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Isolation of Vibrio Species on TSA Media and Comparision of Growth Percentage of Vibrio Species in Sea Water, Pond Water, Tap Water, Ground Water and River Water Samples

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Abstract: ***The purpose of this study was to isolate the single colonies of Vibrio species from TCBS media to TSA media. After stereaking of single colonies on TSA media, petriplates were incubated for 24 hrs at 37°C. After incubation period growth of Vibrio species on TSA plates were observed and also cross verify with TCBS plates. The entire samples showing same results in both (TCBS and TSA) media for Vibrio species. Identification of Vibrio species in different types of aquatic habitates (Sea water, River water, tap water, pond water and Ground water) and their percentage of growth was identified.***

Keywords: Vibrio species, TSA, TCBS, Isolation etc.

1. Introduction

Vibrio is a genus of Gram-negative bacteria that possess a curved rod shape and naturally inhabits aquatic environments worldwide [1]. Within the genus *Vibrio*, several species are known to be important human pathogens.

Three species *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*, are well-documented human pathogens [2] is a recognized pathogen with similar characteristics to *V. cholerae*, except an ability to ferment sucrose. Other species within the genus, such as *V. alginolyticus***,** *V. fluvialis* **,** *V. furnissii V. metschnikovii* and *V. hollisae* are occasional human pathogens. *Vibrio* species account for a significant proportion of human infections from the consumption of raw or undercooked shellfish**.**

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 [3].

Vibrio species are gram-negative and largely halophilic. Although, depending on their sodium chloride requirements a few species are nonhalophilic also. Most of the species are also oxidase-positive. Most of them are sensitive to acidic pH, while tolerant to alkaline pH. As pathogenic organisms, the CDC estimates that there are 8,000 infections and 60 deaths each year that are the result of *Vibrio* infections (Centre for disease control) [4].

The *Vibrio* organisms are heterotrophic, obtaining nutrients from their mutualistic, parasitic, or pathogenic relationships with other organisms. *Vibrio* reproduces through asexual division. Many of the *Vibrio* species form relationships with animals. Some of them are mutualistic, as in the case of relationship between Vibrio and fish. *V. fischeri* is a well-known example for such type of relationships. One of the most interesting fact of the relationship between *V. fischeri* and its host (usually a squid) is that the host expells 90% of the *V. fischeri* each day [5].

Toxigenic *Vibrio cholerae* serogroup O141 has been associated with sporadic cholera-like diarrhea and bloodstream infection in the United States. Consumption of seafood and proximity to the coast may increase the risk of infection. All *V. cholerae* isolates recovered from stool samples of patients with diarrhea or from a normally sterile site should be serogrouped and assessed for cholera toxin production. Improved surveillance and case-control studies are needed to further characterize illness and risk factors for *V. cholerae* O141 infection [6].

2. Materials and Methods

2.1 Trypticase soy agar (TSA)

Over development of green and yellow colonies on TCBS plates, single colonies were taken from TCBS media plate and streaked onto Trypticase Soy Agar (TSA) plates for obtaining discrete colonies after the incubation of 24 h at 37^0C. After incubation time all the plates were checked for confirmation of growth observed in TCBS media.

3. Result and Discussion

Collected total 30 water samples for presumptive and culturable 5 different *Vibrio* species (i.e., growing on TCBS specific media) from different area of Himachal Pradesh, Gujarat and Uttar Pradesh. Random colonies from TCBS plates were streaked on TSA media for confirmation of obtained results.

