



Isolation and Identification of *Vibrio* Spp from Different Dye Industry Effluent in Salem District Tamilnadu

Dr. V. Kalaiselvi*

Department of Microbiology, Sriekailash Women's College, Periyar University, Salem, Tamilnadu, India
Email: k9894450198@gmail.com

P. Umamagesh

Department of Microbiology, Sriekailash Women's College, Periyar University, Salem, Tamilnadu, India

D. Ammu

Department of Microbiology, Sriekailash Women's College, Periyar University, Salem, Tamilnadu, India

R. Latha

Department of Microbiology, Sriekailash Women's College, Periyar University, Salem, Tamilnadu, India

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Abstract

Studies were carried out on the Different type of vibrio spp, isolated from dye industry effluent. Among the 90 strains of vibrio spp isolated from effluents of textile industries, three showed optimize at different factors such as pH, Temperature, salinity, carbone source, nitrogen source etc.

Keywords: Vibrio spp; Dye effluent; Pollution; Public health; Optimization etc.

1. Introduction

Vibrios are highly abundant in aquatic environments, including estuaries, marine coastal waters and sediments, dye industry and aquaculture settings worldwide. *Vibrios* (*Vibrionaceae* strains) belong to Gamma proteobacteria, are gram negative, usually motile rods, are mesophilic and chemoorganotrophic, have a facultative fermentative metabolism, and are found in aquatic habitats and in association with eukaryotes.

They are generally able to grow on the selective medium thiosulfate-citrate-bile salt-sucrose agar (TCBS) and are mostly oxidase positive. The 74 species of this group are distributed among four different families, i.e., *Enterovibrionaceae*, *Photobacteriaceae*, *Salinivibrionaceae* and *Vibrionaceae*. Two new genera, i.e., *Enterovibrio norvegicus* and *Grimontia hollisae* and 20 novel species, i.e., *Enterovibrio coralii*, *Photobacterium eurosenbergii*, *V. brasiliensis*, *V. chagasii*, *V. corallilyticus*, *V. crassostreae*, *V. fortis*, *V. gallicus*, *V. hepatarius*, *V. hispanicus*, *V. kanaloaei*, *V. neonatus*, *V. neptunius*, *V. pomeroyi*, *V. pacinii*, *V. rotiferianus*, *V. superstes*, *V. tasmaniensis*, *V. ezurae* and *V. xuii* have been described in the last few years.

In recent years, molecular techniques have been used for analysis of intraspecies genetic diversity among *Vibrio* spp., for examples, lipopolysaccharides typing, total protein profiling (sodium dodecyl-sulfate-polyacrilamide gel electrophoresis), DNA sequencing, plasmid profiling, pulsed field gel electrophoresis, amplified-fragment length polymorphisms (AFLP) and randomly amplified polymorphic DNA (RAPD). Of these methods, RAPD has been widely used for typing both Gram-positive and Gram-negative bacteria [1-4] and more specifically *Vibrio* spp. [5, 6].

2. Materials and Methods

2.1. Sample Collection

The present study was carried out in different effluent samples of dye industry Salem district. Collected samples were transferred aseptically into sterile containers for microbiological analysis.

2.2. Composition of TCBS (Thiosulfate Citrate Bile Salt Sucrose) Agar

Sucrose- 20.0g, Sodium chloride-10.0g, Sodium citrate -10.0g, Na₂S₂O₃ -10.0g, Yeast Extract - 5.0g, Pancreatic Digest of Casein - 5.0g, Peptic digest of Animal Tissue - 5.0

Oxgall - 3.0g, Sodium Chololate - 3.0g, Ferric citrate - 1.0g, Thymol Blue - 0.04g,

Bromothymol blue - 0.04g, pH- 8.4, Agar- 14.0g Distilled water - 1000ml.

2.3. Growth Optimization

To find out growth optimization parameters *Vibrio* strains alone were selected and grown in nutrient broth (i.e.,) pH 3-12 with the interval of 2.0, salt (NaCl) concentration from 0.5- 4.0 % with the interval of 0.5 % and temperature from 30°C - 60°C with the interval of 10°C. Culture flasks were kept in a water bath shaker at 150rpm for 48 hrs. Growth was measured at 600 nm in a UV Spectrophotometer.

