Single Cell Protein (SCP) Production using Waste Milk as a Substrate by Flower Yeasts

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（花酵母による廃棄乳からのシングルセルプロテイン（SCP）の生産）

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Abstract

Waste milk is a major by-product of the dairy industry and includes low-quality colostrum, transitional milk, and unqualified commodity milk. Waste milk contains a lot of nutrients such as fat, lactose, casein, vitamins, and minerals. Hence, the large amount of discarded milk generates environmental pollution and represents a significant loss of a valuable resource. Attempts to use waste milk including as Liquid feed and for methane fermentation. However, the former is not suitable for long-term storage and transport, while the latter inhibits biogas production because of the high milk fat content. Thus, this study aimed to address the waste milk problem by bioconverting waste milk to Single-cell protein (SCP) using unexplored natural yeast isolates.

For public approval purposes, the isolation source was restricted to local flowers in Tsukuba city, Japan. The isolates were identified by sequencing of the D1/D2 domain of the 26S rDNA encoding gene. To reduce pollution, flower yeasts capable of decomposing the main nutrients of waste milk (fat, lactose, and casein) were screened and selected for mixed culture. The isolates were also evaluated by total organic carbon (TOC) removal efficiency. Further, the isolate that can produce bioactive compounds (carotenoid) was selected to increase product value. The effect of monoculture, mixed culture, and sequential culture on SCP production was also studied. Also, the nutrient content and amino acid profile of the selected yeasts biomass were analyzed. *Kluyveromyces lactis* TY-98 and *Rhodotorula graminis* TY-99 were selected for a mixed culture. These two strains were isolated from baby blue eyes flower (*Nemophila menziesii*) and Tsubaki flower (*Camellia* spp.), respectively. TOC reduction of waste milk by the mixed culture (88%) was higher than that of TY-99 monoculture (33%) and TY-98 monoculture (73%). Although the cell count of TY-98 was relatively higher in mixed culture than in the TY-98 monoculture, TY-99 growth was inhibited in mixed culture. Thus, some attempts such as optimization of incubation temperature, initial pH, mixed population inoculum with different ratios of TY-98: TY- 99, and sequential culture were made. The sequential culture was found to be effective for the growth of

the selected yeasts, but, low in TOC reduction (67%). The results indicated that mixed culture under optimized conditions (incubation temperature 30 °C, initial pH 6.0, and 1:10 ratio of a mixed TY-98: TY-99 inoculum) produced high SCP amount (43.8 g/L) containing carotenoid (1.8 mg/L) and also high in TOC reduction (88%).

To increase TY-99 carotenoid production, attempts to optimize the culture conditions such as initial pH, aeration rates, temperature were made. The effect of irradiation and activator on carotenoid production were also studied in flask cultures. Further, carotenoid production in laboratory fermenter was studied and it produced 3 fold higher carotenoid than that of flask culture. According to the nutrient analysis, the essential amino acids profile and protein content of selected isolates biomass meet the requirements recommended by FAO. Further, they contained a considerable amount of essential amino acids especially Lysine and Threonine (limiting essential amino acids in natural feed such as wheat and corn). Therefore, they can be used as Lysine and Threonine rich -feed or feed additives. Besides protein, yeast biomass also contained some amount of fat, ash, and fiber which also contribute to the overall nutritional product value. Hence, SCP of this study may be beneficial to some special animal feed application depending on the nutritional requirements. Since the cell mass of selected isolates also contained carotenoids, it can be used not only as a Lysine rich-protein source or coloring compound but also as a vitamin supplement because of the provitamin A function of carotenoids. Furthermore, the dairy wastewater problem can be solved together with the bioconversion of casein, fat, and lactose of waste biomass to high-grade feed.