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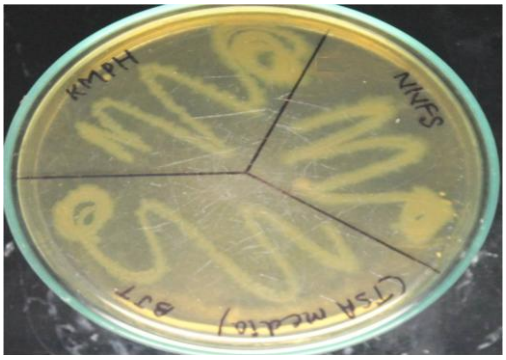

Figure 1: Locations: BJT, KMPH, NNFS
Colony taken for streaking: Green color

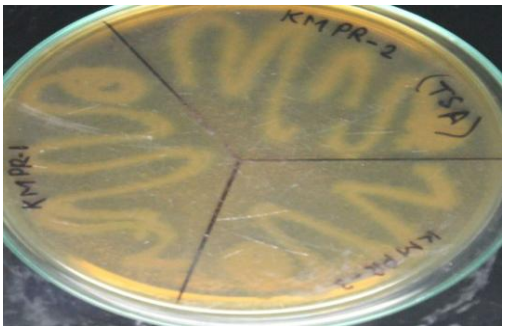

Figure 2: Locations: KMPR (1, 2, And 3)
Colony taken for streaking: Green & Yellow color

Figure 3 Locations: PR1, PRW, GN
Colony taken for streaking: Green color

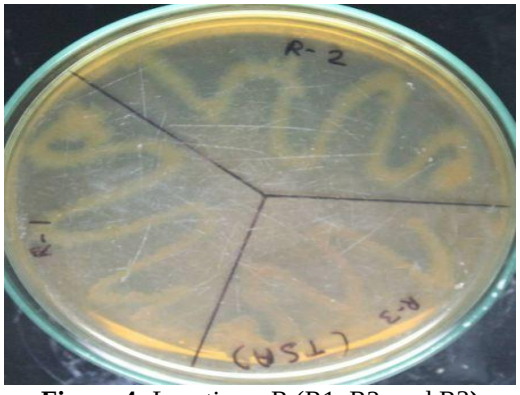

Figure 4: Locations: R (R1, R2, and R3**)**
Colony taken for streaking: Green color

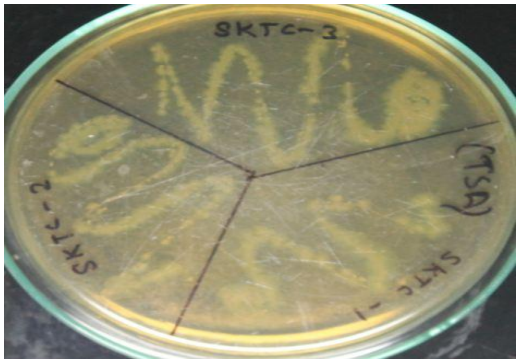

Figure 5: Locations: SKTC (1, 2 and **3)**
Colony taken for streaking: Green color

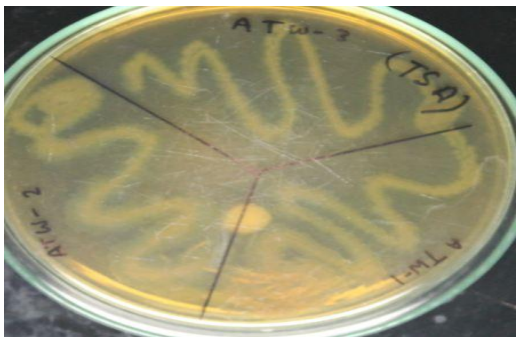

Figure 6: Locations: ATW (1, 2 and **3)**
Colony taken for streaking: Green color

Figure 7: Locations: GHR (1, 2 and 3)
Colony taken for streaking: Green color

Figure 8: Locations: GO (1, 2 and 3)
Colony taken for streaking: Yellow color

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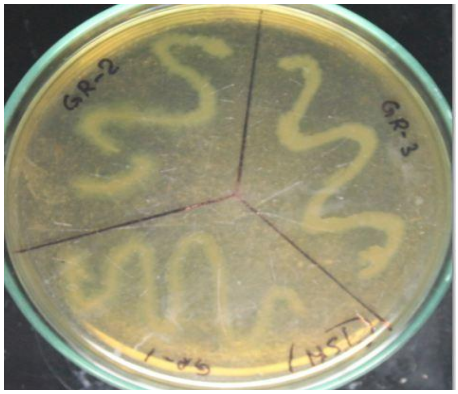

