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**Growth Characteristics of Uncultured Nitrite–Oxidizing Nitrospira**

HOIROTANIGI, YOSHIJITAN AO, SATOSHI TSUNEDA

1Department of Life Science and Medical Biobio, Waseda University, 2Department of Biology, Northeastern University

Key Word: Cultivation, Nitrification, Cell Sorter, Growth Rate

Nitrospira species are numerically dominant and carry out nitrite oxidation in both engineered system and natural environment. Nevertheless, the knowledge of Nitrospira species is limited because the genus Nitrospira contains only three described and cultured species. The cultivation and isolation of various phylotype are still required for more comprehensive understanding of physiological properties. In this study, a new approach to obtain highly enriched culture of Nitrospira was conducted, that is a combination of 1) cultivation of Nitrospira using continuous feeding reactor, and 2) separation of Nitrospira cells using the “cell sorting system”. The Nitrospira species from activated sludge were enriched by continuous feeding of nitrite as substrate. During the enrichment procedure, the abundance of the Nitrospira-like bacteria gradually increased up to over 80% of the total bacterial population. As a result of applying the enriched sample to cell sorting system, Nitrospira microcolonies were successfully separated from planktonic microbial cells (mainly heterotrophic bacteria) under fluorescent microscopic observation. However, separated Nitrospira cells were uncultivated on agar or gelan gum plates again, whereas other microorganisms adherent to microcolonies were cultivated. The results of subculture indicate that the presence of low numbers of contaminants cannot be excluded as the detection limit of FISH is 10^−4 to 10^−6 cells ml^−1. To achieve the final goal of this study, isolation of novel uncultured Nitrospira, growth characteristics of Nitrospira were investigated by applying enriched samples in continuous feeding reactor to batch reactor. In consequence, excess nitrite accumulation was found to inhibit growth activity of Nitrospira.

e-mail: f-hirotsugui@fuji.waseda.jp

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**Characterization of clostridia able to grow in sweet sorghum juice and produce butanol**

OMIHO KANEMOTO, RAHMAN HABINUR, SHIGERU CHOHNAN, YOJII NITTA, YASUROU KURUSU, HIROYUKI OHTA

Ibaraki University College of Agriculture

Key Word: biofuel, sweet sorghum, butanol production, clostridia

Bio-fuels are the promising carbon-neutral materials to reduce the increased level of atmospheric carbon-dioxide due to the huge use of fossil fuel. However, the bio-ethanol production using food crops such as corn and sugarcane has induced the food crisis and also the environmental deterioration such as deforestation. To solve this problem, we have developed a new bio-fuel production system in which the cultivation of sweet sorghum, a non-food crop, in the abandoned farm land is combined with the production of bio-alcohol from its sugar-containing juice. Here we focus on the bio-butanol production by new clostridia isolates grown with sweet sorghum juice because butanol is less corrosive and has higher caloric value than ethanol.

The source of isolation was the upland soil collected from a sweet sorghum field, Ibaraki University College of Agriculture. Enrichment cultures were made by heating the soil suspension at 80°C for 15 min and incubating with the sweet sorghum juice. Isolates were characterized by phenotypic and phylogenetic analyses.

The enrichment resulted in the selective growth of Gram-positive, spore-forming, butanol-producing bacteria. Two strains named SBP1 and SBP2 were isolated from the culture and identified as Clostridium beijerinckii by 16S rRNA gene analysis. The ratio of the amount of butanol produced to that of sugar consumed was 0.48 and 0.51(mol/mol) for strains SBP1 and SBP2, respectively. The optimum temperature for butanol production was 35°C. Noteworthy was that these strains were highly resistant to lignin-derived phenolic compounds (ferulic acid and p-trans-coumaric acid) comparing with a reference strain, Clostridium beijerinckii JCM 1390. To increase butanol production, butanol-tolerant variants are now selected from the strains.

e-mail: 10am207facs.ibaraki.ac.jp