Mem. Fac. Fish., Kagoshima Univ.
Vol. 23 pp. 123~135 (1974)

Study on the Esterase Isozymes of Marine Isolates Belonging to *Vibrio* and *Pseudomonas*

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Abstract

Electrophoretic zymograms of esterase isozymes were determined for 8 strains of marine isolates. In order to compare marine isolates with terrestrial bacteria, some terrestrial bacteria belonging to the same genus as marine isolates were also tes ted. There seems to be a tendency that *Pseudomonas* shows complicated electrophoretic patterns of esterase isozymes with thick and thin bands, on the other hand *Vibrios* show simple electrophoretic pattern with a few thick and clear bands. In this study, we could not obtain the evidence to distinguish the marine isolates from terrestrial bacteria on views of esterase isozymal patterns. It was found that these esterase isozymal patterns varied with cultural conditions, and that the isozymal variation seemed to be correlated to the environmental changes.

Recently, it was frequently observed that an electrophoresis was applied to microbial studies. Colwell *et al*[1] studied esterase isozymes and applied the study to classification of *Vibrios*. Morichi *et al*[2] proved a common feature of electrophoretic patterns of esterases in lactic acid bacteria of 113 strains. Goullet[3] analysed the intracellular esterases of *Escherichia coli* of 25 strains by the acryl amide-agarose zymogram technique, and defined five kinds of esterase band. Lund[4] studied soluble proteins and esterases of some streptococci by gel electrophoresis. This study was done to investigate the relation between environmental changes and isozymal variety for bacteria.

Materials and Methods

Organisms. The strains tested in this experiment were mainly marine isolates, their properties being published elsewhere[5]. Marine isolates belonging to *Vibrio* C-12, C-13, C-28, C-29, and these belonging to *Pseudomonas* C-1, C-7, C-8, C-32, and 1055-1 were used. For comparison, the terrestrial bacteria *Pseudomonas aeruginosa* IFO 3080, *Pseudomonas ovaris* IFO 3738, *Pseudomonas fragi* IFO 3458 (from Institute for Fermentation, Osaka) and halophiles *Vibrio parahaemolyticus* sero type o-5 (from the Faculty of Fisheries, Hokkaido University) and *Vibrio alginolyticus* 370 (from Ocean Research Institute, University of Tokyo) were used. All of these bacteria were incubated at their optimal temperatures 25 C for marine isolates and 30 C for terrestrial bacteria.

Bacteriological methods. Subcultures for all marine bacteria were made on the agar slant of ZoBell 2216 E at 25 C for 16 hours. They were incubated at the

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same temperature above with shaking for 3 days in ZoBell 2216 E of 250 ml in each 500 ml flask, immediately these cultures were extracted referring to the method of Colwell *et al.*[1] To study the relationship between growth conditions and esterase isozymal synthesis, the following subjects were determined: effect by NaCl concentration; effect by incubation temperature; effect by pH; effect by bivalent cationic salts. The effect of NaCl was determined, in accordance with their NaCl tolerance, in various NaCl concentrations within 9 % for marine *Pseudomonads* and within 6 % for marine *Vibrios*. Salts media were prepared by various modifications of NaCl concentration in ZoBell 2216 E medium. The temperature effect was determined at 30 C for marine *Pseudomonads* and 37 C for marine *Vibrios*, comparing with their optimal temperature 25C. Effect of pH was determined at pH 6.8, comparing with pH 8.0. Except temperature effect the incubation was carried out at 25 C for 3 days. The effect of bivalent cationic salts was determined by using two media; one was ZoBell 2216 E for control, another being modified ZoBell 2216 E containing various amounts of bivalent cationic salts. The three bacteria *P. aeruginosa*, *P. ovaris* and *P. fragi* were compared with marine *Pseudomonads* on their esterase isozymes at optimal growth. The incubation was done for 3 days.

