SHORT COMMUNICATION

The Halophilic Properties of *Vibrio parahaemolyticus*

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The halophilic properties of 18 strains of *Vibrio parahaemolyticus*, including the type strain, were studied. The ability of the strains to grow in 0.1% trypticase containing isomolar concentrations of 10 different salts was tested. The results indicate that *V. parahaemolyticus* is capable of growing equally well in media containing salts other than NaCl and that it has no specific requirements for Na⁺ or Cl⁻.

INTRODUCTION

Vibrio parahaemolyticus is widely reported to be halophilic, requiring NaCl for its growth. All media used for its isolation and identification are supplemented with NaCl, but there have been reports of its isolation from fresh water sources (Cholera Research Centre, Calcutta, 1975). These reports prompted us to investigate the halophilic requirements of this organism.

METHODS

Strains and their characterization. Eighteen strains of *V. parahaemolyticus*, including the type strain and another reference strain, were used in this study. Strains 1 (NCTC 10885) and 2 (NCTC 10903 – the type strain) were obtained from the National Collection of Type Cultures, Public Health Laboratory Service, Colindale, London. Strains 3 to 18 were isolated in this laboratory: strains 3 to 7 were from the faeces of cases of gastroenteritis and strains 8 to 18 from sea fish. These strains were identified as *V. parahaemolyticus* on the basis of the results obtained with the following tests: large colonies with dark green centres on thiosulphate/citrate/bile salt/sucrose (TCBS) agar; oxidase positive; good overnight growth at 37 °C in 1% (w/v) trypticase peptone water, when supplemented with 6 or 8% (w/v) NaCl but not with 10% NaCl or with no supplementary NaCl; good overnight growth at 42–43 °C in the same medium with 2% NaCl; fermentative reaction (acid, no gas) in the Hugh and Leifson oxidation/fermentation test; alkaline slant, yellow butt, no H₂S in triple sugar iron agar; acetoin (Voges–Proskauer) negative; indole positive; lysine decarboxylase positive; and arginine dihydrolase negative. (Except where indicated, the above tests were made in media supplemented with 3% NaCl.)

Growth in the presence of salts and sugars. Tests were made of the ability of the strains to grow in 0.1% (w/v) trypticase containing isomolar concentrations (0.1 M and 0.2 M) of the following inorganic and organic salts: Na₂SO₄; NaNO₃; KCl, KI, K₂SO₄, MgSO₄, MgCl₂, sodium citrate and sodium acetate. Ability to grow in isomolar concentrations of glucose and sucrose was also examined. As controls, 0.1% trypticase containing 0.1 M- or 0.2 M-NaCl or no NaCl were used. The pH of the salt solutions without adjustment was 6.9–7.2. Isomolar concentrations were used so that the osmotic pressure of the different salt and sugar solutions would be similar, thus permitting comparison of their specific growth-promoting properties.

Precautions were taken to minimize mineral contamination: Analar-quality salts and sugars were used, solutions were prepared with distilled water, and glassware was thoroughly rinsed with distilled water before use.

Cultures from nutrient agar were streaked on to TCBS agar and incubated overnight at 37 °C. A single colony was picked into 1% (w/v) peptone water containing 3% (w/v) NaCl and incubated overnight at 37 °C. This culture was used to inoculate, with a straight wire, 0.1% trypticase containing 0.1 M and 0.2 M concentrations of the various salts and sugars. The inoculated media were incubated for 24 h at 37 °C and then examined for evidence of growth. Growth was determined by measuring the turbidity with a Klett–Summerson colorimeter, using filter 64.

Table 1. *Growth of V. parahaemolyticus strains in 0.1% trypticase containing different salts*

Turbidity readings were taken after 24 h incubation at 37 °C in the various media, with a Klett-Summerson colorimeter (filter 64). No growth was observed in 0.1% trypticase without added salts.

Salt	Concn (M)	Growth (turbidity) of strain:																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
NaCl	0.1	28	22	50	37	23	39	38	30	21	30	28	37	27	23	30	22	34	20
	0.2	40	41	55	57	36	46	52	45	40	38	40	46	41	35	54	42	47	35
Na ₂ SO ₄	0.1	21	22	33	20	23	28	33	18	21	28	20	28	27	34	25	26	25	22
	0.2	55	49	65	48	45	64	50	40	48	50	46	42	45	59	39	45	46	50
NaNO ₃	0.1	20	20	27	12	36	20	28	20	11	15	25	29	25	24	22	20	24	23
	0.2	28	35	49	35	55	45	43	36	32	31	39	46	40	41	38	33	35	37
K ₂ SO ₄	0.1	14	11	60	36	25	44	52	31	10	11	25	28	18	29	31	29	22	17
	0.2	36	35	75	54	46	72	60	59	21	24	46	58	36	48	50	53	63	40
MgCl ₂	0.1	12	25	18	22	15	32	28	30	12	16	21	17	22	20	17	28	27	28
	0.2	40	46	45	54	46	60	58	64	42	15	50	31	52	57	43	53	50	48
KCl	0.1	0	6	2	0	2	3	0	0	1	0	7	3	11	16	8	9	14	5
	0.2	15	25	8	4	14	18	15	12	8	12	10	13	22	28	19	20	25	18
MgSO ₄	0.1	3	5	0	6	8	10	0	5	0	2	4	6	8	7	7	9	8	8
	0.2	10	13	8	14	12	14	3	15	1	7	10	10	18	13	15	10	16	15
Sodium citrate	0.1	0	1	2	0	0	0	2	0	0	0	0	0	0	0	0	1	2	0
	0.2	8	7	6	6	2	5	7	6	4	5	3	3	6	6	6	8	7	8

RESULTS AND DISCUSSION

The results are shown in Table 1. No growth occurred in 0.1% trypticase without added salts. There was good growth in the 0.2 M concentration of Na₂SO₄, NaNO₃, K₂SO₄ and MgCl₂, comparing favourably with the growth produced in 0.2 M-NaCl. These findings would indicate that *V. parahaemolyticus* has neither a specific requirement for NaCl nor for Na⁺ or Cl⁻ for its growth, contrary to the views generally held at present. The growth in KCl and MgSO₄ was poor but it would appear that growth could be enhanced by increase in concentration since there was a definite increase in turbidity when the concentration was raised from 0.1 M to 0.2 M. There was no growth in KI except for strain 3 which showed scanty growth in the 0.2 M concentration. This finding would support to some extent the observation of Macleod & Onofrey (1956) who found that Br⁻ could substitute for Cl⁻ and not I⁻ as far as the halophilic requirements of some marine bacteria were concerned. None of the strains showed growth in sodium acetate, while some degree of growth was produced by all the strains in 0.2 M-sodium citrate. This observation would suggest that apart from inorganic salts some organic salts too were capable of supporting the growth of *V. parahaemolyticus*, though growth might be slow in media containing them. The absence of growth in glucose and sucrose would confirm that *V. parahaemolyticus* is not a saccharophile.

Larsen (1962) grouped halophiles into 3 categories: group 1, consisting of bacteria which have specific requirements for Na⁺ and Cl⁻, group 2, bacteria with a specific requirement for Na⁺ but not for the anion, and group 3, bacteria with no specific requirement for the cation or the anion. The results of the present study would indicate clearly that *V. parahaemolyticus* belongs to group 3. The present findings could also lend support to the reports of its isolation from fresh water sources.

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