1P-228 Analysis of pathogenic bacteria from the microbial community of organic hydroponic culture

○ Sakuntala Saijai[1], Akinori Ando[1,2], Ryuya Inukai[1], Hisanori Mizobuchi[1], Narumi Ikemoto[1], Shoko Usami[1], Jun Shima[3], Kenji Miyamoto[4], Yasuo Kato[5], Susumu Asakawa[6], Makoto Shinohara[7], Jun Ogawa[1,2]
([1]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., [2]Res. Unit Physiol. Chem. Kyoto Univ., [3]Ryokoku Univ., [4]Keio Univ., [5]Biotechnol. Res. Center, Toyama Pref. Univ., [6]Grad. Sch. Bioagric., Sci., Nagoya Univ., [7]NARO)
sakuntala.saijai.23m@st.kyoto-u.ac.jp

Recently, organic hydroponic becomes one of the interesting techniques for hydroponic culture because it decrease environmental load and provide an efficient cultivation. Organic hydroponic consists of two processes. The first process is nitrification resulted from enrichment of nitrifying microorganisms. The second process is crops cultivation with maintaine the enriched nitrifying microbs in hydroponic solution. However, enriched microbes have to be assessed the biological safety in term of pathogenicity. Therefore, pathogenic bacteria in encriched microbes was investigated in this study. The commercial testing agar plates for pathogenic bacteria such as *Escherichia coli* (O157, O111, O27), *Staphylococcus aureus*, *Campylobacter* sp., *Salmonella* sp. and *Bacillus cereus* were used for evaluation. As a result, *S. aureus* and *B. cereus* were detected from enriched microbes as well as from soil microoganism and the others were not detected. To examine a pathogenic bacteria in organic hydroponic, *E. coli* (O157) was added to hydroponic solution and the growth of the *E. coli* strain was evaluated. It was found that the cell amount of *E. coli* decreased and no *E. coli* cells was deteced after 9 days of nitrification and 4 days of crop cultivation. These results provided enough evidence of safety in organic hydroponic culture.

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([1]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., [2]Res. Unit Physiol. Chem. Kyoto Univ., [3]Ryokoku Univ., [4]Keio Univ., [5]Biotechnol. Res. Center, Toyama Pref. Univ., [6]Grad. Sch. Bioagric., Sci., Nagoya Univ., [7]NARO)

Key words pathogenic bacteria, nitrification, organic hydroponic

1P-229 有機質肥料活用型養液栽培に有用な微生物群集からの亜硝酸酸化菌の単離

○宇佐美 晶子[1], 安藤 晃規[1,2], 犬飼 龍矢[1], 溝渕 久恭[1], 池本 成美[1], Saijai Sakuntala[1], 島 純[3], 宮本 憲二[4], 加藤 康夫[5], 浅川 晋[6], 篠原 信[7], 小川 順[1,2]
([1]京大院・農・応用生命, [2]京大院・生理化学, [3]龍谷大, [4]慶大・理工, [5]富山県大・工・生工研セ, [6]名大院・生命農学, [7]農研機構)
usami512@gmail.com

【目的】有機養液栽培は環境負荷を低減し、効率的な栽培を実現しうる水耕栽培である。本技術は有機態窒素の硝化に有用な微生物群を馴養する耕水工程と、植物定植後に硝化と栽培を同時に行う栽培工程で構成される。我々は微生物源として硝化能が優れた土壌を選抜し、集積を繰り返すことで安定した硝化能を示す微生物群集を開発した。本研究では、高機能微生物群集の人工的再構築を目指して、土壌由来の微生物群集より亜硝酸酸化菌の単離培養を行った。
【方法、結果】硝化菌群を馴養する耕水工程に亜硝酸ナトリウム（5 g/L）を添加し、亜硝酸酸化菌群の集積培養を行った。集積した微生物群を、2% ゲランガム培地（Na_2HPO_4･$2H_2O$, 3.2 g/L, KH_2PO_4, 0.272 g/L, $FeSO_4$･$7H_2O$, 1 mg/L, $MgSO_4$・$7H_2O$, 10 mg/L, $NaNO_2$, 5 g/L）に播種し、コロニーを形成した菌を単離した。さらに亜硝酸酸化酵素遺伝子での増幅が確認できた菌を対象に、上記と同様の組成の液体培地にて亜硝酸酸化活性を確認したところ、亜硝酸酸化活性を示す菌を見いだした。現在、単離した菌の詳細な性状の解析を行っている。

Isolation of a nitrite-oxidizing bacterium from the microbial community for the organic hydroponics.