Enzyme extraction. Preparation of enzyme extract for electrophoresis was done by collecting the bacterial cells of 3 days incubation, successibly the cells were washed twice with 0.25 M NaCl solution. The pellet obtained was resuspended in the Tris-citrate buffer, pH 8.65, being subjected to sonication at 20 KHz for 7 min in cold temperature. The debris yielded was removed by centrifugation at 14,000 r. p. m. for 30 min. at 0 C, then supernatant was obtained. To remove various contaminants the supernatant was put in Visking tube and dialysed in cold temperature for one day. Immediately after dialysed the extract was condensed with collodion bag of Satorius Membrane Filter Goettingen SM 13200.

Disc electrophoresis. Disc electrophoresis was performed with pH 8.0 gel method developed by Williams.[5] The zymograms for the esterase were obtained in the following stains; 1 % (W/V)α-naphthyl acetate in 2 ml of 50: 50 acetone/water mixture, and 50 mg of Fast blue RR salt in 50 ml of 0.1 M Tris-malate buffer, pH 6.4. After staining the gel was washed and preserved in 7 % acetic acid solution. Ozumor 77 densitometer with linear transport was applied to analyse the stained gels. The speed of sample carrier was 20 mm/min and wavelength was at 570 nm.

Results and Discussion

The diagrams described from the zymograms of various marine isolates and authentic strains are shown in Figs 1-40. The figures were arranged in order of subjects; optimal growth, high NaCl concentration, high growth temperature, low pH and bivalent cationic salts deficiency. In order to understand esterase isozymal distribution in various bacteria, a simple modification for diagrams was done, namely electrophoretic mobilities for isozymes were expressed with symbol marks **a**, **b**

and **c** in order of mobile distance, esterase activity being expressed by simple ratio of integral number. The result is shown in Table 1.

Table 1. Esterae isozymal patterns of various bacteria

Growth condition	optimal condition			high NaCl concentration			high growth temperature			low pH			bivalent minerals deficiency		
	a*	b	c	a	b	c	a	b	c	a	b	c	a	b	c
Pseudomonas															
1055-1	7	10	—	9	5	—	10	12	—	10	11	—	5	3	—
C-1	5	2	2	10	2	1	9	3	12	10	3	3	8	1	1
C-7	5	3	5	5	3	2	0	1	3	0	6	4	0	6	2
C-8	5	1	—	5	3	—	8	11	—	8	2	—	0	3	—
C-32	9	5	3	8	1	0	9	3	10	11	3	4	13	0	4
Vibrio															
V. P.	3	1	10	1	2	10	0	3	13	0	4	12	1	1	10
V. A.	1	2	—	1	1	—	0	4	—	2	8	—	2	0	—
C-12	11	10	11	6	3	2	12	2	3	6	2	7	14	7	5
C-13	3	3	—	1	3	—	5	2	—	2	4	—	3	3	—
C-28	2	2	—	0	2	—	0	4	—	2	10	—	0	5	—
C-29	4	4	—	1	1	—	4	11	—	3	7	—	1	1	—

* The symbol marks **a**,**b** and **c** are arranged in order of distance of electrophoresis. The activity is shown by the relative ratio of integral number.

As shown in the table and the figures, the isozymal changes affected by environmental factors were not genera specific. Among these bacteria, marine *Pseudomonas* C-1 seemed to be distinguished for isozymal pattern, namely, in an optimal condition, main band **a** and other two subbands **b** and **c** were observed, the density of both **b** and **c** being half of **a**, to the contrary in high temperature the density of **c** became higher, and that of **b** became very low. In low pH the density of both **b** and **c** showed one forth of that of **a**. In high NaCl concentration only **a** was very high, while **b** was very low, and **c** disappeared.

In general, in the case of *Pseudomonads*, mutual changes in isozymes were easy to occur by cultural conditions. To the contrary in the case of *Vibrios*, these were not. We supposed that there were some relations between esterase isozymes and bacterial adaptability in some environments. In other words, it could guess that isozymes may a suppressive or stimulative role through bacterial growth.

It may be possible to suppose that adaptability of bacteria in sudden enviromental changes depends upon the existence of esterase isozymes. We could not ascertain the difference between marine isolates and terrestrial bacteria.

Acknowledgements

We wish to thank Dr H. Kawamura of the faculty of Medicine, Kagoshima

University for his excellent technical help and useful advice.

