海洋細菌の酵素に関する電気泳動的研究 II

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Studies on the Electrophoresis of Marine Bacterial Enzymes-II Esterase, Malate Dehydrogenase, and Lactate Dehydrogenase

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Electrophoretic zymograms of the three enzymes, esterase, malate dehydrogenase (MDH), and lactate dehydrogenase (LDH) were determined for 11 strains of marine isolates. For comparison, some terrestrial bacteria of the same or similar genera were also analyzed. The results obtained indicated that there were zymographic differences between the various genera, irrespective of the enzymes concerned. Clear differences in both esterase and MDH isozyme-zymograms were noticed between the marine and terrestrial specimens of *Pseudomonas*.

Among various studies on the metabolic control of living form, there are many studies of multiple form of enzymes about terrestrial bacteria, but a few about marine bacteria. Of applied studies the bacterial classification based on their characteristics of isozymal distribution has been proposed: COLWELL *et al.*,[1] suggested that marine Vibrios might be divided into two groups, toxic and nontoxic, by means of esterase isozymal zymogram. And MORITA *et al.*[2,3] reported that specific characters of the marine bacteria are observed electrophoretically on the zymograms of the three enzymes, esterase, MDH, and LDH. The authors could demonstrate in this present study that various marine isolates especially *Pseudomonads* possess their own electrophoretic isozymal patterns discriminable from terrestrial counterparts, and that, of the three enzymes used in the study, both esterase and MDH are more genus specific than is LDH.

Materials and Methods

Bacteria and incubation Marine isolates used are given in Table 1. In addition to the bacteria analysed in the previous paper,[4] *Vibrio metchinikovii* IAM 1039 from a stock collection at the Faculty of Fisheries, Hokkaido University, *Bacillus cereus var. mycoides* IFO 3836 from Institute for Fermentation, Osaka. *Bacillus subtilis* 6051, *Bacillus subtilis* 168, and *Bacillus cereus* from Faculty of Agriculture, Kyoto University, were also used in this study. The media used were ZoBell 2216 E for marine isolates, and Nutrient broth for terrestrials. Stock culturet of marine isolates were made on ZoBell 2216 E agar slope and those of terrestrials on Nutrient agar, both being stored at 10°C. Crude enzyme solutions for electrophoresis was prepared as follows. The cells were incubated for 24 hr in the brothes usually 250 m*l* at 25°C for marine isolates and at 30°C for terrestrial bacteria.

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Table 1. Bacterial strains used

No.	Bacteria		Habitat
1	*Pseudomonas*	C-1	Marine
2		C-2	
3		C-4	
4		C-5	
5		C-19	
6	*Photobacterium*	C-6	
7		C-31	
8	*Vibrio*	C-13	
9		C-18	
10		C-20	
11		C-29	
12	*Pseudomonas fluorescens*	I. F. O. 3081	Terrestrial
13	*chlororaphis*	I. F. O. 3904	
14	*aeruginosa*	I. F. O. 3080	
15	*Vibrio metschnikovii*	I. A. M. 1039	
16	*Bacillus cereus var. mycoides*	I. F. O. 3836	
17	*subtilis* 6051		
18	*cereus*		
19	*megaterium* QMB		
20	*subtilis* 168		

Then, the cells were harvested by centrifugation at 7500 rpm for 20 min, and washed twice with large amounts of 0.25 M NaCl solution. The bacterial pellet obtained was resuspended in the Tris-citrate buffer, pH 8.65, and subjected to sonic vibration at 10 Kc and 1.5 A for 3 to 10 min. After completion of the sonification, the suspension containing disrupted cells centrifuged at 7500 rpm for 40 min. The clear supernatant obtained was analyzed electrophoretically.