Figure 9: Locations: GR (1, 2 and 3)
Colony taken for streaking: Yellow color

Figure 13: Locations: SN (1, 2 and 3)
Colony taken for streaking: Green color

Figure 10: Locations: JTW (1, 2 and 3)
Colony taken for streaking: Yellow color

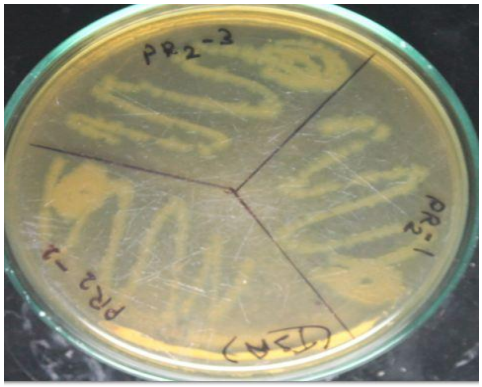

Figure 14: Locations: PR2 (1, 2 and 3)
Colony taken for streaking: Green color

Figure 11: Locations: N (1, 2 and 3)
Colony taken for streaking: Green color

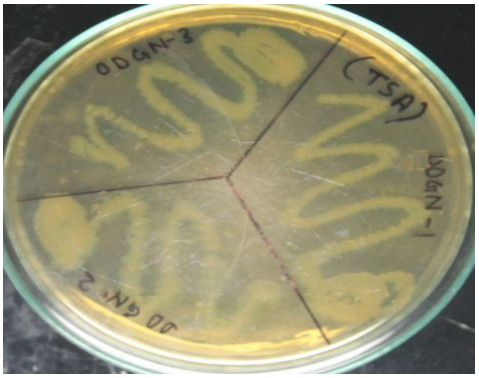

Figure 15: Locations: DDGN (1, 2 and 3)
Colony taken for streaking: Green & Yellow color

Figure 12: Locations: WR (1, 2 and 3)
Colony taken for streaking: Green color

Figure 16: Locations: ILN (1, 2 and 3)
Colony taken for streaking: Green color

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Figure 17: Locations: K (1, 2 and 3)
Colony taken for streaking: Green & Yellow color