*Corresponding Author

3. Results

3.1. Distribution of Vibrios

Totally 90 strains were isolated and identified up to species level using standard manuals. Among 63 strains 42 strains were found to be *Vibrio parahemolyticus*, 37 strains belonged to *V. cholerae* and 10 strains belonged to *V. vulnificus*. Among the 90 strains samples have the maximum number of *Vibrio parahemolyticus*, *Vibrio vulnificus* and very less number of *Vibrio cholerae*. The samples have large number of *Vibrio parahemolyticus*, *Vibrio vulnificus* and few *Vibrio cholerae*, *Vibrio cholerae* followed by *Vibrio parahemolyticus*.

Picture-1. Biochemical identification of Vibrios *Vibrio parahemolyticus*



Vibrio spp

Table-1. Biochemical identification of *Vibrio parahemolyticus*

S.no	Tests	<i>Vibrio parahemolyticus</i>
1	Hydrogen sulphide(TSI agar)	-
2	Urea hydrolyzed	+
3	Indole	+
4	Methyl Red	-
5	Voges- proskauer	-
6	Citrate	+
7	Catalase	+
8	Motility	+
9	Lactose	+
10	Maltose	+
11	Grams reaction	-
12	Oxidase	+
13	Nitrogen reduction	+
14	Simmons citrate	+
15	Urea broth	+

Vibrio cholera

Table-2. Biochemical identification of *Vibrio cholerae*

S.no	Tests	<i>Vibrio cholerae</i>
1	Hydrogen sulphide(TSI agar)	-
2	Urea hydrolyzed	+
3	Indole	+
4	Methyl Red	+
5	Voges- proskauer	+
6	Citrate	+
7	Catalase	+
8	Motility	-
9	Lactose	+
10	Maltose	+
11	Grams reaction	-
12	Oxidase	-
13	Nitrogen reduction	+
14	Simmons citrate	+
15	Urea broth	+

Vibrio vulnificus

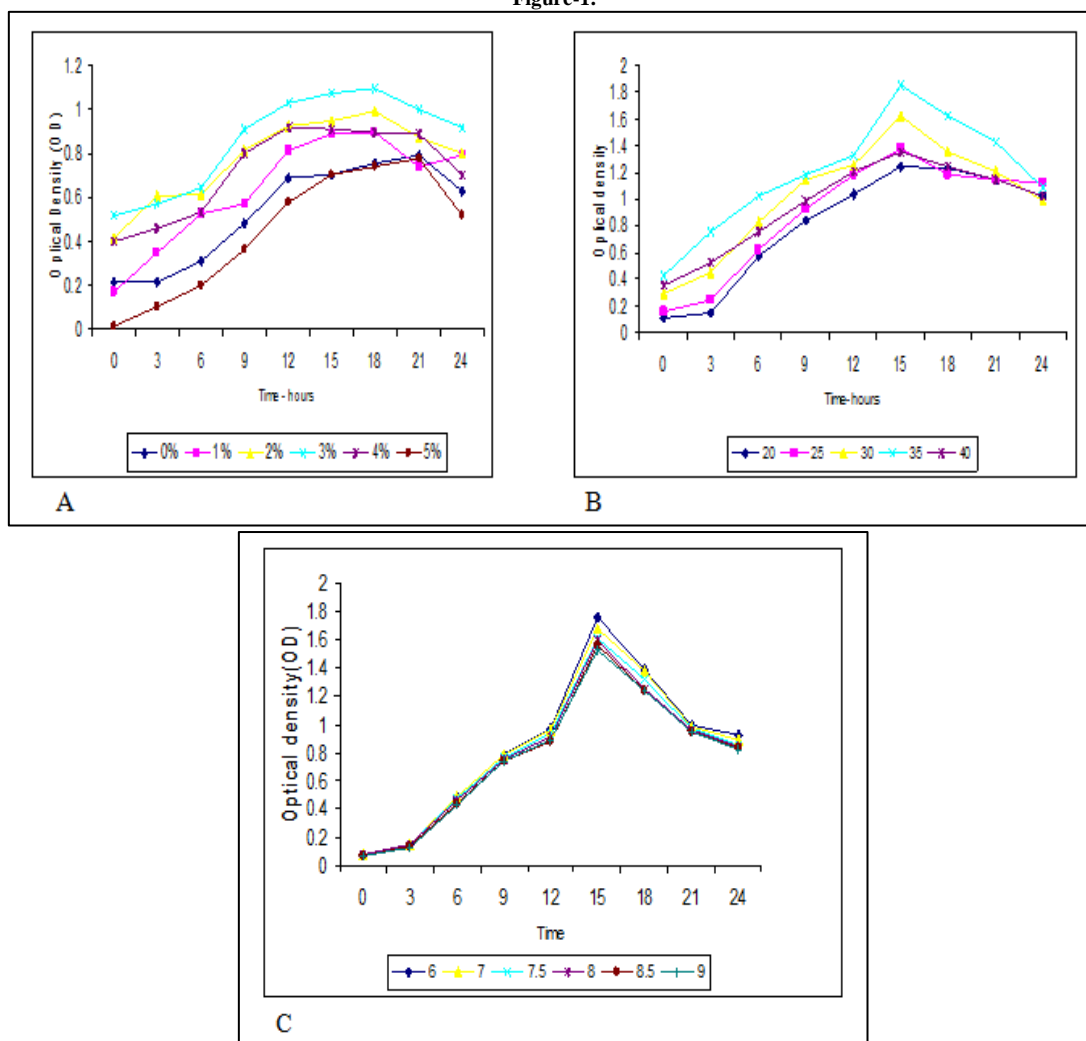
Table-3. Biochemical identification of *Vibrio vulnificus*