Electrophoresis Disc electrophoresis was performed by the method developed by DAVIS[5] and the pH 8.0 gel was used for esterase isozyme and the pH 9.2 gel for both MDH and LDH. The zymograms for each enzyme were obtained by the following stains; for esterase, 1% (w/v) α-naphthyl acetate in 2 m*l* of 50: 50 acetone/water mixture, and 50 mg Fast blue BBsalt in 50 m*l* of 0.1 M Tris-malate buffer, pH 6.4. For MDH and LDH,[6] NAD 15 mg, phenazine methosulfate 5 mg, nitro blue tetrazolium (2 mg/m*l*) 5 ml, and 0.63 m*l* of 50% *dl*-sodium lactate for LDH, or 250 mg of DL-sodium malate for MDH, dissolved respectively in 25 m*l* of 0.1 M Tris-HCl bufier, pH 8.7.

Densitometry An Ozumor 77 densitometer with a linear transport attachment was applied to analyse the stained gels. The carrying speed of sample was fixed at 20 mm/min and the wavelength was set at 570 nm.

Results

Various zymograms of esterase isozymes for both marine and terrestrial bacteria are diagrammatically shown in Fig. 1. In Fig. 2 are shown the zymograms and densitograms of esterase isozymes of marine and terrestrial representatives. As seen in these figures, distinctive differences of zymograms were observed not only between the bacteria of the same genus from both habitats, but also among the various genera irrespective of the origin. Within the same genus, the two *Photobacteria* analyzed have showed an almost identical isozymal pattern. The marine *Pseudomonas* bacteria showed comparatively similar pat terns, and so were the case with the marine *Vibrios* except C-29 strain (No. 11). On the other hand, *Bac. subtilis* 6051 (No.17)was very similar to both *Bac. megaterium* (No. 19) and *Bac. subtilis* mutant strain (No. 20) in the isozymal pattern. To the contrary, the patterns of *Bac. cereus* (No. 18) and *Bac. cereus var. mycoides* (No. 16) were dissimilar to the remainder. In comparison with the isozymes of marine isolates, these of terrestrial bacteria, in general, was greater in mobility. By those examinations, it was found that there is a distinctive character common to marine isolates: the appearance of a marked band of esterase isozyme at near Rf 0.6.

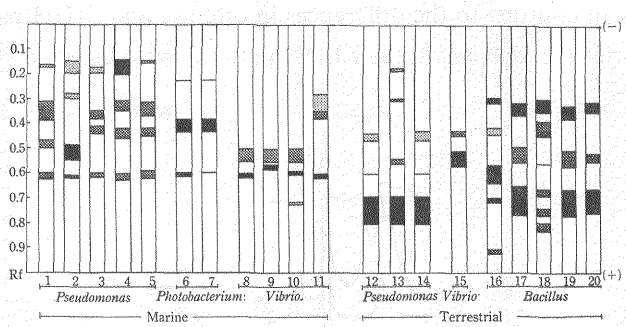

Fig. 1. Electrophoretic patterns of esterase isozymes in various bacteria. Intensities of enzyme activity: ■ strong, ■ moderate, ▨ weak

The densitograms for MDH are shown in Fig. 3, and the diagrams for LDH in Fig. 4. A marked differences between marine *Pseudomonas* and terrestrial counterparts for MDH was found: A distinct band with Rf about 0.9 was presented by the former alone. Similar differences between *Pseudomonas*

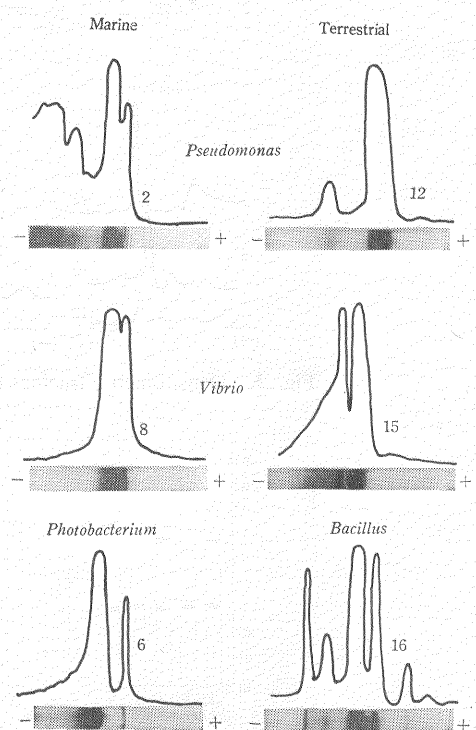

Fig. 2. Polyacrylamide gel electrophoresis: esterase isozyme patterns and their densitometric tracings in typical strains.

bacteria of both origins were observed previously with the electrophoretic patterns of soluble proteins.[7] In the two *Photobacteria* the MDH was completely identical to each other, both having an intense band near Rf 0.8. On the other hand, MDH isozymes of marine *Vibrios* differed a little from those of marine *Photobacteria* and *Pseudomonads*. Marine *Vibrios* possessed less bands of MDH isozymes than *Pseudomonads*. MDH isozyme patterns of *Bacillus* species were extremely variable.