References

1) Hogan, M. A. and Colwell, R. R. (1968) : *J. Appl. Bacteriol.*, **32**, 103-111.
2) Morichi, T., Sharpe, M. E. and Reiter, B. (1968) : *J. gen. Microbiol.*, **53**, 405-414.
3) Goullet, P. (1972) : *J. gen. Microbiol.*, **77**, 27-35.
4) Lund, B. M. (1965) : *J. gen. Microbiol.*, **40**, 413-419.
5) Williams, D. E. and Reisfeld, R. A. (1964) : *Ann. New York Acad. Sci.*, 121 Art. **2**, 373.

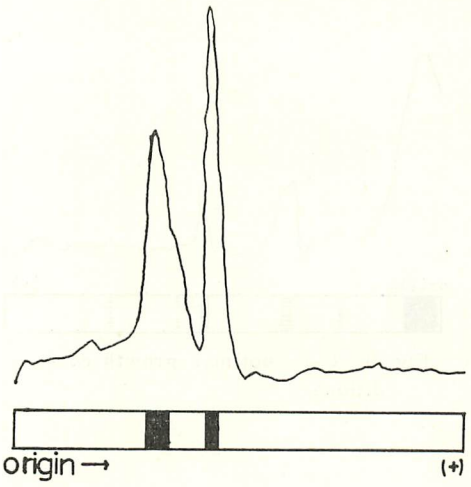

Fig. 1. Densitometric tracing of electrophoretic gel on *Pseudomonas* 1055-1 (optimal growth condition)

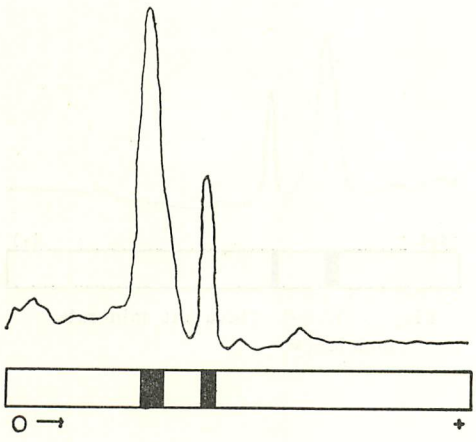

Fig. 2. 1055-1 (high NaCl concentration)

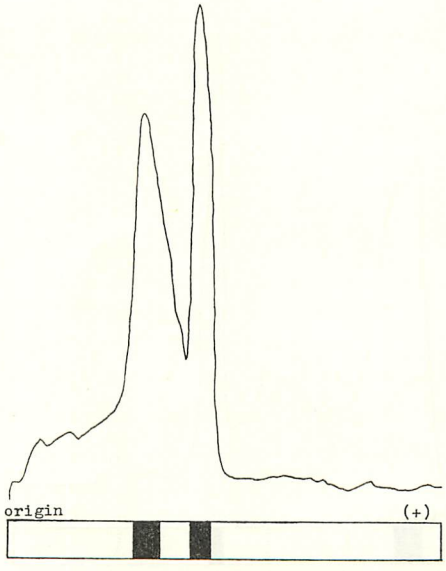

Fig. 3. 1055-1 (High growth temperature)

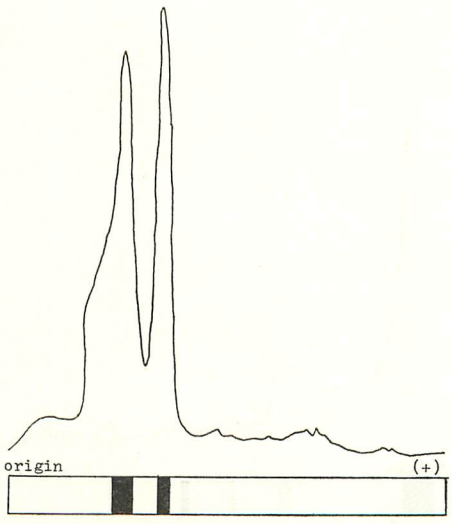

Fig. 4. 1055-1 (Low pH)

