

PATHOGENIC *VIBRIO ALGINOLYTICUS* STRAINS FROM BATHING WATERS OF CONERO RIVIERA, ITALY

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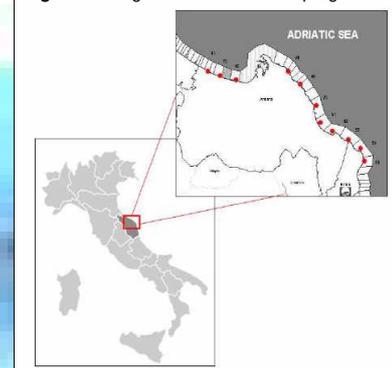
INTRODUCTION

Vibrio alginolyticus is the prevalent species in the marine environment and it has been recognized as pathogenic to both human and marine animals. In humans most of the clinical isolates of *V. alginolyticus* have been isolated from patients with extraintestinal infections (otitis or cellulitis) and this organism has rarely been reported as a cause of acute gastroenteritis. In marine organisms *V. alginolyticus* has been associated with lethality, septicemias, ascites and ulcers, exophthalmia and corneal opacities. The aim of this work was to investigate the potential pathogenicity of *V. alginolyticus* isolates from bathing waters along the Conero Riviera (Adriatic Sea, Central Italy)

MATERIALS AND METHODS

During the 2004-2005 bathing season from April to September, water was monthly sampled at eleven locations (Figure 1). Seawater temperatures were measured in situ with a digital thermometer. Using the membrane filter method, *Vibrio* strains were isolated on thiosulfate-citrate-bile salts-sucrose (TCBS) agar and *V. alginolyticus* was confirmed by using biochemical standardized protocol (Ottaviani et al., 2003). All *V. alginolyticus* isolates were tested for cytotoxicity on Vero cells, elongations on CHO cells, protease, lipase, elastase, gelatinase urease, haemolytic activity by conventional methods (Masini et al., 2007); *ctx*, *tdh* and *trh* genes by PCR (Ottaviani et al., 2005; Koch et al., 2001). Moreover, all strains were tested for the virulence on mouse, by intraperitoneally inoculum of bacterial cells extract in ethanol with 1% acetic acid (Matsumura, 1995).

Figure 1: Geographical location of sampling sites



RESULTS

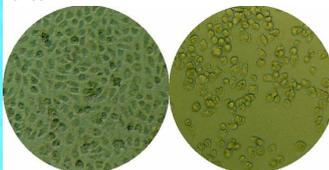
57 *V. alginolyticus* strains were isolated in the period June-September, when water temperature exceeded 20°C, out of 132 samples of water analyzed. All strains were cytotoxic and virulent for mouse (Figures 2-3), one strain showed strong elongation activity on CHO cells and some of them had protease, gelatinase, lipase, elastase, urease and haemolytic activity (Table 1). In one isolate PCR detected the *trh* gene.

Table 1: Molecular and enzymatic characterisation of *V. alginolyticus* isolates

| Strains No. | <i>tdh</i> | <i>trh</i> | <i>ctx</i> | Haemolytic activity | Protease | Lipase | Gelatinase | Elastase | Urease |
|-------------------------|------------|------------|------------|---------------------|----------|--------|------------|----------|--------|
| <i>V. alginolyticus</i> | 0% | 2% | 0% | 0% | 0% | 70% | 100% | 91% | 2% |
| 34 | - | - | - | - | - | + | + | + | - |
| 1 | - | + | - | - | - | + | + | + | + |
| 5 | - | - | - | - | - | + | + | - | + |
| 11 | - | - | - | - | - | - | + | + | - |
| 4 | - | - | - | - | - | - | - | + | - |
| 2 | - | - | - | - | - | - | + | + | - |

Cytotoxicity

Figure 2: Morphological changes of Vero cells induced by *V. alginolyticus*. (1) untreated cells, (2) treated cells. Magnification is 100x



Mouse test

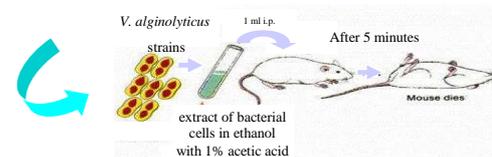


Figure 3: Mouse virulence of *V. alginolyticus* strains

CONCLUSIONS

These results demonstrate:

- 1) the presence of potentially pathogenic *V. alginolyticus* strains in the Conero Riviera;
- 2) *V. alginolyticus* strains as a potential reservoir of many known virulence genes of other *Vibrio* species in the aquatic environment.

On the basis of our data, we may presume that pathogenic *V. alginolyticus* strains present in bathing water may contribute to the onset of wound infections, enteric pathologies and septicemia in humans by exposure to seawater. The role of these micro-organisms should be further investigated.

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