S.no	Tests	<i>Vibrio vulnificus</i>
1	Hydrogen sulphide(TSI agar)	-
2	Urea hydrolyzed	+
3	Indole	+
4	Methyl Red	+
5	Voges- proskauer	-
6	Citrate	+
7	Catalase	+
8	Motility	+
9	Lactose	+
10	Maltose	+
11	Grams reaction	-
12	Oxidase	+
13	Nitrogen reduction	+
14	Simmons citrate	+
15	Urea broth	+

3.2. Factors Influencing the Growth Study of Vibrios

Vibrio cholerae selected from samples, and the effect of pH 3-12 with the interval of 2.0 was also carried out. In this present study the effect of pH was varied between the samples. *Vibrio cholerae*, it was observed that the OD was maximum (2.498) at pH 8.5 and the minimum was 0.081 at pH 6.0. Effect of temperature 20- 50⁰C with the interval of 5.0⁰C was also carried out. *Vibrio cholerae*, it was observed that the maximum was 1.856 at 35⁰C and the minimum was 0.108at 20⁰C. Salt concentration 0- 5% with the interval of 1 % was also carried out. Observed that the maximum was 2.498 at 4 % of salt concentration and the minimum was 0.241 at 1% salt concentration.

Figure-1.



- A. The average Growth rate of *Vibrio cholerae* at different salinity
- B. The average Growth rate of *Vibrio cholerae* at different temperature
- C. Average Growth rate of *Vibrio cholerae* at different pH

4. Discussion

The detection and quantification and identification of pathogenic bacteria is one of the most challenging problems in environmental microbiology and has consequently earned increasing attention of microbiologist in recent years. The present study with the study of *Vibrio* spp. In different dye effluent samples. Results of the present study showed that the ubiquitous *Vibrios* are present in all the sampling sites of varied nature. *Vibrio* population level was found to be more or less constant throughout the study except in dye effluent. Okeyo et al., 2018 have reported that the *Vibrio* Species in Wastewater Final Effluents and Receiving Watershed, Implications for Public Health. Thompson and Jean [7], were supported for *Vibrios* are ubiquitous and abundant in the aquatic environment. Isolated 74 species of this group are distributed among four different families. Peele et al., 1981 have reported that the water receiving pollutant from pharmaceutical industries showed dominance of *Vibrio* sp. The influence of salinity on the survival of a halophilic bacterium like *Vibrio* was also documented by Kaneko and Colwell [8] and Martin [9], Froelich and Noble [10]. The influence of salinity on the survival of a low temperature suitable for *vibrio* spp growth.

5. Summary and Conclusion

The primary goal of the present study was to find out the distribution and population level of *Vibrio* spp. in dye industry effluent samples were collected and analyzed. Totally 90 strains were isolated from samples and they were identified up to special level species like *V. parahemolyticus*, *V. cholerae* and *V. vulnificus* were found in all the samples. *V. cholerae* from each station were selected for growth study to identify the physicochemical factors (pH, temperature and salinity) influencing their survival. In the present study *V. cholerae* present more in dye industry effluent samples and the physicochemical factors also influencing the *V. cholerae* growth patterns.

On the whole, present data sustains a high abundance of *Vibrio* in dye industry effluent. The fact that this environment offers an ideal environment for *Vibrios* is reflected in the abundance of the organism and the frequency of isolation. Based on the present study it is hereby concluded that there is an urgent need to make a thorough survey on the distribution of *Vibrio*. From this survey one can get the occurrence of *Vibrio* and sudden change of environmental condition and environmental living things which influences the distribution of *Vibrios*.

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