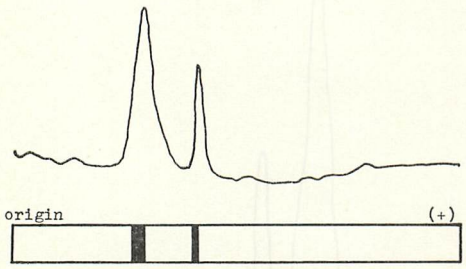

Fig. 5. 1055-1 (bivalent minerals deficiency)

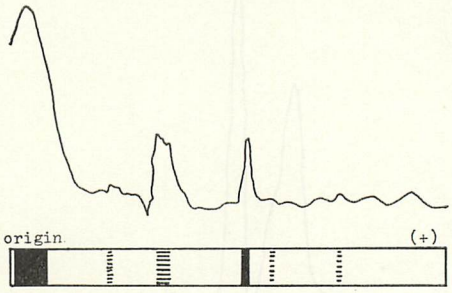

Fig. 6. C-1 (optimal growth condition)

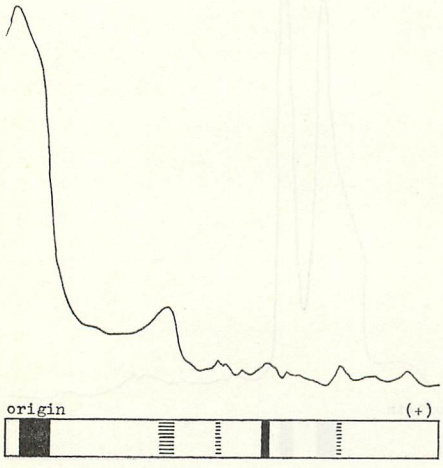

Fig. 7. C-1 (High NaCl concentration)

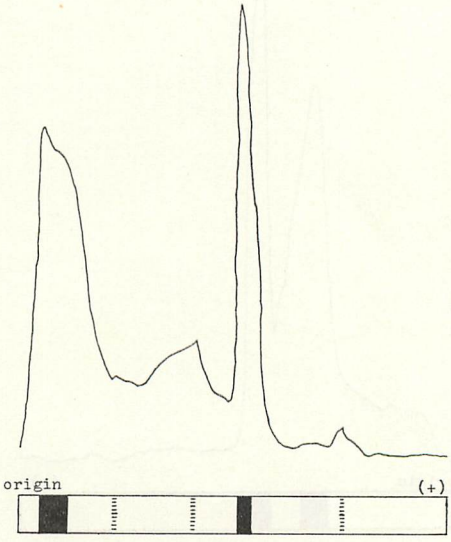

Fig. 8. C-1. (High growth temperature)

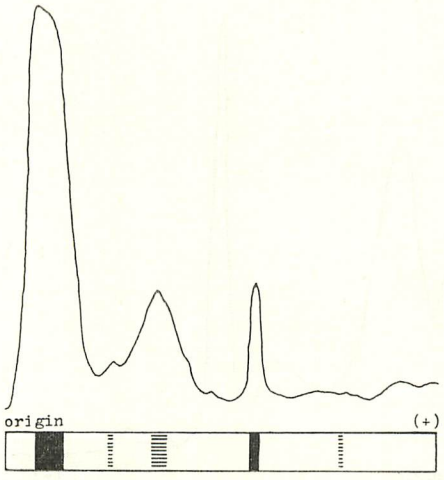

Fig. 9. C-1. (Low pH)

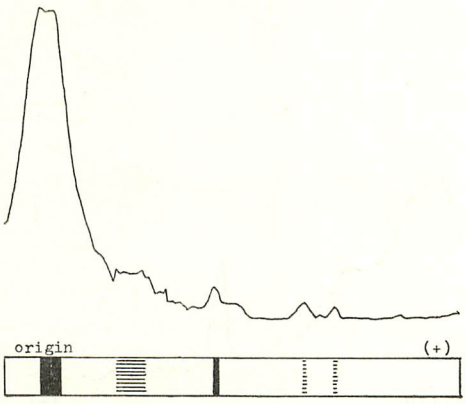

Fig. 10. C-1 (Bivalent minerals deficiency)

