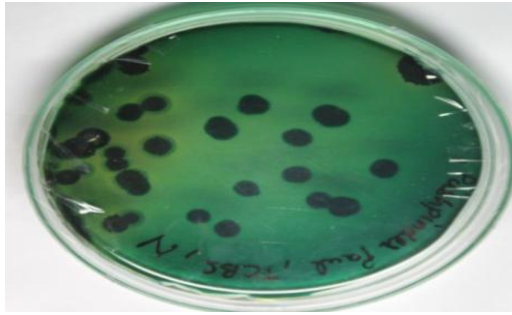


Figure 14: Location: N

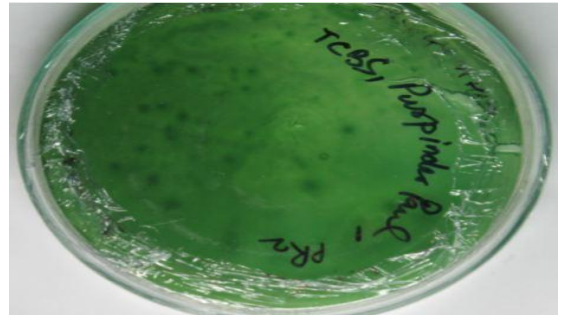


Figure 19: Location: PR2

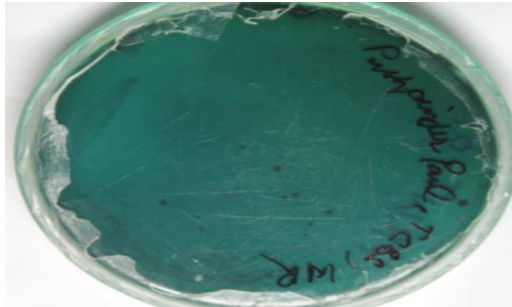


Figure 15: Location: WR

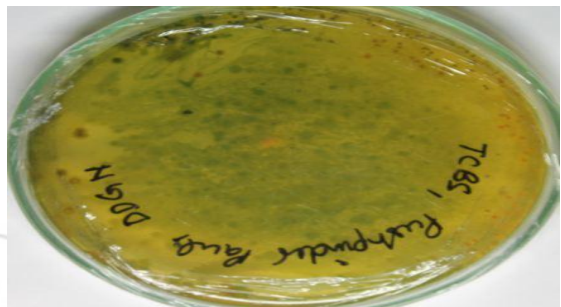


Figure 20: Location: DDGN

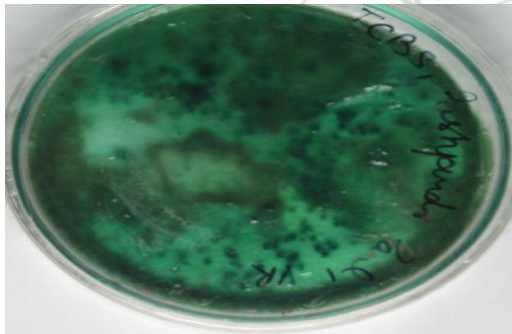


Figure 16: Location: VR

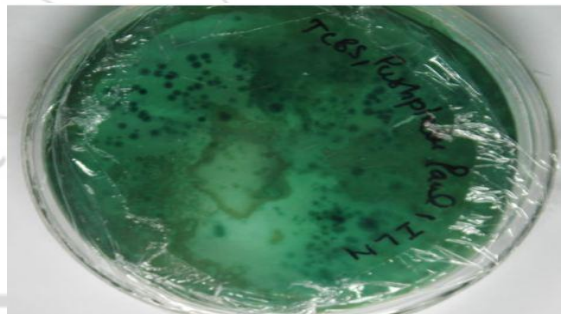


Figure 21: Location: ILN

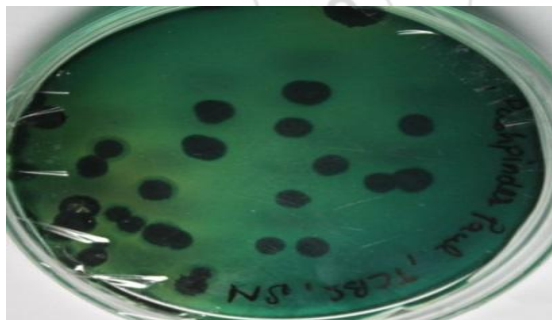


Figure 17: Location: SN

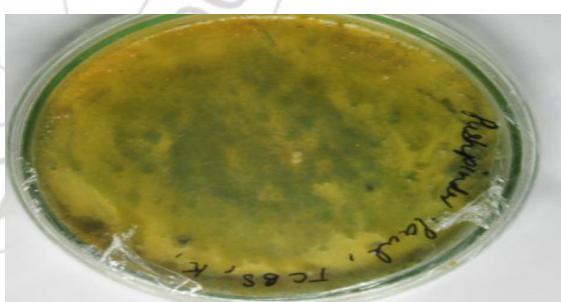


Figure 22: Location: K

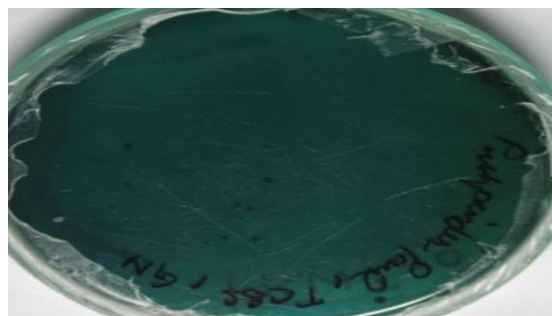


Figure 18: Location: GN

4. Conclusions

For identification of *Vibrio* species, we collected total 30 water samples from different locations of Himachal Pradesh, Gujarat and Uttar Pradesh. Our result showed that 73% of water samples (n=22) had the presence of *Vibrio* species whereas, 27% of water samples (n=08) showed negative i.e. absent of test organisms on TCBS Media. With respect to the observations made in the present study most literature reported a similar results [9] ranged fall between 21.4% - 99.6% showed positive samples on TCBS agar plate media for *Vibrio* species. 22 no samples were identified for the presence of *Vibrio* species contamination at different location.

References

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Author Profile



Pushpinder Paul received the M.Sc. degree in Biotechnology from Punjabi University, Patiala in 2008. From 2009 to till date he has worked with various pharmaceutical companies. His core area is sterile operations and validations.

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