In contrast to the differences in esterase and MDH patterns between marine and terrestrial *Pseudomonads*, their LDH patterns were hardly distinguishable from each other.

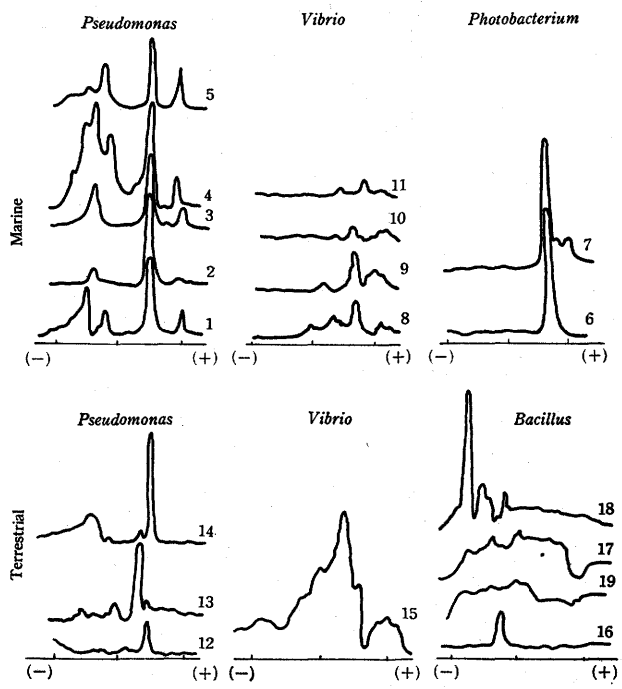

Fig. 3. Densitometric tracings of electrophoretic patterns for isozymes.

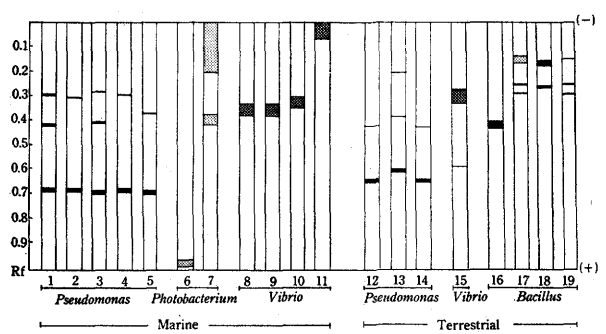

Fig. 4. Electrophoretic patterns of LDH isozymes in various bacteria. Intensities of enzyme activity:
■ strong, ■ moderate, ▩ weak

Discussion

HOGAN and COLWELL[8] suggested that by applying the isozyme analysis combined with assessment of GC %, an information could be obtained which is useful for not only taxonomy of deep sea bacteria, but also for comparison with terrestrial counterparts. We would agree with them, since according to the present examinations, many marine *Pseudomonads* are distinguishable from the same terrestrial genus by analyzing esterase isozyme. BURTO and MORITA[9] observed the rapid inactivation of MDH for a certain psychrophile at 30°C, and EDWARDS and RETTER[10] reported similar phenomena for methophiles slightly above the maximum growth temperature, both evidencing that their MDH isozymes are temperature sensitive. The incubation temperature adopted in the present examinations was different between marine and terrestrial bacteria because of the difference of their optimal growth temperature. Accordingly, we could not obtain further information which might be derived from the experiment done at the same incubation temperature. However, it could be concluded from the present results that marine and terrestrial bacteria are satisfactorily classified on the basis of characteristic isozymal patterns of MDH and esterase.

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