

Isoprenoid Quinone Profiles of the *Leclercia adecarboxylata* KCTC 1036^T

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The isoprenoid quinone composition of *Leclercia adecarboxylata* KCTC 1036^T was determined by using high-performance liquid chromatography. *L. adecarboxylata* KCTC 1036^T are characterized by their production of both ubiquinone-7, ubiquinone-8 and menaquinone-8 as major quinones. It is clear that the analysis of isoprenoid quinone profiles provides a new criterion of great promise for identifying *Leclercia* strains.

The genus *Leclercia* first isolated from foods is a member of the family *Enterobacteriaceae*, formerly named *Escherichia adecarboxylata*, on the basis of its IMViC test and yellow pigment production (9). In 1972, *E. adecarboxylata* was described as a synonym of *Enterobacter agglomerans* (4). On the other hand, *E. agglomerans* is very heterogenous in terms of biochemical reactions (5) and DNA hybridization studies (2). In 1986, *Escherichia adecarboxylata* was phenotypically and genetically distinguished from *Enterobacter agglomerans* and transferred to the newly created generic name *Leclercia* which has only one species, *L. adecarboxylata* (12). However, identification of genus *Leclercia* remains problematic due to limited taxonomic information.

Chemotaxonomic analyses have proved of value in the characterization of a wide variety of prokaryotes (6). These characteristics are of increasing importance in the delineation of prokaryotic taxa (11). The value of isoprenoid quinone analyses had been discussed by Collins and Jones (3). In this report, the isoprenoid quinone composition of *L. adecarboxylata* KCTC 1036^T (T=type strain) was analyzed, and the results are presented and discussed.

L. adecarboxylata KCTC 1036^T (=ATCC 23216) was obtained from the Korean Collection for Type Cultures (KCTC), Korea Research Institute of Bioscience and Biotechnology, Korea Institute of Science and Technology (Taejon, Korea). The strain used for quinone analysis was cultured aerobically on nutrient broth (Difco 0003-17-8) at 30°C.

Cells were harvested from cultures at the early stationary phase of growth and washed with 1% saline solution. Quinones were extracted with chloroform/methanol (2:1, v/v) from fresh wet cells (7), evaporated in vacuum, and purified by thin-layer chromatography (TLC) on Merck Kieselgel 60 F₂₅₄ plates (20×20 cm, 0.5 mm thickness) using a mixture of petroleum ether and diethyl ether (9:1, v/v) as the developing solvent. Quinone bands were detected under short wavelength ultraviolet, scraped from the TLC plate, and recovered in acetone as previously described (7, 8). High-performance liquid chromatography was used to determine the isoprenoid quinone composition of the strain. The analytical systems used were as follows: instrument, Hitachi L-5000 LC Controller; pump, Hitachi L-6000; column, YMC-Pack ODS-AM (4.6×150 mm, YMC Co., Ltd., Japan); column temperature, 30°C; eluent, methanol/isopropyl ether (3:1, v/v) for ubiquinone, methanol/isopropanol (7:5, v/v) for menaquinone; flow rate, 1ml/min. Ubiquinones and menaquinones were detected by monitoring at 275 nm and 270 nm, respectively, with a Tosoh UV-8011 detector. Data were analyzed by using a Hitachi D-2000 chromato-Integrator. In this study, ubiquinones and menaquinones with *n* isoprene units were abbreviated Q-*n* and MK-*n*, respectively.

Authentic ubiquinones were obtained from Sigma Chemical Co. or prepared from known bacterial cultures. Menaquinone standards were prepared from known bacterial cultures.

Fig. 1 shows the separation of ubiquinones (a) and menaquinones (b) by HPLC. The quinone compositions determined by HPLC are shown in Table 1. *L. adecarboxylata* KCTC 1036^T were characterized by their

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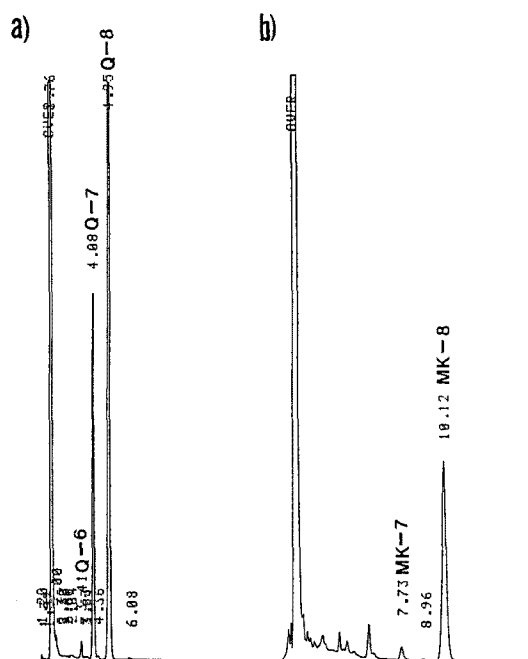


Fig. 1. HPLC chromatogram of (a) ubiquinones and (b) menaquinones from *Leclercia adecarboxylata* KCTC 1036^T.

