Journal of Fish Diseases 1991, **14**, 359–365

The use of API 20E for the identification of *Vibrio anguillarum* and *V. ordalii*

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Abstract. Sixty-eight strains of *Vibrio anguillarum*, five of *V. ordalii* and the type strains of *V. alginolyticus*, *V. carchariae*, *V. damsela* and *V. parahaemolyticus* were compared using the API 20E gallery. Within the *V. anguillarum* strains, distinct groups could be separated mainly on the basis of their reaction on indole production and the fermentation of amygdalin and arabinose. *Vibrio ordalii*, the former *V. anguillarum* biotype 2, could easily be separated from *V. anguillarum* and from the other fish pathogenic *Vibrio* spp.

Introduction

Vibriosis is known to be one of the most common diseases of marine organisms. Besides the 48 or more marine fish species in which the disease has been described, *Vibrio anguillarum* and *V. ordalii* have also been identified in oysters (Bolinches, Toranzo, Silva & Barja 1986), lobsters (Bowser, Rosenmark & Reiner 1981) and several freshwater fish species (Rucker 1959; Muroga 1975). Because of the world-wide occurrence of these bacteria and the large economic losses related to the disease, a rapid identification of the responsible organisms is of extreme importance.

A standardized and rapid identification system is offered by the API 20E system (BioMérieux S.A., Marcy-l'Etoile, France). This miniaturized multitest system was originally developed for the identification of Gram-negative enteric bacteria in clinical laboratories. However, during the last decade, this system has been increasingly used for the identification of marine and fresh water fish pathogens, (Baudin-Laurencin 1981; MacDonell, Singleton & Hood 1982; Kent 1982; Maugeri, Crisafi, Genovese & Scoglio 1983; Fujioka, Greco, Cates & Schroeder 1988).

Unfortunately, apart from the reaction profiles for *V. alginolyticus, V. parahaemolyticus* and *V. damsela*, none of the other fish pathogenic vibrios (Colwell & Grimes 1984) are incorporated in the *Analytical Profile Index*, 3rd edn (1989) for this teststrip.

Therefore, and because of the large biochemical variability existing between strains of *V. anguillarum*, as shown in several taxonomic studies (Egidius & Andersen 1977; West, Lee & Bryant 1983; Bryant, Lee, West & Colwell 1986), 68 named strains of this species together with five strains of *V. ordalii* and the type strains of the other fish pathogenic vibrios were tested with the API 20E gallery.

Material and methods

Bacterial strains

The strains used in this study are listed in Table 1.

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Table 1. List of strains

Number of strains	Source*	Country
V. anguillarum		
20	T. Jørgensen	Norway
4	K. Andersen	Norway
1	R. Wiik	Norway
7	I. Dalsgaard	Denmark
3	T. Wiklund	Finland
2	F. Baudin-Laurencin	France
2	A. Toranzo	Spain
9	T. Kitao	Japan
6	K. Muroga	Japan
2	Y. Ezura	Japan
3	M. Tolmasky	USA
1	T. Cipriano	USA
NCIMB 6†; −571; −829; −1873; −1874; −1875; −1876; −2131; −2286;	NCIMB	Scotland
V. ordalii		
1	T. Jørgensen	Norway
1	F. Baudin-Laurencin	France
2	T. Cipriano	USA
NCIMB 2167†	NCIMB	Scotland
V. damsela		
NCIMB 2184†	NCIMB	Scotland
V. tubiashii		
NCIMB 1340†	NCIMB	Scotland
V. carchariae		
NCIMB 12705†	NCIMB	Scotland
V. parahaemolyticus		
LMG 2850	LMG	Belgium
V. alginolyticus		
LMG 4409†	LMG	Belgium

* Source of strains: NCIMB: National Collections of Industrial and Marine Bacteria, Aberdeen, Scotland, UK; LMG: Labo voor Microbiologie Rijksuniversiteit Gent, Gent, Belgium; Dr T. Jørgensen, University of Tromsø, Norway; Dr K. Andersen, Institute of Marine Research, Norway; Dr R. Wiik, Institute of Marine Research, Norway; Dr T. Wiklund, Åbo Akademi, Finland; Dr I. Dalsgaard, Fiskepathologisk Laboratorium, Denmark; Dr F. Baudin-Laurencin, Laboratorie de Pathologie d'Animaux Aquatique, France; Dr A. Toranzo, Universidad de Santiago, Spain; Dr T. Kitao, Miyazaki University, Japan; Dr K. Muroga, Hiroshima University, Japan; Dr Y. Ezura, Hokkaido University, Japan; Dr M. Tolmasky, Oregon Health Sciences University, USA; and Dr T. Cipriano, U.S. Fish and Wildlife Service, West Virginia, USA.
† Type strain.

