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〈TECHNICAL NOTE〉

A New Synthetic Medium for Lactic Lactococci: Application to Marine Lactic Acid Bacteria

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Lactococcal cells are nutritionally fastidious and thus, generally cultured either in milk or M17 medium (Terzaghi and Sandine, 1975). In this study, *Lactococcus cremoris* wild-type (KH) and its less-proteolytic mutant (KHA1) cells were grown on the M17 medium or with modified M17 medium by replicated parallel experiments. The modified M17 medium had the same composition as M17 medium, except that lactose was replaced by glucose. Analyses of culture-broth samples, in which the M17 and the modified M17 media were used, were conducted by high-performance liquid chromatography (HPLC). But, working with these media created noisy problems in analyses of samples. Therefore, a new semi-synthetic medium was developed on the basis of nutritional requirements (Morishita et al., 1981). The composition of the semi-synthetic medium determined on the basis of the nutritional requirements and the composition of milk, is presented in Table 1. The composition of M17 medium is also presented and compared in the table.

L. *cremoris* KH and KHA1 cells were grown again on the new synthetic medium containing glucose or lactose. The broth samples were then drawn and analyzed by HPLC. Clearer separations of fermented products were achieved from the new medium than those with the M17 and the modified M17 media. In comparison with the M17 or the modified M17 media, growth on the new medium was good (Kim et al., 1993). Additional fermentations were also carried out at a controlled pH of 7.0, where enhanced growth of lactococcal cells was obtained. In the fermentations, samples were also analyzed for the concentrations of sugar and lactic acid. The results showed that the new synthetic medium was as good as or better than the M17 and the modified M17 media. This is because casein hydrolysate in the synthetic medium provided a ready supply of amino acids and peptides for L. *cremoris* KH and KHA1 cells.

Lactic acid bacteria (LAB) including Lactococcal cells have been known to be an effective means of preserving foods, at the same time as giving particular tastes in fields of dairy products. LAB also have always occupied an important place in the technology of sea products, and marine LAB have known to be present in traditional fermented products (Ohhira et al., 1988). To apply the new synthetic medium to marine LAB, two different LAB were isolated from pickled anchovy and pollacks caviar and were grown on the new media in which various concentrations of NaCl (3, 5, 7 and 10%) added. They were also grown on the medium solution in natural seawater (35‰ salinity) and on the solution of natural seawater itself, too. As seen in Fig. 1, Marine LAB were grown best on the synthetic medium solution in natural seawater and the higher concentrations of NaCl were added to the medium, the longer lag-phase of growth profile appeared. Marine LAB in natural seawater were not grown well. From these results, the synthetic medium seems good to cultivate cells which are essential to get salted fish aged.

In this study, it showed that the new synthetic medium provided adequate nutrition for *L. cremoris* KH and KHA1 cells, which have been used as cheese starters (Stadhouders et al., 1988). Using this new medium, the acid production capability of starter cultures could be also measured quantitatively. Thus, this new medium was inferior to the M17 or the modified M17 medium in culturing the cheese starters and in measuring fermentation characteristics of the starter cells. Moreover, this new medium found to be good for selected and well-identified marine LAB which are used in rapid fermentations of low-salted fish.

Key words : synthetic medium, marine lactic acid bacteria, fermentation, low-salted fish

Table 1. The compositions of a new semi-synthetic medium and the M17 medium (per liter of solution in distilled water)

New medium	M17 medium
Lactose, 5.0 g	Lactose, 5.0 g
Yeast extract, 1.0 g	Yeast extract, 2.5 g
NaH_2PO4, 147.10 mg	β−disodium glycerophosphate, 19.0 g
$MgSO_4.7H_2O$, 76.02 mg	1 M−$MgSO_4.7H_2O$, 1 ml
Ascorbic acid, 6.75 mg	Ascorbic acid, 0.5 g
Nicotinic acid, 1.0 mg	Beef extract, 5 g
$FeSO_4.7H_2O$, 0.622 mg	Polypeptone, 5 g
Thiamine, 0.25 mg	Phytone peptone, 5 g
$CaCl_2.2H_2O$, 18.34 mg	
Casein hydrolysate, 2.5 g	

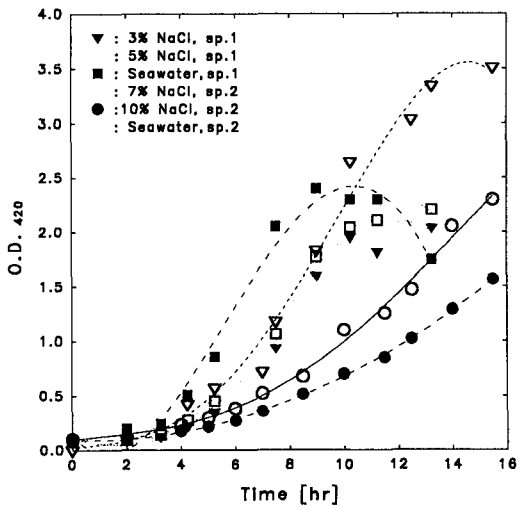

Fig. 1. Optical density of marine LAB on new synthetic medium at 420 nm.

References

Kim, J. K., R. K. Bajpai and R. T. Marshall. 1993. Characterization of growth and enzymes produced by prt$^+$lac$^+$and prt$^-$ lac$^-$ *Lactococcus lactis* cells. Appl. Biochem. Biotechnol., 39, 265.

Morishita T., Y. Deguchi, M. Yajima, T. Sakurai and T. Yura. 1981. Multiple nutritional requirements of lactobacilli : Genetic lesions affecting amino acid biosynthetic pathways. J. Bacteriol., 148, 64.

Ohhira, L., M. C. Chan, T. Miyamoto, K. Kataoka and T. Nakae. 1988. Identification of lactic acid bacteria isolated from traditional side-dish fermented foods in southeast Asia. Jap. J. Dairy Food Sci., 37, 185.

Stadhouders, J., L. Toepoel and J. T. M. Wouters. 1988. Cheese making with prt$^-$ and prt$^+$ variants of N-streptococci and their mixtures. Phage sensitivity, proteolysis and flavour development during ripening. Neth. Milk Dairy J., 42, 183.

Terzaghi, B. E. and W.E. Sandine. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol., 29, 807.

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