101 Identification of Lactobacillus plantarum and Lactobacillus pentosus by photobiotin labeling DNA-DNA hybridization

°Somboon Tanasupawat, Takayuki Ezaki,* Ken-Ichiro Suzuki,** Sanae Okada,*** Kazuo Komagata,*** and Michio Kozaki (Department of Agricultural Chemistry and *Nodai Research Institute, Tokyo University of Agriculture, Sakuragaoka 1-1-1, Setagaya-ku, Tokyo 156; **Department of Microbiology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500; ***Japan Collection of Microorganisms, RIKEN, Wako-shi, Saitama 351-01)

The homofermentative lactobacilli were found in many kinds of fermented foods in Thailand (1). Of 35 isolates, 32 contained meso-diaminopimelic acid (meso-$A_2$pm) in the cell wall and were similar to Lactobacillus plantarum. Recently, Lactobacillus pentosus was separated from Lactobacillus plantarum by DNA-DNA homology (2). Our study deals with identification of the isolates based on chemosystematic characteristics and photobiotin labeling DNA-DNA hybridization. The result reveals that 10 isolates were designated as Lactobacillus plantarum and 21 isolates were Lactobacillus pentosus. One isolate containing meso-$A_2$pm in the cell wall and 3 isolates containing lysine in the cell wall could not be identified as any known species. Differential characteristics including cellular fatty acid composition and DNA base composition of the isolates will be reported. The ecological distribution of these rod-shaped lactic acid bacteria from fermented foods in Thailand will be also discussed.

References: 1) S. Tanasupawat. J. Graduate School. Chulalongkorn University. 5, 84 (1984).
2) P. Zanoni, J. A. E. Farrow, B. A. Phillips, and M. D. Collins. Int. J. Syst. Bacteriol. 37, 339 (1987).

102 **Protoplast formation and regeneration in *Haematococcus pluvialis***

°Agus Eko Tjahjono, Yachiyo Hayama, Toshihide Kakizono, Naomichi Nishio and Shiro Nagai (Dept. of Fermentation Technology, Fac. of Engineering, Hiroshima University)

A unicellular green alga *Haematococcus pluvialis* has recently been focused as astaxanthin producer. Astaxanthin, a ketocarotenoid is widely employed as a red colorant for fish aquaculture. As one of the possible candidates for the industrial production, several growth characteristics such as growth rate and temperature, and light requirement of the alga have to be improved. Protoplast fusion between homothallic alga like *Haematococcus* provides a method of combining favorable genomes for the carotenoid biosynthesis. It is thus of interest whether protoplast formation and regeneration system of the alga can be established for the genetic improvement.

*Haematococcus* cells were mixotrophically grown in an acetate medium under 12 h L/ 12 h D illumination cycle using either white light or blue light. Protoplast formation was carried out in the reaction mixture composed of the intact algal cells, sorbitol and mannitol mixture as an osmotic regulator, and commercial protease, pH 7.8, and incubated at 35°C. The frequency of the protoplast formation was determined as cell number decrease upon transfer of the enzyme treated cells into 0.02 % Triton X-100. Protoplast regeneration was conducted by step-wise reduction of the osmotic regulator concentrations in the protoplast formation mixture. Then, the protoplasts were spread on agar plate containing various concentrations of the osmotic regulator.

Proteinase K (0.06 %) was found to be most effective for the protoplast formation. When the algal cells were grown under white light, the frequency of protoplast formation was about 50-55%. Blue-light grown culture exhibited higher yield of protoplast formation up to 70 %. The presence of 40-60 mM L-arginine stabilized the protoplast upon reduction of the osmotic regulator. The protoplasts were most successfully regenerated into the vegetative cells on the agar plates containing 0.05 M sorbitol and mannitol. Without the osmotic regulator, only a small number of colony were formed on the plate. The step-wise reduction of the osmotic regulator concentrations enabled the protoplasts to regenerate at the higher frequency.