Morphology, motility and cultural conditions

All strains were maintained on a long-term preservation medium (LPM) (Colwell 1987) in screw-cap vials at room temperature from which they could be directly transferred to brain heart infusion agar plates (Difco) supplemented with 1·5% NaCl (BN). Cultures grown overnight on BN at 26°C were used for the inoculation of all the tests.

Salt requirement was tested in tryptone water 1% (Difco) supplemented with 0 and 1·5% NaCl.

Motility was determined by the hanging drop method.

Gram reaction was tested by suspending a colony from an overnight BN culture in a drop of 1·5% saline, which gave better results than suspensions made in distilled or deonized water.

Biochemical characteristics

Oxidative versus fermentative metabolism was tested on Hugh-Leifson glucose medium (Hugh & Leifson 1953) supplemented with 1·5% NaCl.

Citrate utilization was tested on Simmons citrate agar (Difco) supplemented with 1·5% NaCl.

Sensitivity to the vibriostat 0/129 (2,4-diamino-6,7-diisopropylpterydine) and Novobiocin was determined by using discs impregnated with 10 and 150 μg 0/129 (Oxoid), and discs with 5 μg Novobiocin (Pasteur Diagnostics), placed on sheep blood agar plates (Difco) seeded with an overnight BN-broth culture.

The oxidase test was performed by streaking a colony onto a disc impregnated with dimethyl-p-phenyldiamine: a positive test is indicated by a purple coloured reaction.

API 20E strip

A few alterations to the prescribed method for the inoculation of the test strip were made in order to adapt the system to marine bacteria:

(1) The incubation time was increased to 48–72 h and the incubation temperature was lowered to 26°C.
(2) A suspension in 1·5% saline was used as inoculum.
(3) In the carbohydrate tests, only the fermentation of sugars was allowed by sealing these cups with sterile mineral oil.

Computer analysis

All the data obtained from the strips, except the citrate reaction, were used for computer analysis. The citrate reaction was discarded because a large variability occured on the strip, whilst all strains were citrate positive to the classical test. The oxidase reactions, performed separately from the API 20E gallery, were also included in the computer analysis.

The data were computed with the SAS UPGMA clustering method using the Euclidean distances. The tree was constructed with the R-squared units.

Results and discussion

Morphology, motility and biochemical characteristics

The *Vibrio* strains tested were all motile, Gram-negative, slightly curved rods (pleomorphic rods occurred), except for the strains ET-1 and PT 7680, which were non-motile.

All were sensitive to the vibriostat 0/129 except PT 493, which was resistant to 150 μg 0/129. *Vibrio parahaemolyticus* was the only strain resistant to Novobiocin.

Vibrio damsela, NCIMB 2184 was oxidase negative. This is surprising because the type strain is supposed to be oxidase positive (Austin & Austin 1987). All the other strains were oxidase positive, from which it can be concluded that the method used is appropriate for testing the oxidase reaction in vibrios.

All strains tested had a fermentative metabolism towards the Hugh & Leifson (1953) glucose medium. There was no problem in culturing the strains on BN, and all strains could be directly plated from LPM to BN, on which they all grew well overnight. All strains grew better in tryptone water supplemented with NaCl than in tryptone water with no sodium cloride added.

API 20E reactions

MacDonell *et al.* (1982) suggested the use of a 2% marine salt solution for the inoculation of the API 20E strip for the characterization of marine and estuarine bacteria. However, for standardization reasons and the possible comparison of the gallery with classical tests, the present authors decided to use a 1·5% sodium cloride solution as the suspending fluid for the inoculation of the test strip. This 1·5% saline solution seemed appropriate because *V. alginolyticus* and *V. parahaemolyticus* gave 98·2 and 99·9% correct identification values, respectively, according to the *Analytical Profile Index*. The profile obtained for *V. damsela* was not present in the profile index because the present authors' strain fermented amygdalin and was oxidase negative.