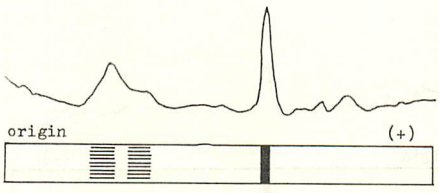

Fig. 11. C-7. (High growth temperature)

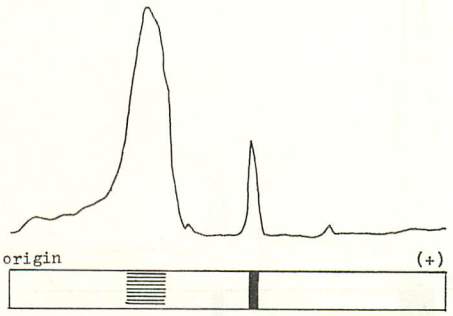

Fig. 12. C-7. (Low pH)

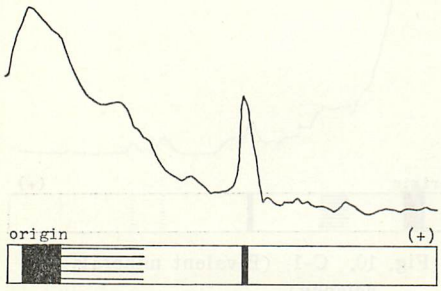

Fig. 13. C-8. (High NaCl concentration)

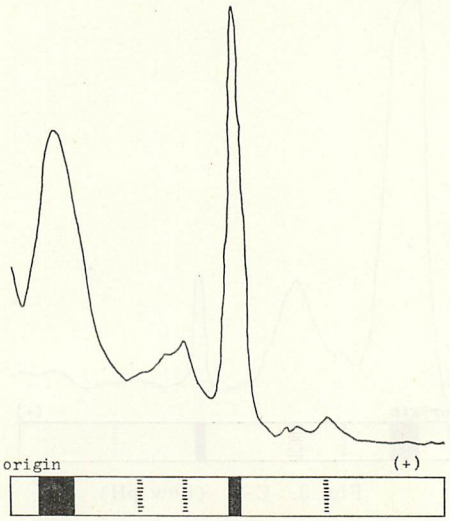

Fig. 14. C-8. (High growth temperature)

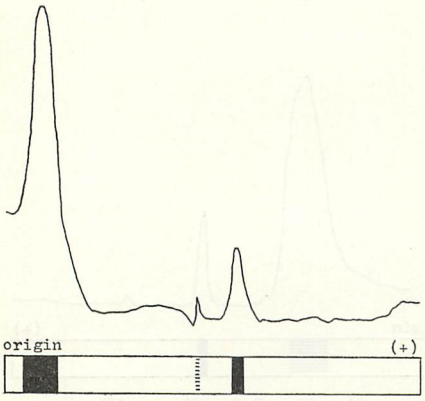

Fig. 15. C-8. (Low pH)

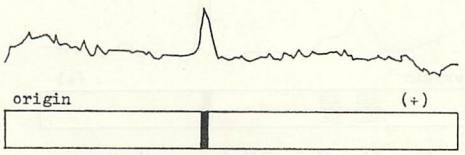

Fig. 16. C-8 (Bivalent minerals deficiency)

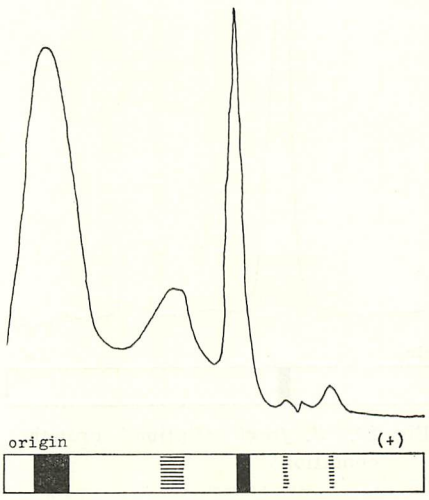

Fig. 17. C-32. (High growth temperature)

