O_Plant Microbiology

O-1

Nematicidal Activity of Aureothin Family Isolated from Actinomyces

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Plant parasitic nematodes are parasitic on all parts such as plant growth points, leaves, stems, and roots, and ingest cell fluid as nutrients through bulbous needles. Parasitic nematodes can be classified into external parasitics, which are inflicted by the outside according to their infestation habits, internal parasitics that enter the tissues and take up nutrients by inserting the head into the tissues. In domestic facility cultivation, the damage of root-knot nematodes is very severe. In the case of melon, the detection rate is higher than 80%, and the detection rate is high and the density is high, so most of the fields cannot be cultivated without nematode control. There is also pine wilt disease (PWD), which is caused by pine wood nematode among plant diseases. Abamectin, a compound currently used as a nematode control, is used in agriculture, but it is time to take other measures due to problems such as water insolubility and resistance. Therefore, we tried to find compounds with nematicidal activity from actinomyces, which are known to produce many antibiotics. The active compounds were isolated from the selected strains by screening for bactericidal activity against *Bursaphelenchus xylophilus* from actinomyces. The nematode active compound was isolated and purified from extracts from two strains. Compounds were identified and properties were confirmed through MS/MS and NMR analysis. It was confirmed that a total of three compounds had nematicidal activity, and it was confirmed that the structure is an aureotin derivative. Therefore, the efficacy of the strain extract containing the active compound was evaluated in greenhouse conditions. Since it showed excellent efficacy, it is suggested that it could be developed as a potential pesticide of aureotin-based compounds.

Keywords : Plant disease control, actinomyces, aureothin

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O-2

Identification and Characterization of a New Fungal Pathogen Causing Root Rot Disease of *Gastrodia elata* in Korea

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Gastrodia elata (*G.elata*), a myco-heterotrophic orchid family (Orchidaceae), depends on two fungal partner: *Mycena* spp. and *Armillaria mellea*, seed germination and plant growth, respectively. Recently, more than 70% of *G.elata* has loss in the actual yield in Korea due to root rot disease. In this study, we aim to identify the new fungal pathogen causing root rot of *G.elata*. We have observed necrotic spots of *G.elata* from Anseong (37˚0´N 127˚16´E), one of the largest *G.elata* production areas in Korea. Symptoms showed round and grayish brown spots, which eventually coalesced into larger black lesions on tubers. The six pure cultures were recovered from necrotic lesions of symptomatic tubers. For fungal identification and characterization, fungal genomic DNA was isolated from tuber of *G.elata*, and amplified from two fungal loci, the internal transcribed spacer regions 1 and 4 and 5.8S nrDNA (ITS), and the translation elongation factor alpha (TEF-α). Three pathogenic fungi were identified the new fungal pathogen through phylogenetic analysis, morphological observation, and the pathogenicity test, while other 3 fungi were identified and formally described as *Clonostachys rosea*. Therefore, these results may assist to make the proper method to control root rot disease in the fields of *G.elata* in Korea.

Keywords : *Gastrodia elata* (*G.elata*), Internal Transcribed Spacer (ITS), Translation Elongation Factor alpha (TEF-α)

O-3

Isolation, Characterization and Culture Conditions for Multifunctional Biocontrol Agents against Phytopathogenic *Botrytis cinerea* and *Ralstonia solanacaum*

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Botrytis cinerea and *Ralstonia solanacearu*m are critical pathogens which causes bacterial wilt and gray mold, respectively in various crops, including pepper, tomato, and potato. Once infected, it is very important to block the infection early because the both pathogens rapidly spreads to healthy crops. There are many microbial agents capable of controlling bacterial wilt and gray fungi, respectively, but simultaneous management strategies of mitigate both strains are very limited. In this study, 500 strains were isolated and characterized from soil and the surface of plants for simultaneous controlling Botrytis cinerea and Ralstonia solanacearum infections. Firstly, evaluation of anti-pathogenic activity of the isolates was performed by using paper disk diffusion assay. As a result, we found 3 bacterial isolates showing strong antifungal activity against the plant pathogens. 16S rDNA analysis revealed that they are members of *Bacillus subtilis, Bacillus velezensis* and *Bacillus siamensis*. Among three strains, *B. subtilis* JC-6 showed the best anti-pathogenic activity. Especially, this strain showed simultaneous mitigation of Botrytis cinerea and *Ralstonia solanacearum*. Prior to agricultural field application, we investigated medium optimization for mass cultivation of B. subtilis JC-6 with low costs. 5 carbon sources (glucose, sucrose, glycerol, maltose and soluble starch) and 5 nitrogen sources (soy bean flour, yeast extract, soytone, peptone and tryptone) were tested. The optimized carbon and nitrogen source was glucose and yeast extract. As a result, B. subtilis JC-6 showed 2.5x109 total cells/ml and the endospore cells were 2.0x109 under the optimized culture conditions using 100L fermenter. It is obvious that B. subtilis JC-6 will be effectively used for multifunctional biological agent of bacterial wilt and gray mold disease.

