Ecological significance of siderophore production in marine bacteria

Anh Van Pham, Steven Nearhos and Gordon H. G. Davis

Department of Microbiology, University of Queensland, St Lucia, Queensland 4067, Australia

Abstract

Using media selective for vibrios, 701 bacterial isolates were obtained from marine plant, animal and abiotic samples. They were sorted on basic characters into vibrios and non-vibrios. All were assayed biologically for siderophore production under limiting iron conditions. About 70% of all isolates produced siderophores with no significant difference between vibrios and others. Bacteria from non-animal samples yielded more siderophore producers than statistically expected. One vibrio isolate from a diseased fish produced two phenolate and four hydroxamate compounds in iron-depleted culture supernatants but no plasmids were detected in the strain. Of twenty two named *Vibrio* strains representing fifteen species, seventeen representing thirteen species produced siderophores in biological assays.

Introduction

Most bacteria require iron for growth. Bacterial siderophores are compounds of various kinds which trap iron. The ability of bacterial animal pathogens to bind iron for their own use in competition with the host's iron trapping systems can contribute to the pathogen's virulence. Suppression or enhancement of growth of one microbe by the released siderophores of another can also occur and may offer possibilities for infection therapy or prevention (Weinberg, 1978; Neilands, 1984).

Siderophore production is known in *Vibrio cholerae* (Payne and Finkelstein, 1978) and other *Vibrio* species pathogenic for humans and in *Vibrio anguillarum*, a fish pathogen (Crosa *et al.*, 1977). In some strains of the latter species the curing of a plasmid which determines siderophore production has been shown to render strains avirulent (Crosa *et al.*, 1980). Vibriosis caused by *V. anguillarum* and related species results in considerable economic losses to the fishing industry (Taranzo *et al.*, 1983).

As part of an ecological study of marine vibrios we have looked for siderophore activity in twenty two named strains of marine *Vibrio* species and in 701 local marine isolates from various sites. One strain isolated from the kidney of a diseased fish (*Acanthopagurus australis*) was examined for plasmids and the number and type of siderophores produced.

Materials and methods

Bacteria

The named strains tested are shown in Table 1. Isolations were made from healthy and diseased fish, invertebrates, plants, sediments and water from estuarine sites on the Queensland coast between Brisbane and Bundaberg. Vibrio and vibrio-like bacteria were selected by enrichment of samples in

alkaline-peptone water and bromothymol blue-teepol broth prior to growth on TCBS and Simidu media. Vibrios (*i.e.* Vibrionaceae) were differentiated from 'non-vibrios' as Gram-negative, polar flagellate glucose fermenting rods.

Siderophore bioassay

The method of Miles and Khimji (1975) was used. A weak iron-chelating marine vibrio isolate was chosen from 135 local isolates after determining their relative inhibition in agar pour plates by deferrated ethylenediamine-dihydroxyphenyl acetic acid (EDDA, K and K Laboratories) at various concentrations. The most sensitive strain, UQM 2849, was a local isolate of *V. parahaemolyticus*. This indicator strain was grown overnight on nutrient agar and a cell suspension (0.75 A_{650}) prepared in PBS containing 2% NaCl. Supplemented mineral salts medium (K_2HPO_4, 7 g; KH_2PO_4, 3 g; sodium citrate, 2 g;

Table 1 Siderophore production in twenty two named strains representing 15 *Vibrio* species

Species	Strain	Origin	Siderophore activity
V. alginolyticus	UQM 2770	ATCC 1749	+
V. anguillarum	UQM 2771	ATCC 19264	+
V. cholerae	UQM 2772	(a)	+
V. cholerae	UQM 2773	ATCC 14035	+
V. cholerae	UQM 2742	(b)	+
V. campbellii	UQM 2779	ATCC 25920	+
V. diazotrophicus	UQM 2780	ATCC 33466	+
V. fluvialis	UQM 2744	NCTC 11327	+
V. furnissii	UQM 2774	NCTC 11378	+
V. harveyii	UQM 2781	ATCC 14126	−
V. metchnikovii	UQM 211	(c)	+
V. natriegens	UQM 2782	ATCC 14048	+
V. nigripulchritudo	UQM 2784	ATCC 27043	+
V. parahaemolyticus	UQM 2776	ATCC 17802	+
V. pelagius	UQM 2785	ATCC 25916	−
V. splendidus	UQM 2777	ATCC 33125	+
V. splendidus	UQM 2786	ATCC 25914	+
V. vulnificus	UQM 2740	(b)	−
V. vulnificus	UQM 2741	(b)	−
V. vulnificus	UQM 2743	(b)	−
V. vulnificus	UQM 2745	(b)	+
V. vulnificus	UQM 2778	ATCC 27562	+ +

(a) From M. O'Brien, University of Maryland, U.S.A. (b) From D. Callinan, Regional Veterinary Laboratory, Wollongbar, Australia. (c) Unknown.