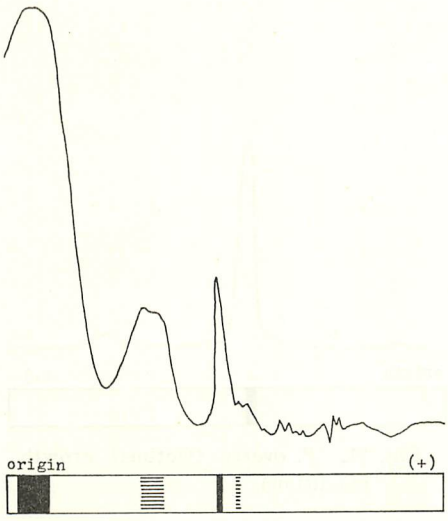

Fig. 18. C-32. (Low pH)

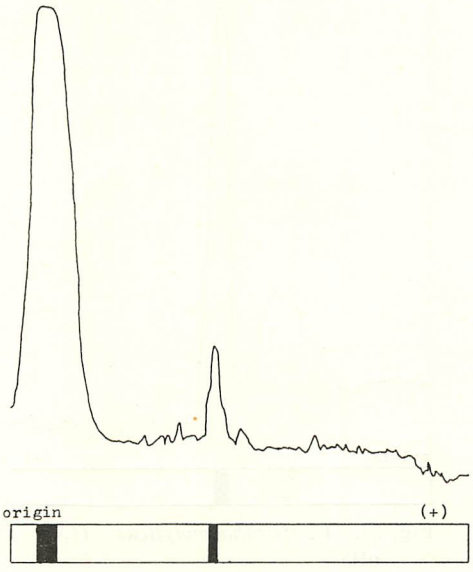

Fig. 19. C-32 (Bivalent minerals deficiency)

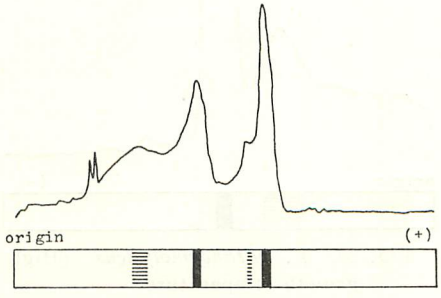

Fig. 20. *P. aeruginosa* (Opimal growth condition)

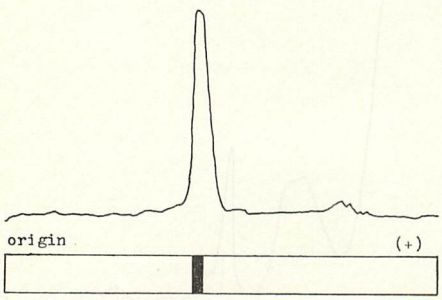

Fig. 21. *P. ovaris* (Optimal growth condition)

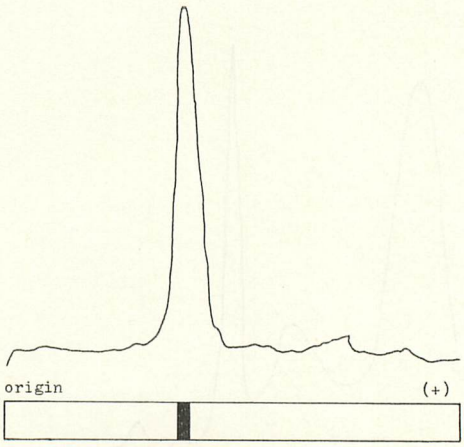

Fig. 22. *P. fragi* (Optimal growth condition)

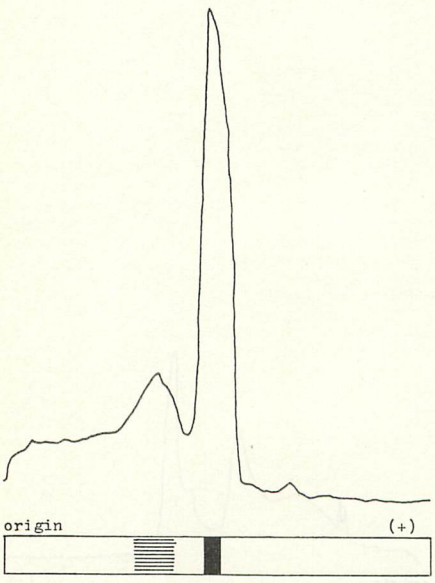

Fig. 23. *V. parahaemolyticus* (High growth temperature)