Table 1. Quinone composition of *Leclercia adecarboxylata* KCTC 1036^T.

Strain	Ubiquinone homologue (%) ^a			Menaquinone homologue (%)	
	Q-6	Q-7	Q-8	MK-7	MK-8
<i>L. adecarboxylata</i> KCTC 1036 ^T	1	24	75	5	95

^aData were represented as percentage of total peak area.

production of both Q-7, Q-8 and MK-8 as major quinones. The family *Enterobacteriaceae* strains contained Q-8 and MK-8 as major quinones. Gram-negative marine rod bacteria, *Shewanella* contained Q-7, Q-8, MK-7, and methylmenaquinone-7 as major quinones, apart from *S. hanedai* IAM 12641^T (Q-7, Q-8, and MK-7) and *S. putrefaciens* IAM 13596 (Q-7 and Q-8) (1, 10). Therefore, the presence of Q-7, Q-8 and MK-8 has not been observed in any members of the family *Enterobacteriaceae* except *L. adecarboxylata*. It is concluded that the analysis of isoprenoid quinone profiles provides rapid and clear criteria to distinguish *Leclercia* from other

species of *Enterobacteriaceae*.

REFERENCES

1. Akagawa-Matsushita, M., T. Itoh, Y. Katayama, H. Kurish, and K. Yamasato. 1992. Isoprenoid quinone composition of marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J. Gen. Microbiol.* **138**: 2275-2281.
2. Brenner, D. J., B. R. Davis, A. G. Steigerwalt, C. F. Riddle, A. C. McWhorter, and G. R. Fanning. 1982. Atypical biogroups of *Escherichia coli* found in clinical specimens and description of *Escherichia hermannii* sp. nov. *J. Clin. Microbiol.* **15**: 703-713.
3. Collins, M. D. and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* **45**: 316-354.
4. Ewing, W. H. and M. A. Fife. 1972. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the Herbicola-Lathyri bacteria). *Int. J. Syst. Bacteriol.* **22**: 4-11.
5. Farmer III, J. J., M. A. Asbury, F. W. Hickman, D. J. Brenner, and the *Enterobacteriaceae* Study Group. 1980. *Enterobacter sakazakii*, a new species of "*Enterobacteriaceae*" isolated from clinical specimens. *Int. J. Syst. Bacteriol.* **30**: 569-584.
6. Goodfellow, M. and D. E. Minnikin. 1985. Introduction to chemosystematics. p. 1-16. In Goodfellow, M. and D. E. Minnikin. (ed.), *Chemical Methods in Bacterial Systematics*. Academic Press, London.
7. Hiraishi, A., Y. K. Shin, and J. Sugiyama. 1992. Rapid profiling of bacterial quinones by two-dimensional thin-layer chromatography. *Lett. Appl. Microbiol.* **14**: 170-173.
8. Hiraishi, A., Y. K. Shin, J. Sugiyama, and K. Komagata. 1992. Isoprenoid quinones and fatty acids of *Zoogloea*. *Antonie van Leeuwenhoek* **61**: 231-236.
9. Leclerc, H. 1962. Etude biochimique d'enterobacteriaceae pigmentees. *Ann. Inst. Pasteur* **102**: 726-741.
10. Moule, A. L. and S. G. Wilkinson. 1987. Polarlipids, fatty acids, and isoprenoid quinones of *Alteromonas putrefaciens* (*Shewanella putrefaciens*). *Syst. Appl. Microbiol.* **9**: 192-198.
11. Murray, R. G. E., D. J. Brenner, R. R. Colwell, P. De Vos, M. Goodfellow, P. A. D. Grimont, N. Pfennig, E. Stackebrandt, and G. A. Zavarzin. 1990. Report of the Ad Hoc committee on approaches to taxonomy within the *Proteobacteria*. *Int. J. Syst. Bacteriol.* **40**: 213-215.
12. Tamura, K., R. Sakazaki, Y. Kosako, and E. Yoshizaki. 1986. *Leclercia adecarboxylata* gen. nov., comb. nov., formerly known as *Escherichia adecarboxylata*. *Curr. Microbiol.* **13**: 179-184.

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