○Shoko Usami[1], Akinori Ando[1,2], Ryuya Inukai[1], Hisanori Mizobuchi[1], Narumi Ikemoto[1], Sakuntala Saijai[1], Jun Shima[3], Kenji Miyamoto[4], Yasuo Kato[5], Susumu Asakawa[6], Makoto Shinohara[7], Jun Ogawa[1,2]
([1]Div. Appl. Life Sci., Grad. Sch. Agric., Kyoto Univ., [2]Res. Unit Physiol. Chem., Kyoto Univ., [3]Ryukoku Univ., [4]Dept. Biosci. Inform., Keio Univ., [5]Biotechnol. Res. Center, Toyama Pref. Univ., [6]Grad. Sch. Bioagric., Sci., Nagoya Univ., [7]NARO)

Key words nitrite-oxidizing bacteria, nitrification, organic hydroponics

1P-230 Effect of magnet field on bacterial enrichment culture in a column-type bioreactor

○ Hak jin Song[1], Yong-Keun Choi[1], Ye Chan Kwak[1], Yung-Hun Yang[1], Sang Hyun Lee[1], Kwang Jin Kim[2], Ho-Hyun Kim[3], Jung Yea Kim[4], Hyung Joo Kim[1]
([1]Dept. Bioeng., Konkuk Univ., [2]Natl. Horticultural Res. Inst., Rural Development Administration., [3]Inst. Environ. Res., Yonsei Univ. Coll. Med., [4]GARDEN4U Co., Ltd)
hyungkim@konkuk.ac.kr

In this study, the effect of magnetic field on bacterial diversity in an enrichment culture was investigated. For the enrichment culture system, an attachment (carbon felt, 5 x 5 x 0.5cm) for bacteria and a magnet (30 mT) were installed in a column-type bioreactor (working vol. 1.3 L). For the operation of the system, freshly collected activated sludge (26 ml) from a wastewater treatment system was inoculated to the bioreactor, and 10 fold diluted nutrient broth (NB) medium was fed to the bioreactor (1.0 ml/min). During the cultivation, dissolved oxygen (DO) concentration of the effluent from the system was monitored. After 32 days of enrichment culture, the bacterial attachment was harvested, and analyzed using the Denaturing Gradient Gel Electrophoresis (DGGE). The DGGE results showed the bacterial diversity of the attachment was highly affected by the presence of magnetic field. The DO monitoring result of the effluent also showed that the presence of magnetic field in the system induces the increase of oxygen concentration surrounding the attachment. Various bacterial strains including as *Janthinobacterium lividum*, *Sphingobacterium psychroaquaticum*, and unculturable strains were identified from the sample. These results indicate that the enrichment culture using magnetic field could be possible for the isolation of novel bacterial species for industrial applications.

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○Hak jin Song[1], Yong-Keun Choi[1], Ye Chan Kwak[1], Yung-Hun Yang[1], Sang Hyun Lee[1], Kwang Jin Kim[2], Ho-Hyun Kim[3], Jung Yea Kim[4], Hyung Joo Kim[1]
([1]Dept. Bioeng., Konkuk Univ., [2]Natl. Horticultural Res. Inst., Rural Development Administration., [3]Inst. Environ. Res., Yonsei Univ. Coll. Med., [4]GARDEN4U Co., Ltd)

Key words magnetic force, enrichment culture, dissolved oxygen, *Janthinobacterium lividum*

1P-231 *Burkholderia* sp. 由来 deoxy inosose 還元酵素遺伝子のクローニングと発現

伊藤 伸哉[1], ○黒川 純司[1], 戸田 弘[1], 小西 一誠[2]
([1]富山県大・工・生物工, [2]旭化成ケミカルズ株式会社)
z16072@st.pu-toyama.ac.jp

(-)-*vibo-quercitol*は血糖値低下効果等の生理学的機能や医・農薬品中間体として有用であり、また燃料電池冷却液の添加剤、太陽熱や低廉な夜間電力を利用する為の蓄熱材としての工業的利用も期待されている糖アルコールである。この *vibo*-quercitol は 2-deoxy-*scyllo*-inosose (DOI) の不斉還元により生産する事ができ、原料となる DOI はグルコースより生産する方法が知られている。DOI の還元を触媒する酵素の取得を目的としてラセミ体クエルシトールを炭素源に土壌より quercitol 資化性菌 Burkholderia sp. AKC-020 株を得た。本菌より DOI 還元酵素 (DOIR) を精製し、酵素特性を決定した。次に精製した DOIR の N 末端･内部アミノ酸配列をもとに PCR により DOIR 遺伝子のクローニングと大腸菌による組換え酵素の発現を行い、80% d.e. 以上の高いジアステレオマー過剰率で *vibo*-quercitol を生産できる事を確認した。DOIR の相同性検索の結果、inositol 2-dehydrogenase に分類される脱水素酵素と高い同一性を示した。そこで他の inositol dehydrogenase が DOI 還元を触媒するかどうかを調べるために 6 種の細菌由来 inositol dehydrogenase をクローニングし、大腸菌で発現させた。その結果、AKC-020 株由来 DOI 還元酵素と比較的相同性の高い酵素では DOI 還元が確認でき、*vibo*-quercitol の生産が可能であった。

Cloning and heterologous expression of the deoxy inosose reductase gene

Junji Kurokawa[1], ○Junji Kurokawa[1], Hiroshi Toda[1], Kazunobu Konishi[2]
([1]Dept. Biotechnol., Fac. Eng., Toyama Pref. Univ., [2]Asahi Kasei Chemicals Corp.)

Key words quercitol, deoxy inosose reductase, *Burkholderia* sp.