

# Isolation and Screening of potential Exopolysaccharide producing Bacteria from Marine Ecosystem of Gujarat

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### Abstract:

In recent studies many bacteria in the marine environment secrete Exopolysaccharides (EPSs), which comprise a substantial component of the extracellular polymers surrounding bacterial cells. In recent years, the increasing demand for natural polymers for pharmaceutical, food and other industrial applications has led to a remarkable interest in EPSs produced by marine bacteria. Gujarat contains 1600 km long costal area, which is very diverse in concern with bacterial diversity. Soil sampling was done from various coastal areas of Gujarat. Isolation and Screening of exopolysaccharide producing bacteria were carried out in Glucose Yeast Extract Broth with Glucose as a carbon source. Congo red Plate assay was carried out to check the nature of Exopolysaccharide. On the basis of Colonial and Morphological characteristics, total 57 different bacterial isolates were obtained in primary screening. Among these 10 good EPS producers were selected in Secondary screening and their EPS quantification was done by using Acetone as a solvent. In future, the EPS production will be optimized and their applications will be studied.

### Introduction:

EPSs are high molecular weight polymers composed of saccharides subunits and are secreted by a microorganism into the surrounding environment. Microorganisms synthesize large spectrum multifunctional polysaccharides including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides (EPSs). Exopolysaccharides generally consist of monosaccharide and some non carbohydrate substituents (such as protein, nucleic acids, lipids, acetate, pyruvate, succinate, and phosphate). Microbial EPS plays an important task in interaction between bacteria and their environment. More than 70% of the surface of the planet is covered by sea water, which contains an exceptional biological diversity, representing more than 95% of the whole biosphere. The deep sea is, therefore, the largest ecosystem of our planet: about 50% of the surface of the planet is below that 3000 m depth. However, it is one of the less studied. Marine bacteria are commonly found in intertidal zones, deep seas and extreme places, such as hydrothermal sources or polar seas. Despite the incredible capacity of marine bacteria to develop different adaptation mechanisms, including the synthesis of EPS, not many in-depth studies have been conducted yet. Thus, the oceans are an open and very promising research field for EPS discovery

### Aims and Objectives:

Collection of Samples from various sites of costal areas of Gujarat in form of sediment and water.  
Isolation of Exopolysaccharide producing bacteria  
Primary and Secondary screening of bacteria  
Quantification of different Isolates using Acetone.

### Materials and Methods:

- Collection of Soil and Sediments samples nearby Seashore in sterile container and sterile zipper plastic bag from marine Ecosystem of Gujarat.
- Acclimatization of collected sample into Glucose yeast extract broth with different NaCl salt concentration.
- Isolation of Marine Bacteria using different media.(Zobell Marine agar, Glucose Yeast extract Agar with 3.5% NaCl)
- Screening of Exopolysaccharides producing bacteria.
- Selection of best Isolates by Congo red agar plate assay.
- Phenotypic Characterization of Best Isolates.
- Quantification of EPS by Precipitation method with Acetone

### Results and Discussion:

Total 57 isolates were isolated from various sites of costal areas of Gujarat. On the basis of colony morphology 12 isolates were selected as best isolates for further study.  
**Figure 1.1: Best Isolates on Glucose Yeast Extract Agar with 3.5% NaCl**



Table 1.1: Morphological Characteristics of the Best Isolates

Colony Morphology	VHP-1	VHP-22	VHP-24	VHP-28	VHP-29	VHP-33	VHP-34	VHP-36	VHP-38	VHP-39	VHP-45	VHP-46
SIZE	Small	Small	Small	Small	Small	Small	Small	Small	Small	Small	Small	Small
SHAPE	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
EDGE	Wavy	Entire	Wavy	Wavy	Entire	Entire	Entire	Wavy	Entire	Wavy	Entire	Entire
ELEVATION	Raised	Raised	Convex	Convex	Flat	Convex	Convex	Raised	Convex	Raised	Convex	Convex
SURFACE	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
CONSISTANCY	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous	Viscous
OPTICAL	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Translucent	Opaque	Translucent
PIGMENT	Red	-	-	-	-	-	-	-	-	-	-	-