Fig. 24. *V. parahaemolyticus* (Low pH)

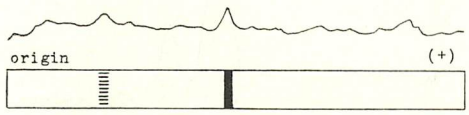

Fig. 25. *V. alginolyticus* (High NaCl concentration)

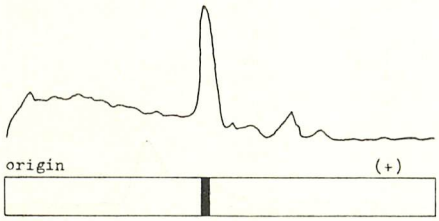

Fig. 26. *V. alginolyticus* (High growth temperature)

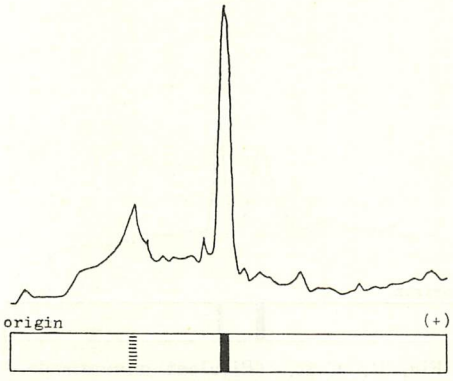

Fig. 27. *V. alginolyticus* (Low pH)

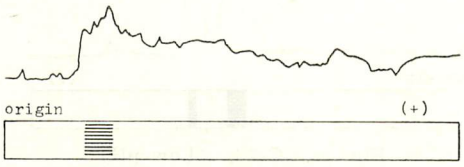

Fig. 28. *V. alginolyicus* (Bivalent minerals deficiency)

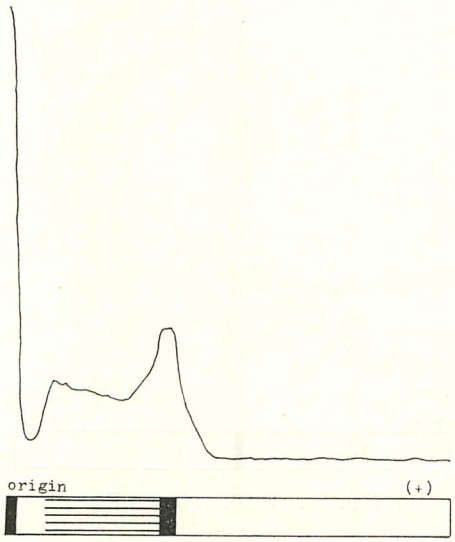

Fig. 29. C-12. (High NaCl concentration)

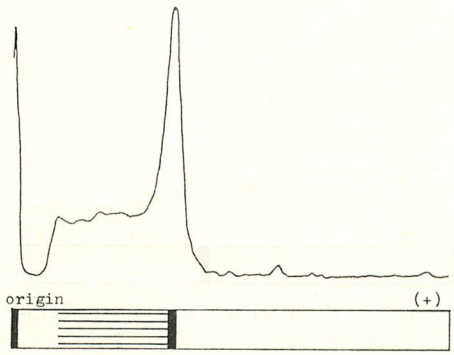

Fig. 30. C-12. (Low pH)

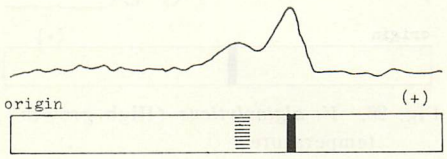

Fig. 31. C-13 (High NaCl concentration)

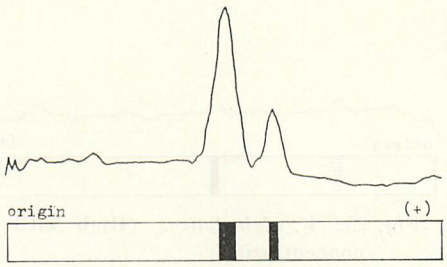

Fig. 32. C-13. (High growth temperature)