Keywords : *Botrytis cinerea, Ralstonia solanacaum*, biocontrol agents

O-4

A cocktail of Bacteriophage Suppresses Bacterial wilt in Tomato Plant and Alters Rhizosphere Microbiome Structure

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Bacteriophages (phages) have been proposed as an alternative to pesticides to kill bacterial pathogens of plants. However, the effective strategy of phage biocontrol is variable and poorly understood. Here, we characterized phages infecting *Ralstonia pseudosolanacearum,* for their biocontrol efficacy and their effect on microbiome structure in tomato plants. Among 33 isolated phages, two distinct phages RpT1 and RpY2 having comparable genomic and similar morphological features within the *Autographiviridae* family. Both phages had a broad host range and persisted in a wide range of natural environmental conditions such as various soils, temperatures, and pH over a prolonged period. Two phages were treated in tomato plants at various times with several concentrations, either individually or in cocktail. Treatment of phages 3 days later of pathogen inoculation with 10-fold higher than pathogen density demonstrated biocontrol activity against bacterial wilt both in cocktail and separately. Additionally, bacterial community analysis in the rhizosphere revealed that the composition and abundance of phage-treated plants were altered with selective microbiota. Results suggest that the decreased disease incidence was explained by lysis of *R. pseudosolanacearum* in rhizosphere soil and possible enrichment of plant-beneficial bacterial species. Altogether, our results provide insights into developing the effective phage therapy to manage bacterial wilt and its effect on microbiome composition.

Keywords : Bacteriophage, biocontrol, *Ralstonia pseudosolanacearum*

O-5

Characterization of Newly Isolated Bacteriophage PhiPC01 to Control *Pectobacterium carotovorum* subsp. *carotovorum*

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Pectobacterium carotovorum subsp. *carotovorum* is one of the most important phytopathogens causing bacterial soft rot. The disease brings huge economic loss as it occurs on diverse crops such as carrot, cabbage, and potato during storage and distribution processes as well as cultivation. To control the pathogen, we newly isolated *Pectobacterium*-targeting bacteriophage PhiPC01 from sewage sampled from Tancheon Water Treatment Center. Based on the morphological and genomic analysis, phage PhiPC01 having icosahedral head and short tail is assumed to be classified into the family *Autographiviridae*. Total 50 open reading frames (ORFs) were predicted from the 39.9-kb dsDNA genome, and most of the functional proteins were similar to other autographiviruses PPWS4, PP81, and PP47 infecting *Pectobacterium* spp.. PhiPC01 was able to infect not only *P. carotovorum* but also *P. brasiliense*, and stably retains its infectivity at a wide range of temperatures (4-50 ℃) and pH levels (5-11). Currently, we are trying to evaluate other bacteriolytic properties of PhiPC01and its putative endolysin (ORF4) to develop them as useful tools in controlling phytopathogenic *P. carotovorum*.

Keywords : Bacteriophage, *Pectobacterium carotovorum,* phytopathogen

O-6

Species Diversity of Endophytic Fungi Isolated from Root of *Quercus* spp. in the Limestone Area

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Endophytic fungi live in all tissues of plant and do not cause disease symptoms. They secrete secondary metabolites such as alkaloids which can defend plants from herbivore or external pathogens. Recently, endophytic fungi are becoming more important because of the study results that these metabolites showed antimicrobial or anticancer effects. Limestone refers to a rock that contains at least 50% of the carbonate minerals. Soils formed from limestone differ in physical and chemical properties from non-limestone soil. In this study, we tried to confirm the difference of endophytic fungal diversity according to the Ca^{2+} concentration and the pH level in the limestone area. We collected the root samples from the rhizosphere of *Quercus* spp., and isolated the endophytic fungi from the roots. We observed the morphological characteristics of the fungal strains, and analyzed internal transcribed spacer (ITS) rDNA sequences. As a result, 48 fungal species from 29 genera were identified. We confirmed that the species diversity and the dominant species differ according to the Ca^{2+} concentration and the pH level of the soil. This result suggested that the soil environment of limestone areas can affect the endophytic fungal diversity in plant roots.