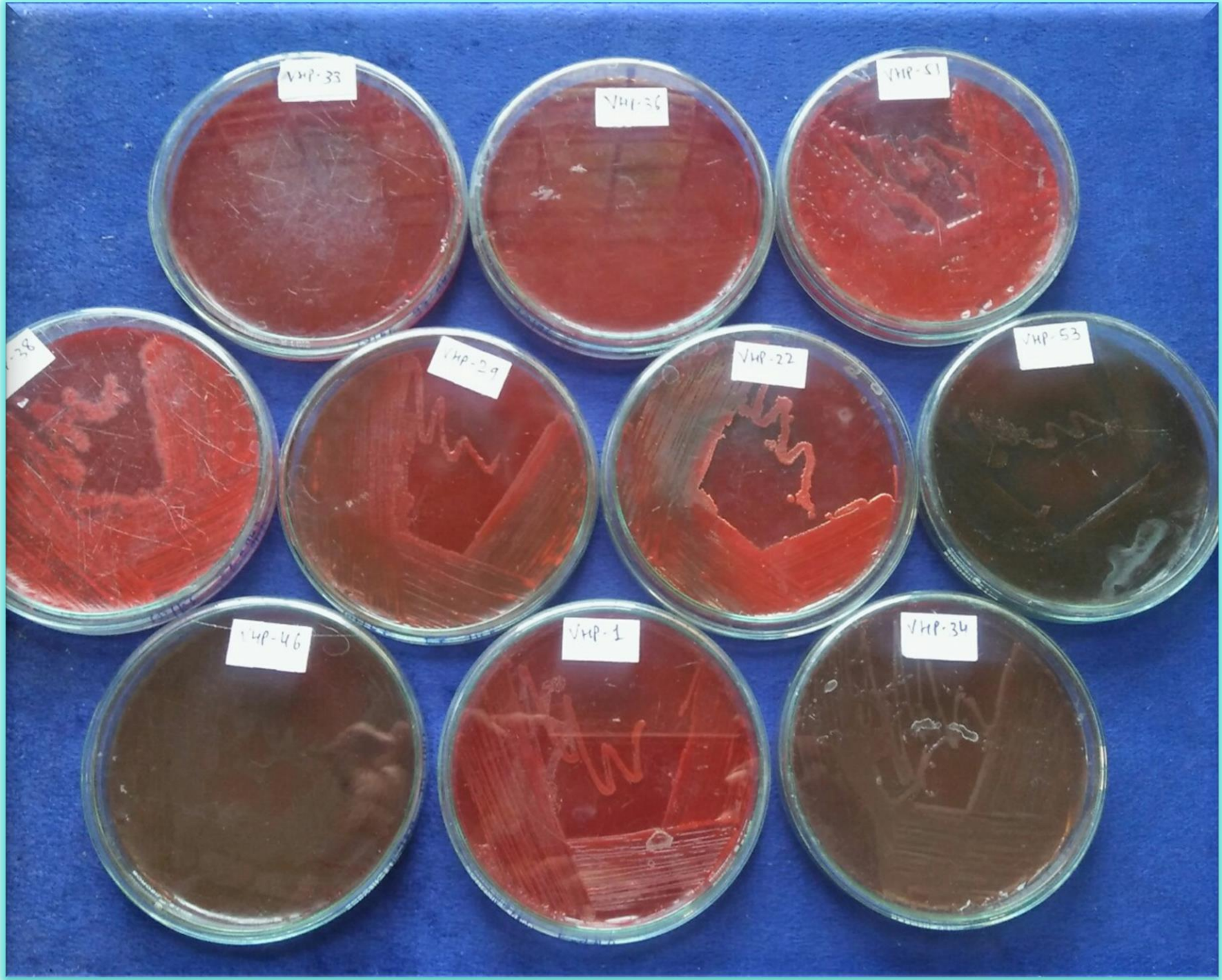
Table 1.1 shows Colony Characteristics of different potential Exopolysaccharide producing Bacteria isolated from different Costal sites of Gujarat. Furthermore Biochemical characterization done including different Biochemical Tests showed in Table 1.2

Table 1.2: Biochemical Characterization of the Best Isolates

BIOCHEMICAL TEST	VHP-1	VHP-22	VHP-24	VHP-28	VHP-29	VHP-33	VHP-34	VHP-36	VHP-38	VHP-39	VHP-45	VHP-46
MR	-ve	-ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
VP	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
Amylase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Lipase	-ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
Protease	+ve	+ve	-ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Gelatinase	-ve	+ve	-ve	-ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
Citrate Permease	-ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve

Further analysis has done to check the nature of Exopolysaccharide by Congo red Agar Assay. In this assay we can get to know that EPS has Acidic or Alkali nature. Glucose yeast extract with NaCl is incorporated with 0.08% Congo red dye. After 24 hrs incubation out of 12 isolates 10 isolates have given good result on Congo red Agar. Total 3 isolates with Black colored colony on Congo red Agar shows positive indication for Biofilm formation.

Figure 1.2: Congo Red Agar Assay



Finally Quantification was done by Acetone solvent with 1:2 volume Broth and Solvent ratio. At 72 h Broth was withdrawn and centrifuged at 10000 rpm for 20 min. After centrifugation supernatant was taken into centrifuge tube and double volume of acetone was added. Naturally present extracellular polysaccharide precipitated in solvent. Then it was filtered with Pre-weighted filter paper. On the following day dry Filter paper's weight was considered for the final calculation of EPS produced.

Table 1.3: Quantification of EPS of the Best Isolates

Isolate	EPS in Mg/100 ml
VHP-1	37.5
VHP-22	38.8
VHP-24	19.6
VHP-29	33.9
VHP-33	34.9
VHP-34	43.3
VHP-36	32.3
VHP-38	40.0
VHP-46	30.0
VHP-51	37.7
VHP-53	31.6

### Conclusion:

From the performed set of an experiment it can be concluded that VHP-34 is the most potential EPS producing bacteria. Further study on this bacteria will be done as part of the future planning.

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