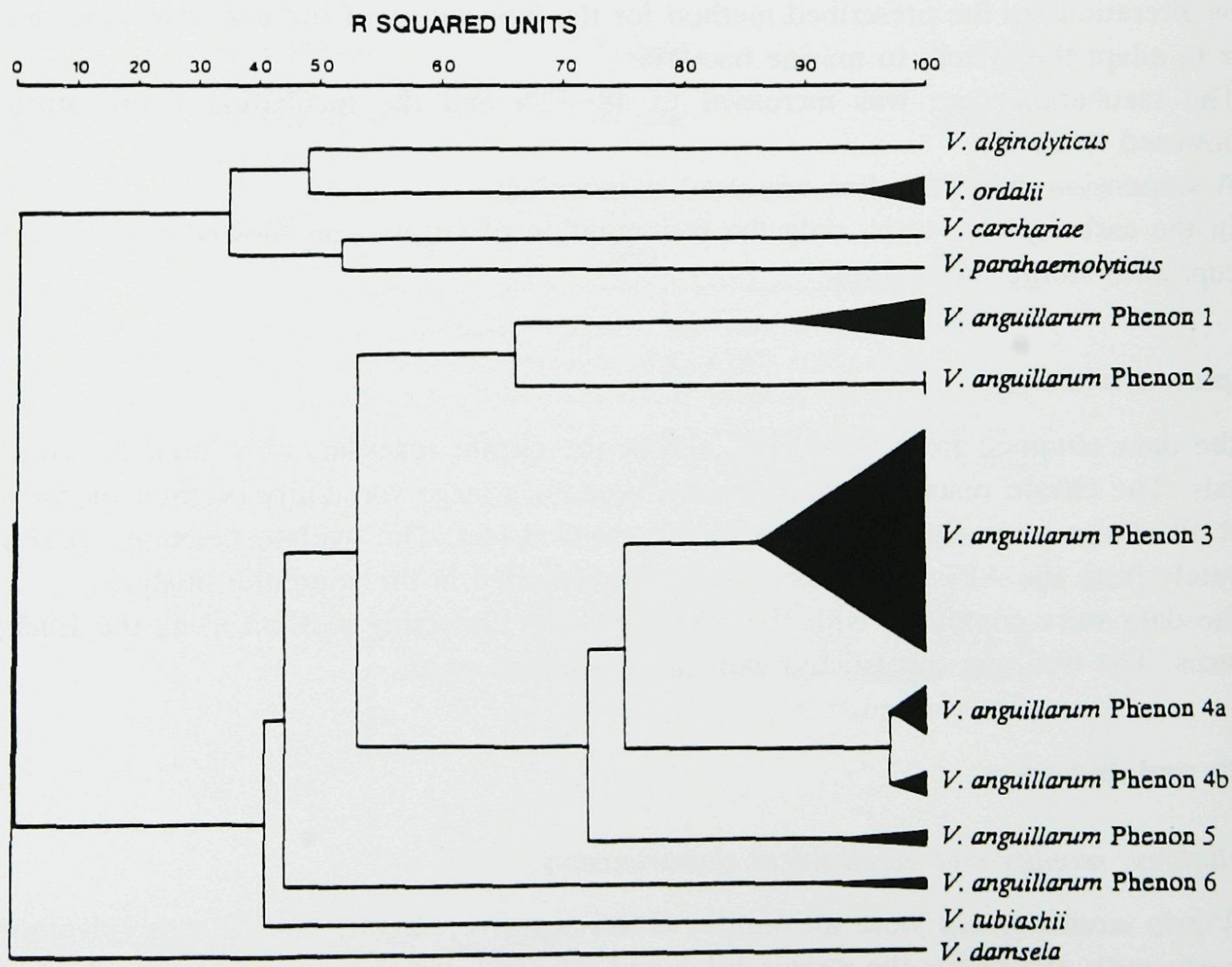

Figure 1. Simplified dendrogram, based on the API 20E profiles and the oxidase reaction, minus the citrate reaction. The data were computed with the SAS UPGMA clustering method using the Euclidean distances.

Figure 1 shows a simplified dendrogram, based on the API 20E reactions obtained for the strains.

In Table 2, the corresponding reactions for the different phena and species are shown.

Phenon 1, V. anguillarum

This phenon contains five Scandinavian strains which are all negative for the indole reaction, and for the fermentation of amygdalin and arabinose. Two more strains are incorporated in this phenon: NCIMB 2286, which is indole and arabinose negative but amygdalin positive; and PB 28, which reacts like the five Scandinavian strains but is also able to ferment sorbitol.

Phenon 2, V. anguillarum

Four strains are grouped here because they react negatively to the arginine dihydrolase of the strip, and are negative for the fermentation of amygdalin and arabinose.