Keywords : Endophytes, fungi, symbiosis

O-7

Culturable Bacterial Composition of Rhizosphere Soil of Alpine Leek underneath Palmate Maple

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Alpine leek (*Allium victorialis*) is a medical plant with anti-cancer and anti-oxidant functions and used as a food ingredient due to unique taste. It was reported that habitats of alpine leek was closely related with neighboring plants such as hardwood; ecological important value of alpine leek was higher in maple layer. Root-associated microbial composition was also affected by maple layer; out of these, some bacteria could play an useful role in plant growth promotion. In this study, we isolated bacterial strains from rhizosphere of alpine leek surrounded or non-surrounded by palmate maple, and investigated their composition. As a result, we identified 21 strains from alpine leek grown in the bottom of palmate maple, the strains were belonging to different 11 genera including *Streptomyces*, *Curtobacterium* and *Lelliottia*, whereas, *Pseudomonas* (25%) and *Bacillus* (57.1%) were commonly abundant in rhizosphere soils of alpine leek regardless surrounding plants. These findings demonstrated that bacterial compositions in rhizosphere of alpine leek were could be related to neighboring plant including palmate maple. Further study, we will elucidate the effects of 21 strains on alpine leek growth and productivity.

Keywords : Alpine leek, palmate maple, identification

O-8

The Interaction between PGPB and Tannin on the Growth and Early Development of Plants and Its Implication on Tannin-Mediated Allelopathy in Temperate Forests

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Tannins are highly abundant compounds in ecosystems and have long been assumed to be involved in allelopathic interaction among plants. Despite well-known importance of ecological interactions between plants and microbes, the potential role of tannin in mediating these interactions has not been well addressed. In this study, we investigated the influence of tannin on the growth of plant growth-promoting bacteria (PGPB) that are found under the oak and pinetrees and their plant-growth-promoting (PGP) effects. We isolated 67 bacteria in the soil under the oak and pine trees in South Korea, and selected eight species of PGPB and tested their combinatory effect with tannin on the early development and the growth of *Arabidopsis thaliana*. We found that the PGP effect in response to tannin depended on the species of PGPB. In particular, two species belonging to *Lysinibacillus* promoted the growth and early development of plants only when tannin was absent in the soil, whereas two species belonging to *Paenibacillus* showed visible PGP effect only when tannin was present. Our results suggest that tannin, an allelochemical, has a strong effect on plant-microbial interaction in a temperate ecosystem. The mechanism of species-specific effects of PGPB in response to tannin needs further investigation.

Keywords : PGPB, tannin, allelopathy

O-9

The Ideal Stretagy for Powerful Antioxidant Producing Microalga Biomass (*Haematococcus lacustris*) Increment by Co-Cultivation with Key-Species of Bacterium Identified by Metagenomic Analysis