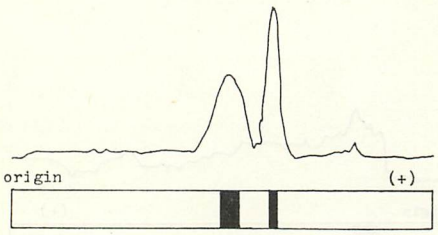

Fig. 33. C-13. (Low pH)

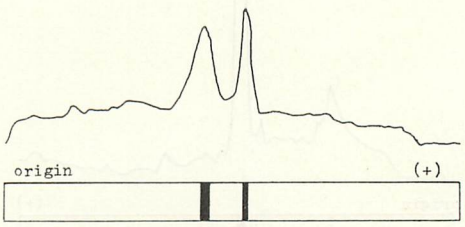

Fig. 34. C-13. (Bivalent minerals deficiency)

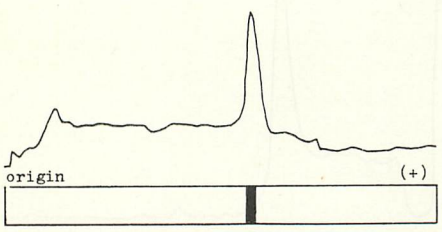

Fig. 35. C-28 (High growth temperature)

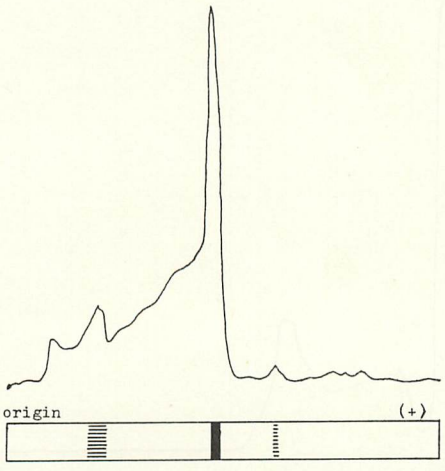

Fig. 36. C-28. (Low pH)

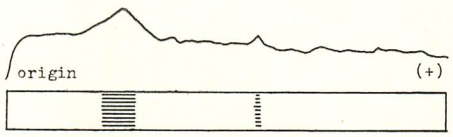

Fig. 37. C-29. (High NaCl concentration)

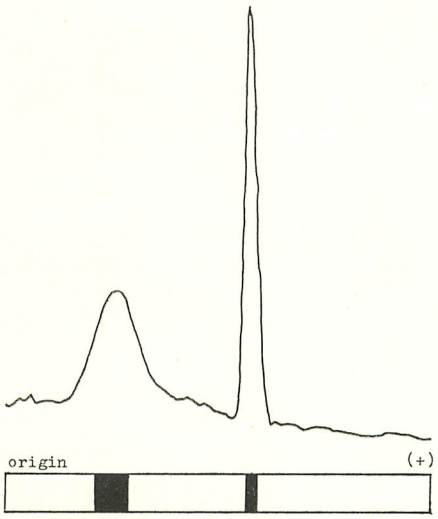

Fig. 38. C-29. (High growth temperature)

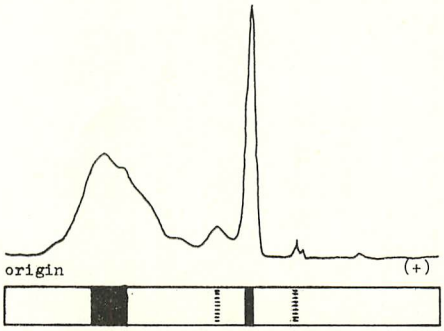

Fig. 39. C-29. (Low pH)

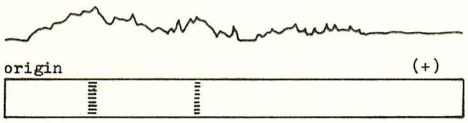

Fig. 40 C-29. (Bivalent minerals deficiency)