Table 2. Biochemical characteristics of the different *Vibrio* sp. and phena of *V. anguillarum*, grown at 25 °C on the API 20E strip

	V. alginolyticus	*V. ordalii*	*V. carchariae*	*V. parahaemolyticus*	*V. damsela*	*V. tubiashii*	*V. anguillarum* Phenon 1	*V. anguillarum* Phenon 2	*V. anguillarum* Phenon 3	*V. anguillarum* Phenon 4a	*V. anguillarum* Phenon 4b	*V. anguillarum* Phenon 5	*V. anguillarum* Phenon 6	*V. anguillarum* 13 A5
Number of strains	1	5	1	1	1	1	7	4	37	9	5	3	2	1
ONPG	−	−	−	−	−	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	−	−	−	−	+	+	+	−	$+^{36}/_{37}$	+	+	+	+	+
Lysine decarboxylise	+	−	+	+	−	−	−	−	−	−	−	−	−	+
Ornithine decarboxylise	−	−	+	+	−	−	−	−	−	−	−	−	−	−
Citrate Simmons	−	−	+	+	−	−	v	v	v	v	v	v	v	−
H_2S	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease	−	−	+	+	+	−	−	−	−	−	−	−	−	−
Tryptophan deaminase	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Indole	+	−	+	+	−	+	−	+	$+^{36}/_{37}$	+	+	+	+	+
Voges Proskauer	−	$+^{3}/_{5}$	−	−	+	+	+	+	$+^{36}/_{37}$	+	+	+	+	−
Gelatinase	+		+	+	−	+	+	+	+	+	+	+	+	+
Acid from:														
glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
mannitol	+	+	+	+	−	+	+	+	$+^{36}/_{37}$	+	+	+	+	+
inositol	−	−	−	−	−	−	−	−	−	−	−	−	+	−
sorbitol	−	−	−	−	−	−	$+^{6}/_{7}$	+	+	+	+	−	−	−
rhamnose	−	−	−	−	−	−	−	−	−	−	−	−	−	−
sucrose	+	+	+	−	−	+	+	+	$+^{36}/_{37}$	+	+	+	+	+
melibiose	−	−	+	−	−	+	−	−	−	−	−	−	−	−
amygdalin	+	+	+	+	+	+	$-^{6}/_{7}$	−	$+^{36}/_{37}$	+	−	+	−	+
arabinose	−	−	−	+	−	−	−	−	+	−	−	$-^{2}/_{3}$	½	−
Oxidase	+	+	+	+	−	+	+	+	+	+	+	+	+	+

Phenon 3, V. anguillarum

This phenon represents 37 of the 68 *V. anguillarum* strains tested, and therefore, is the predominant type of this bacterium. The reaction pattern observed here is the same as that found by Baudin-Laurencin (1981) for the strain VA 408 and very much resembles the pattern described by Kent (1982). The only differences from the observations of Kent (1982) are the positive reactions for arginine dihydrolase, indole formation and arabinose fermentation. Kent (1982) indicates variable results for these characteristics, but the present authors selected only those strains with positive reactions on these tests in this phenon. In this study, the strains with negative reactions on these tests are placed in other phena. The reaction profile for this phenon is identical to the profile given by Austin (1988) for *V. anguillarum*.

Phenon 4, V. anguillarum

This phenon comprises 14 strains. Nine of these are able to ferment amygdalin (phenon 4a), including the type strain, NCIMB 6. The other five are negative for this characteristic (phenon 4b).

Phenon 5, V. anguillarum

These three strains are placed apart from the rest because they share a negative reaction to the fermentation of sorbitol

Phenon 6, V. anguillarum

The two strains in this phenon are different from all the others by reason of their ability to ferment inositol.

One strain leans towards *V. alginolyticus*, namely *V. anguillarum* 13A5. Distinct from all the other *V. anguillarum* strains, this strain is able to decarboxylise lysine.

The results the present authors obtained for *V. anguillarum* closely resemble those of Baudin-Laurencin (1981), Kent (1982) and Austin (1988). None of the reactions reported by Maugeri *et al.* (1982) resemble the profiles the present authors obtained. All 20 strains tested by Maugeri *et al.* (1982) were negative for the fermentation of sorbitol while, in the present tests, only three out of 68 strains were negative for this reaction. Maugeri *et al.* (1982) report several strains which were ONPG negative, but none of the strains we tested were negative for the ONPG reaction.

The profiles obtained for the other fish-pathogenic vibrios very strongly resemble the profiles given by Kent (1982) and by Austin (1988) with one major difference: in the present tests, most of the other vibrios were able to ferment amygdalin, while this reaction was reported as being negative in the tests of the authors cited above.

The present authors conclude that the API 20E system offers a good tool for the characterization of fish pathogenic vibrios and that the variability observed within the species *V. anguillarum* is mainly the result of their ability to ferment the sugars amygdalin and arabinose.

Acknowledgments

This study was supported by a personal grant from the I.W.O.N.L. to L. Grisez (no. 880224), and by a grant from the N.F.W.O. (no. 2.0081.87)

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