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A green unicellular alga (*Haematococcus lacustris* NIES144) has been known for its diverse beneficial carotenoid pigments, such as β -carotene, canthaxanthin, and astaxanthin. Specifically, carotenoid pigments such as β-carotene (BC), canthaxanthin (CX), and astaxanthin (AX) synthesized by microalgae are attracting international attention as the world is becoming an aging society. These pigments are used as functional food of antioxidants and anti-aging for humans. They are produced also by plants and fish, but not suitable for foods due to soil contamination and heavy metal accumulation in top predator fish. However, in the case of microalgae, high biomass could be obtained in a small-scale incubator, and it is safer from heavy metals or contaminations. BC, CX, and AX are biosynthesized by different representative microalgae strains. *Dunaliella salina* was recognized first as producing a high concentration of intracellular BC. *Chlorosarcinopsis* and *Dactylococcus* produce CX as a strong antioxidant material. AX, a powerful antioxidant, is also produced by *H. lacustris* under stress conditions such as nutrients depletion. *H. lacustris* use the phytoene as an AX precursor produced by the methylerythritol phosphate pathway in the plastids and mevalonate terpenoid pathway cytoplasm. Therefore, *H. lacustris* can produce various kinds of pigments, thereby being an industrially valuable candidate for diverse antioxidation raw materials without complicated processes. During carotenoid biosynthesis in microalgae, the normal algal plastids are degraded and the chlorophyll-a content is decreased. In this way, diverse carotenoid pigments are produced at different compositions, depending on algal strains or conditions. However, the slow growth of this alga is the major obstacle for producing the industrial scale of astaxanthin. To solve this weak point, algal growth-promoting bacterial strains that co-existed in the algal phycosphere were identified according to the NGS metagenomics analysis. This study focused on defining algal growth positive bacteria community modules, and major abundant taxa that had a positive correlation with algal growth were isolated (*Sphingomonas hankookenesis, Paenarthrobacter ureafaciens,* and three species of *Microbacterium* sp.) and each of algal growth-promoting effect patterns were evaluated. For the astaxanthin industrialization, this metagenomic data analysis will give solving the ideal strategy for the slow growth of *H. lacustris* some insight.

Keywords : NGS-metagenome, *Haematococcus lacustris*, Key-role species

O-10

Comparative Proteomic Analysis of *Erwinia amylovora* Cultured in Three Different Conditions

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Erwinia amylovora is a Gram-negative plant pathogenic bacterium causing fire blight disease on apple and pear. Although the disease is a serious threat to Rosaceae plants worldwide, in 2015, the disease was firstly reported in Korea. Therefore, most studies regarding the virulence of the bacterium have been focused on foreign strains. Here, we report differentially abundant proteins from Korean strain TS3128 isolated from Anseong grown three different conditions: LB (rich medium), HMM (*hrp* inducing medium), and MBMA (amylovoran inducing medium). Using a label-free shotgun proteomic analysis, a total of 969, 998, and 1033 proteins were commonly detected in the three biological replicates cultured in LB, HMM, and MBMA, respectively, and were subjected to comparative analysis and clustering the orthologous groups (COGs) classification. In LB vs. HMM, the abundance of proteins belonging to groups E, F, H, J, M, and P were mostly changed. Specifically, proteins related to the type III secretion system and iron-related proteins were identified. In the second set (LB vs. MBMA), the expression of proteins associated with amylovoran production and carbohydrate metabolism were changed. In addition, many proteins related to cell wall/membrane/capsule, including TonB-dependant receptors and porins, were also found. In HMM vs. MBMA, the numbers of proteins whose abundance was changed were lower than the other two sets (LB vs. HMM and LB vs. MBMA). These results lead to new insights into understanding biological and cellular mechanisms that responded to different conditions. It also provides fundamental information to elucidate virulence functions in *E. amylovora* isolated in Korea.

Keywords : Fire blight, proteomics, *Erwinia amylovora*

O-11

Optimal Culture Conditions of Antagonistic *Bacillus thuringiensis* BC-046 against Gray Mold caused by Botrytis cinerea on Ginseng

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Gray mold disease is one of the major diseases of ginseng. We selected *Bacillus thuringiensis* BC-046, a gray mold antagonist, in a previous study. The control of gray mold disease was mainly chemical control using disinfectants. However, research on biological control has been actively conducted in recent years due to the occurrence of resistant strains and environmental pollution problems due to the abuse of chemical pesticides. Therefore, for the development of an antagonist microbial agent, the culture characteristics of the BC-046 strain were tested. The growth curve of BC-046 was measured, and the time of the stationary phase was confirmed based on the growth curve. The culture characteristics of BC-046 were measured for growth according to the influence of the optimum medium, temperature, stirring speed, pH, carbon source, carbon source concentration, nitrogen source, nitrogen source concentration, and inorganic salts. As a result of setting the optimal culture conditions of BC-046, the growth induction period was shorter than that of the existing culture conditions, and the cell mass increased by 42% compared to the previous one after 10 hours of culture. This result is thought to be helpful in establishing a mass production system for the practical use of BC-046. In addition, it is thought that there is a need for follow-up studies, such as selection of cryoprotectants for long-term storage and antagonistic activity.