Table 2 Siderophore activity in *Vibrio* and non-vibrio isolates from marine-sources

Isolation source	Vibrios	Non-vibrios	Total	
Water	10/11*	7/7	17/18	(94)
Sediments	23/33	10/11	33/44	(75)
Oysters	13/24	1/2	14/26	(54)
Copepods	30/37	1/6	31/43	(72)
Mussels	11/16	6/10	17/26	(65)
Crabs	24/33	3/3	27/36	(75)
Prawns	17/21	5/10	22/31	(71)
Algae	10/10	0	10/10	(100)
Errant polychaetes	7/9	0	7/9	(77)
Pseudolana dactylosa	5/6	0	5/6	(83)
Cymothoa indica	10/12	0	10/12	(83)
Mugil cephalus	108/159	98/149	206/308	(67)
Sillago ciliata	80/98	10/15	90/113	(80)
Acanthopagurus australis	1/1	15/18	16/19	(84)
Totals	349/470 (74)	156/231 (67)	505/701 (72)	

* Fractions show number of strains producing siderophores over number tested. Percentages in parentheses.

$MgSO_4.7H_20$, 0.1 g; $(NH_4)_2SO_4$, 1 g; NaCl, 10 g; glucose, 0.2 g; peptone (Oxoid L37), 0.1 g; agar (Oxoid L11), 10 g; distilled water, 1,000 ml; pH 7.5) stored for 3 days or more at 4°C after the addition of 10 μg/ml of filter sterilised EDDA to chelate available iron, was melted in 100 ml lots, cooled to 45°C, 0.3 ml of indicator bacterial suspension added per 100 ml and six plates poured. Test strains were patch inoculated by loop, four per plate, incubated 24 h at 25°C and the degree of indicator growth under or around the test inocula recorded.

Extraction and chromatography of siderophores of UQM 2855

Vibrio strain UQM 2855 showed strong chelating activity in the Miles and Khimji test and was originally isolated from the kidney of a diseased bream (*Acanthopagurus australis*). Iron rich and iron depleted cultures were prepared using one litre volumes of mineral salts medium (above), without the agar, glucose or peptone, containing 0.1 mM ferric chloride ($FeCl_3$) or 0.1 mM EDDA to create iron rich and depleted media, respectively. After 24 h incubation at 25°C the culture supernatants were extracted by the method of Rogers (1973). The extracts were chromatographed on Merck cellulose TLC

medium for 7 h using 5% ammonium formate containing 0.5% formic acid as solvent (O'Brien *et al.*, 1969). Phenolates were detected under UV and hydroxamates by spraying with 4% ferric chloride in 0.01 N HCl.

Plasmid analysis

Strain UQM 2855 was examined for plasmids by the method of Crosa *et al.* (1977).

Results

Tables 1 and 2 show the results of siderophore bioassays on named vibrios and local marine isolates. Statistically (Chi- square test) the data in Table 2 showed no significant difference between vibrio and non-vibrio strains in terms of the number of isolates showing siderophore production. When sub-populations of strains from various sites were compared against the rest of the strains it was found that the sub-population from exposed sites (*i.e.* water, sediments, algae) showed more siderophore producers than expected whereas the other strain sub-populations associated with filter feeders, invertebrate scavengers, fish, did not.

Extracts of strain UQM 2855 grown in iron rich medium yielded only one phenolate compound (R_f 0.06). The iron deficient culture yielded two phenolates (R_f 0.06 and 0.03) and four hydroxamates (R_f 0.45, 0.53, 0.66 and 0.73). The hydroxamate at R_f 0.73 was violet and the rest blue with $FeCl_3$.

No plasmid DNA was detected in UQM 2855 in three attempts.

Discussion

The results indicate common production of extracellular iron chelators in marine bacteria and their general exchange between producers and non-producers. About 75% of the field strains and named *Vibrio* species strains tested showed siderophore activity. In the case of one very active strain of *V. vulnificus* several potential siderophore moieties were demonstrated but no plasmids detected (*cf* Andrus *et al.*, 1983).

Among our isolates there appeared to be a higher proportion of siderophore-producing strains from non-animal sites than animal sites. This may mean that on animal surfaces marine bacteria unable to produce siderophores are able to take advantage of producer strains to supply iron while in non-animal sites they are not. This in turn could reflect the possible trapping of bacterial chelators in animal surface slimes, where non-producers could accumulate to remain near a source of iron.

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