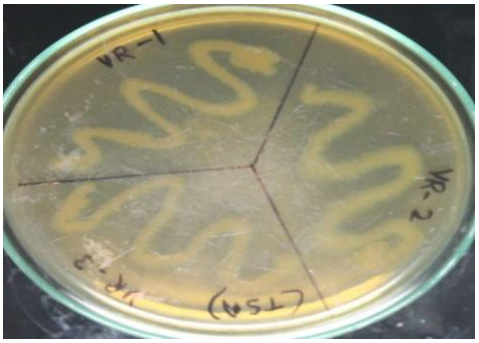

Figure 18: Locations: VR (1, 2 and 3)
Colony taken for streaking: Green Color

Table 1: Results of TCBS & TSA

S. No	*Type of sample*	*Collection Place*	*TCBS Media*	*TSA Media*
1	Ground Water (Natural Resource)	Hamirpur (H.P)	+	+
2	River Water	Hamirpur (H.P)	+	+
3	Ground Water (Natural Resource)	Hamirpur (H.P)	+	+
4	Ground Water (Natural Resource)	Hamirpur (H.P)	+	+
5	River Water	Hamirpur (H.P)	+	+
6	River Water	Hamirpur (H.P)	+	+
7	River Water	Hamirpur (H.P)	+	+
8	Ground Water (Natural Resource)	Hamirpur (H.P)	-	-
9	River Water	Hamirpur (H.P)	+	+
10	River Water	Hamirpur (H.P)	+	+
11	Ground Water (Natural Resource)	Kangra (H.P)	+	+
12	River Water	Hamirpur (H.P)	+	+
13	Sea Water	Bharuch (Gujarat)	-	-
14	Pond Water	Bharuch (Gujarat)	-	-
15	River Water	Bharuch (Gujarat)	+	+
16	River Water	Bharuch (Gujarat)	+	+
17	Tap Water	Bharuch (Gujarat)	-	-
18	Pond Water	Bharuch (Gujarat)	-	-
19	Pond Water	Ankleshwar (Gujarat)	+	+
20	River Water	Bharuch (Gujarat)	+	+
21	Tap Water	Ajamgarh	+	+
22	Tap Water	Jankipuram (Lucknow)	+	+
23	River Water	Kanpur	+	+
24	River Water	Gomti nagar (Lucknow)	+	+
25	Tap Water	Lucknow	+	+
26	Tap Water	Lucknow	+	+
27	River Water	Lucknow	+	+
28	Pond Water	PGI (Lucknow)	-	-
29	Pond Water	Mohanlalganj (Lucknow)	-	-
30	Pond Water	Telebagh (Lucknow)	-	-

From the above 30 sample results it shows that total 06 samples from pond water, 05samples from tap water, 01 samples from sea water, 05 samples from ground water(Natural resources) and 13 samples from river were collected for isolation and identification of Vibrio species. From 30 samples, some showing positive results and some showing negative results for Vibrio species with TCBS media.All the samples showing the same results while single colonies of Vibrio species were isolated from TCBS media and streaked on TSA media. It confirms the earlier results shown with TCBS media.

Out of 06 pond water sample, 01 no. shows positive results and 05 no. shows negative results.From tap water samples, 04 no. shows positive results and 01 no. shows negative results.From river water samples, all samples showing positive results for availability of Vibrio species.Sea water sample showing negative results for Vibrio specie. From ground water samples (Natural resource) 04 no. shows positive results and 01 no. shows negative results. The percentage of Vibrio species contamination in River water, Tap water and Ground water was found more than in Pond water and sea water. However variation observed in the results of water samples collcted from similar source but different location. Pond water sample collected from Gujarat showing some contamination of Vibrio species however pond water sample of Lucknow showing negative results.Similarly Tap water sample of Bharuch showing negative results of Vibrio species however Tap water sample of Lucknow showing positive resuts.However there was similarity found in all the results of river water collected from Gujarat, Lucknow and Himachal Pradesh locations.

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Table 2: %age of Vibrio Species

Water sample	*Location of sample*	*Total samples*	*+Ve*	*-Ve*	*Percentage of Vibrio species*	*Overall Percentage of Vibrio species*
Pond Water	Bharuch (Gujarat)	3	1	2	33.33%	16.66%
	Lucknow (UP)	3	0	3	0%	
Tap Water	Bharuch (Gujarat)	1	0	1	0%	80%
	Lucknow (UP)	4	4	0	100%	
River Water	Bharuch (Gujarat)	3	3	0	100%	100%
	Lucknow (UP)	3	3	0	100%	
	Himachal Pradesh	7	7	0	100%	
Sea Water	Dahej (Gujarat)	1	0	1	0%	0%
Ground Water	Himachal Pradesh	5	4	1	80%	80%

4. Conclusion

From the above study it was clear that Vibrio species growth on TCBS media and TSA media was same.It was also found that sea water sample showing absence of Vibrio species however river water sample showing positive growth.Percentage of Vibrio contamination in River water, Tap water and Ground water was found much higher than Pond water and sea water.Also variation observed in the results of some water samples collcted from similar source but different location (Tap water and Pond water). However there was similarity found in all the results of river water collected from Gujarat, Lucknow and Himachal Pradesh locations.

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Author Profile

Pushpinder Paul received the M.Sc. degree in Biotechnolgy 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. He is ISO 9001-2015 certified QMS Lead auditor from IRCA. He is PhD research scholar at Singhania University, Rajasthan.

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