Keywords : Ginseng, gray mold antagonist, optimal culture conditions

O-12

Establishment of Fire Blight (*Erwinia amylovora*) Inoculation Conditions for Each Part of Apple Seedling

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The injuries caused by the fire blight pathogen, *Erwinia amylovora*, make not only the current crop year's products but also the pear and apple industries extremely destructive and dangerous in a region or country. Management of fire blight is difficult by limitations on use of antibiotics in agriculture, development of antibiotic resistance, and limited efficacy of alternative control agents. Infections commonly occur during blooming or on late blooms during the three weeks following petal fall. Many use of highly susceptible rootstocks to fire blight, such as Malling 9, Malling 26, etc. has increased the danger that infected blocks are able to suffer serious damage. Microorganisms offer the further prospect for application to improve the plant protection because they are generally recognized as "natural" compounds able to influence the safety and quality of fruits. We performed biological assay to find inoculation conditions for each part (flower, branch, leaf, seedling) of M9 and Crab apple (*Malus prunifolia*) seedlings prior to the useful microorganism experiment. As a result, the optimal inoculation concentration of *E. amylovora* for bioassay with flowers, branches, leaves and seedlings was found to be 10^6 CFU/ml. The result showed that this bioassay can be used as basic data for biological analysis to control fire blight pathogens using effective microorganisms.

Keywords : Fire blight, apple, *Erwinia amylovora*

O-13

The Cooperation between *Flavobacterium* sp. and Nnative Rhizosphere Microbiota Enhance the Growth of Tomato Plant

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The rhizosphere microorganisms affect plant fitness such as plant growth and disease resistance. Previously, we showed that a *Flavobacterium* sp. TCH3-2 strain isolated from tomato rhizosphere soil promote tomato growth with indigenous microbial community, but not by alone. In this study, we investigated the effect of changes in rhizosphere and endospheric microbiome by TCH3-2 on tomato growth. The 16S rRNA gene amplicons were sequenced by MiSeq sequencer and analyzed using QIIME2 pipeline. The 16S rRNA amplicon sequencing data showed that there was significant difference between the TCH3-2 treated and untreated control group in tomato rhizosphere and endosphere microbiome at 3- and 5-weeks post inoculation. To validate microbiome data, we constructed a synthetic community (SynCom) with several bacterial isolates from tomato rhizosphere soil and assessed the plant growth-promoting activity of TCH3-2 and SynCom in sterilized nursery soil. The inoculation of TCH3-2 or SynCom did not showed plant growth-promoting activity, but the mixed treatment significantly increased the growth of tomato. Intriguingly, the treatment of heat-killed TCH3-2 with SynCom failed to promote the growth of tomato plants. In addition, we found that the plant growth-promoting compound are secreted by the combination of SynCom and TCH3-2 using a hydroponic system. Taken together, these data showed that *Flavobacterium* sp. TCH3-2 strain might enhance plant growth by collaborating with beneficial rhizosphere microbiota.

Keywords : *Flavobacterium* sp., PGP, SynCom

O-14

Characterization of Potential Plant Growth-Promoting Rhizobacteria as Biological Agents with Antifungal Activity, Plant Growth-Promoting Activity, and Mineral Solubilizing Activity

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The purpose of this study is to confirm the antifungal activity, plant growth-promotion activity, and mineral solubilizing activity of 18 types of bacteria isolated purely from rhizosphere soil. The potential of isolates of the genus *Bacillus* and *Pseudomonas* as biocontrol agents was confirmed through the antifungal activity of these isolates. This activity was judged to be due to the activities of various hydrolytic enzymes on the cell wall of plant pathogenic fungi and the activity to produce siderophores. In addition, most of the isolates have been found to have ACC deaminase production activity, IAA production activity, and nitrogen fixation activity. These characteristics are believed to have a positive effect on root development and growth, and productivity of crops by reducing the concentration of stress ethylene under environmental stress conditions in which plants are commonly exposed. In addition, as a result of confirmation test for the solubilizing activity of the isolates for phosphoric acid, silicon, calcium carbonate, and zinc, some isolates were found to have mineral solubilizing activities. Inoculation of these isolates during plant growth is expected to help plant growth by converting nutrients necessary for plant growth into usable forms that can be absorbed by plants. The availability of 18 isolated strains as a biocontrol agent was suggested through the results of antifungal activity, plant growth-promotion activity, and mineral solubilizing activity.

