NEW POLYENIC ANTIBIOTICS ACTIVE AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

IX. RECLASSIFICATION OF A STRAIN W-315 PRODUCING ENACYLOXINS

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Strain W-315 produces a family of new polyenic antibiotics named enacyloxins[1~3], which are active against Gram-positive and Gram-negative bacteria.

In our earlier paper[4], we identified the producing strain according to the 8th edition of BERGEY's Manual of Determinative Bacteriology[5]. Strain W-315 was shown to be an "intermediate" strain among the genera *Gluconobacter*, *Pseudomonas* and *Acetobacter*[6]. Since strain W-315 had characteristics similar to *Gluconobacter*, except for the absence of ubiquinone-10 (Q_{10}), we tentatively identified strain W-315 as a *Gluconobacter* sp. Recently, we reexamined the generic placement of strain W-315 utilizing the additional data found in the new edition of BERGEY's Manual of Systematic Bacteriology[7].

In this paper, we propose that strain W-315 be transferred to the genus *Frateuria*.

YAMADA *et al.*[8] characterized the isoprenoid quinones of polarly flagellated intermediate strains of acetic acid bacteria; COLLINS *et al.*[9] determined the distribution of menaquinones in actinomycetes and corynebacteria. Both papers pointed out the importance of isoprenoid quinones for the classification of bacteria. We, therefore, analysed the isoprenoid quinones of strain W-315 because this was not done for the earlier paper[4]. Isoprenoid quinones were extracted and purified according to the method of COLLINS *et al.*[10]. The mass-spectrum of the isoprenoid quinones was obtained on a JEOL DX-303HF spectrometer. As shown in Fig. 1, there was a main peak at *m/z* 726 in the higher mass region and two other intensive peaks in the middle mass region, *i.e.* at *m/z* 235 and 197. The latter two peaks correspond to the pyrilium and benzelium ions, respectively, which are produced by the characteristic decomposition of ubiquinone molecule[11].

Since ubiquinone-8 (Q_8) exhibits a parent mass ion of 726, the principal isoprenoid quinone of strain W-315 would be expected to be Q_8. To verify this possibility, we carried out high resolution mass spectrometry and obtained a mass ion of *m/z* 726.5622. This value is in good agreement to that of Q_8 (calcd. 726.5587). In conclusion, we assigned the isoprenoid quinone of strain W-315 to Q_8.

"Intermediate" strains of acetic acid bacteria are divided into two groups, the one having peritrichous flagella and the other having polar flagella[6]. The "intermediate" strains which have polar flagella have Q_8 as the major quinone component, while those which have peritrichous flagella have Q_9 (genus *Acetobacter* except *A. xylinum*), and Q_{10} (genus *Gluconobacter* and *A. xylinum*)[8,12].

SWINGS *et al.* proposed a new genus *Frateuria* for *Acetobacter aurantius* which had polar flagella and Q_8[13] as the major quinone component. Sub-

Fig. 1. Mass-spectrum of isoprenoid quinone isolated from strain W-315.

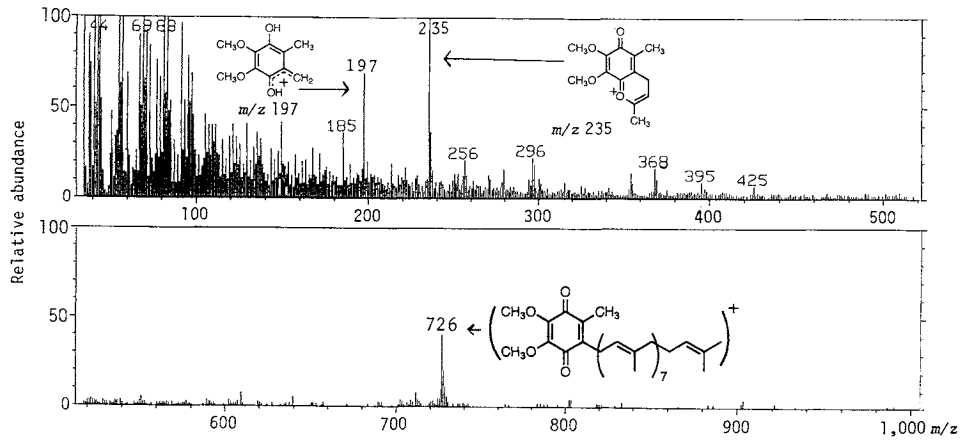

Table 1. Characteristics of strain W-315 compared with those of the genera *Frateuria*, *Gluconobacter* and *Acetobacter*.

Characteristics	W-315	*Frateuria**	*Gluconobacter**	*Acetobacter**
Flagellation	Polar	Polar	Polar or none	Peritrichous
Pigmentation	Yellow	Brown	None or brown	None or brown
Oxidation of lactate to CO_2	+	+	−	+
Production of acetic acid from ethanol	+	+	+	+
Ketogenesis	+	+	+	D
Growth factor required	−	−	+	D
Formation of H_2S	−	+	−	−
Ubiquinone	Q_8	Q_8	Q_{10}	Q_9 or Q_{10}
Growth in presence of 30% D-glucose	+	+[a]	−	−
Growth on FRATEUR's Hoyer mannitol medium	+	+	−	−
Acid produced from:				
D-Arabinose	+	+	+	−
Inositol	+	+	D	−
Maltose	−	−	D	−
D-Fructose	+	D	+	−
Carbon sources for growth:				
D-Mannose, L-arabinose, D-lyxose, L-lyxose	+	+	−	−
GC content (mol%)	64.4	62～64	57～64	51～65

[a] Some strains are negative.
D: Different reactions in different taxa.
* From the BERGEY's Manual of Systematic Bacteriology. Vol. 1, pp. 210～213, 1984.

sequently, BERGEY's Manual of Systematic Bacteriology was published and genus *Frateuria* was established[7]. Strain W-315 closely resembles the new genus *Frateuria*, although some of its physiological properties, *i.e.* oxidase,oxidation of D-lactate and H_2S production, are different from those of published *Frateuria*[7]. We reexamined the physiological properties of strain W-315 by the methods of SHIMWELL *et al.*[14], SWINGS *et al.*[13], YAMADA *et al.*[8] and WATANABE *et al.*[4].

Oxidase activity, detected by test papers containing tetramethyl-*p*-phenylenediamine, was negative and decomposition of D-lactate to carbonate, observed by crystallization of calcium carbonate around the streaks on agar plates, was positive. The strain showed good growth ($OD_{660} > 1.0$) in a medium composed of 5% glucose and 0.5% yeast extract, at pH 3.6. H_2S production, carried out on TSI Agar (Nissui), was not detected. Since the type strain of *Frateuria aurantium* (IFO 3245) produced H_2S[7], we tried to detect H_2S production in both strains under the same conditions. Neither strain W-315 nor strain IFO 3245, produced H_2S. Other physiological properties of strain W-315 were the same as those published in our earlier paper[4].

The results summarized in Table 1 indicate that strain W-315 has the same characteristics as those of genus *Frateuria* except for the absence of H_2S production. From these results, we concluded that strain W-315 belongs to genus *Frateuria*.

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