Keywords : Plant growth-promoting rhizobacteria, mineral solubilizing activity, biocontrol agent

O-15

Improvement of Biomass and Lipid Production under Light Emitting Diodes (LEDs) Wavelength of *Tetraselmis gracilis*

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Microalgae are one of the means to reduce the concentration of carbon dioxide in the atmosphere by absorbing carbon dioxide, the main greenhouse gas in the atmosphere. Microalgae can be used as a feedstock for products such as food, fed and cosmetics. It has also been considered as a promising feedstock for biofuel production. LEDs have advantages that are different from other artificial light sources such as small size of light source, excellent energy efficiency, and being able to irradiate only specific wavelengths. Therefore, it is suitable for culturing microalgae that grows efficiently at a specific wavelength. Experiment was conducted with a two-phase culture system. The first phase experiment optimized nitrate concentration and photoperiod. Nitrate concentration was set at 80, 160, 240 and 320 mg L^{-1}. As a result of the experiment, *T. gracilis* produced 0.45, 0.63, 0,72 and 0.84 g dcw L^{-1} of biomass, respectively, and the productivity (dcw/day) was the highest at 160 mg/L. The cultivation period could be shortened through the photoperiod (12:12, 18:6 and 24:0 h light/dark cycle) experiment. The second phase improves lipid content of microalgae by applying the LED wavelength, nitrate depletion, pH and salt.

Keywords : Microalgae, lipid, light emitting diodes

O-16

The Effect of Light-Emitting Diodes (LEDs) Photoperiod and Light Intensity on Growth and Lipid Production of *Chlamydomonas hedleyi*

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Microalgae are attracting attention as an alternative to grain biofuels due to their high lipid productivity, fast growth rate, and non-edible resources. Without competing with food and feed crops, and growing awareness of global energy issues, microalgae are now at the forefront of research as a sustainable and environmentally friendly alternative. It is the next generation biomass with almost no restrictions on arable land with higher oil yields than currently available agricultural crops. In this study, to find the optimal LED photoperiod and light intensity, the LED photoperiod was set to 12:12 h, 18:6 h and 24:0 h light/dark cycle and light intensity was set to 300, 400, 500 and 600 $\mu molm^{-2}s^{-1}$. The maximum efficiency is achieved by dividing the biomass production phase (the first phase using red LED, 625 nm) and the lipid production phase (the second phase using green LED, 520 nm). In the first phase, *C. hedleyi* produced maximum biomass about 0.95 g dcw/L at 10 days under the 24:0 h light/dark cycle and 500 $\mu molm^{-2}s^{-1}$. In the second phase, the highest lipid production about 41.3% was obtained in the culture after exposing to green LEDs under the 12:12 h light/dark cycle and 400 $\mu molm^{-2}s^{-1}$ at second phase day 2.

Keywords : Microalgae, light-emitting diodes, light conditions

O-17

Enhancement of Growth and Lipid Production of *Pavova lutheri* Using Phytohormones at LED Wavelength in 60 L Bioreactor

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Microalgae have been focused as a possible source of new generation biofuels because of fast growth, high biomass productivity and high lipid accumulation. Furthermore, long chain fatty acids found in microalgae have important functions as health supplements. Plant growth regulators (PGRs) can contribute to plant growth and development. PGRs are chemical messengers involved in a broad spectrum of physiological and biochemical processes of higher plants at very low concentration. In this study, first phase culture was optimized for each concentration using of 2, 4-dichlrophenoxyacetic acid (2, 4-D), indole-3-acetic acid (IAA), and 1-naphthalene acetic acid (NAA). And auxin-based PGRs 2, 4-D, IAA, and NAA were each mixed with gibberellic acid (GA) and cultured for synergistic effects. After that, lipid accumulation by the concentration of abscisic acid (ABA) was optimized in the second phase culture. After optimizing the type and concentration of phytohormones, photoperiods 12:12, 18:6, and 24:0 h were optimized. The optimized condition was a mixture of 5 mg/L of 2, 4-D and 20 mg/L of GA, and the photoperiod was 24:0 h light and dark cycle. At this time, the biomass reached 1.16 g/L, produced 0.54 g/L higher biomass compared to the control without using the phytohormones, and the cultivation period was also shortened by 5 days.

Keywords : Microalgae, phytohormones, light-emitting diodes