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Identification of Alga-lytic Bacterium AK-07 and Its Enzyme Activities Associated with Degradability of Cyanobacterium *Anabaena cylindrica*

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***Anabaena cylindrica* 분해세균 AK-07의 동정과 분해 관련 효소활성 조사.** 김정동 · 한명수* (한양대학교 생명과학과)

부영양화 현상을 나타내는 석촌호수와 팔당호의 표층수와 저니로부터 178개의 균주를 분리한 후, *Anabaena cylindrica* lawn 상에서 plaque를 형성하는 9개의 균주를 선별하였으며 이들 중에서 남조류 생장 억제 능력이 가장 우수한 AK-07를 선발하였다. AK-07의 특성과 16S rDNA의 염기 서열 분석을 기초로 하여 유연관계를 조사한 결과, 형태적, 생리적 생화학적 특징들은 *Acinetobacter* 속의 특성들과 유사하였으며, 16S rDNA의 염기 서열 분석한 결과는 *Acinetobacter johnsonii*와 99.5%의 유사성을 나타내어, *Acinetobacter johnsonii* AK-07로 명명하였다. 남조류 분해 특성을 조사하기 위해서, AK-07를 *A. cylindrica*와 혼합 배양시 접종 2일 후에 남조류의 분해가 관찰되었고, 접종 10일 후에는 남조류가 완전히 사멸하였으며, AK-07의 세포 수는 8×10^8 cfu ml^{-1}까지 증가하였다. 그러나 배양 상등액을 *A. cylindrica*와 혼합 배양 하였을 때에는 남조류의 분해는 관찰 되지 않았다. 따라서 AK-07는 남조류를 직접 접촉하여 분해하는 것으로 사료되어, AK-07에 세포에 존재하는 효소의 활성을 조사한 결과 Protease와 glycanases 중에서 β-Xylosidase의 활성이 가장 높았으며, Alginase, Laminarinase, Lipase, β-Galactosidase 및 β-Glucosidase의 활성도 높은 수준으로 관찰되었다. *A. johnsonii* AK-07은 *A. cylindrica*의 polysaccharides나 peptidoglycans를 monosaccharides이나 저분자 유기물로 분해하는 것으로 여겨진다.

Key words : hydrolytic-enzyme, alga-lytic bacterium, *Acinetobacter johnsonii*, *Anabaena cylindrica*

INTRODUCTION

Many cyanobacteria are ecologically important microorganisms in view of their nitrogen-fixing ability (Khan *et al.*, 1994). They can, however, bring out severe trouble such as water blooms in many freshwater lochs and reservoir around the world due to mass development (Carmichael, 1994). Water blooms by cyanobacteria, particularly in genera *Microcystis* and *Anabaena*, are widely distributed in nature freshwater ecosystems during summer, and have often resulted in a deterioration of water quality with adverse effects on lake ecology, livestock, human water supply, and recreational amenity. They produce the toxic substances (Harada, 1996) and offensive odors (Tsuchiya *et al.*, 1992). Several approaches have been tried to control cyanobacterial blooms from lakes and reservoirs. The frequently used algicidal agents were copper sulfide (McGuire *et al.*, 1984) and simazine, which block photosynthesis (Reyssac and Pletikosic, 1990), but this is expensive and potentially damaging

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on the environment. An alternative approach is to reduce the nutrient required the algal growth, although it is not easy to control the amount of nutrient reaching lakes and reservoirs. None of these methods have been successful so far (Burnham *et al.*, 1976, 1981). Therefore, biological control was on the rise for a more suitable method to regulate cyanobacterial blooms and bacterial pathogens that inhibit growth of cyanobacteria have been isolated (Yamamoto *et al.*, 1998). Only a few cases, however, has been elucidated the nature of the antagonistic mechanisms (Burnham *et al.*, 1976, 1981). Moreover, the role of physiological adaptation and selection of alga-lytic bacterial species with respect for algal degradation are not easily investigated in natural systems due to its obvious interactions that are more complex exist in nature microbial communities (Gonzalez *et al.*, 1996). With the aim of obtaining other microorganisms, which can be used for controlling cyanobacterial blooms, the authors isolated and identified bacterial strains with algal-lytic activities against *Anabaena cylindrica* and investigated enzyme activities of alga-lytic bacterium. The activities of protease (gelatinase and caseinase), lipase, amylase, carboxymethyl-cellulase (CMCase), chitinase, agarose, fucodian hydrolase, laminarinase, alginase, β-glucosidase, α- and β-galactosidase, β-N-acetylglucosaminidase, β-xylosidase, and α-mannosidase were examined.

MATERIALS AND METHODS

1. Algal culture conditions

Anabaena cylindrica M-1 that used as host for alga-lytic bacteria was kindly supported by Institute of Applied Microbiology (IAM) culture collection of Tokyo University, and a clonal axenic culture was cultivated and maintained in BG-11 medium (Castenholz, 1988; Rippka, 1988) under continuous illumination of cool white fluorescent lamps giving an incident light intensity of 35 μmole s^{-1} m^{-2} and at 25~28°C with agitation at 150 rpm on rotary shaker.

2. Isolation of characterization of alga-lytic bacterium

Various surface waters and sediment cores were collected from Pal'tang reservoir and Lake of Sukchon in Korea where are eutrophic lake in which cyanobacteria are dominant phytoplankton during summer season (Kim, 2001). Alga-lytic bacteria were isolated by the soft agar overlayer technique (Shilo, 1970). Axgenic cultures of *A. cylindrica* M-1 were grown BG-11 medium for 1 week, and 1 ml of *A. cylindrica* M-1 cultures was mixed with 1 ml of filtered (200 μm filter) suspension of surface waters or sediment samples and molten BG-11 soft agar equilibrated to 50°C. The mixture was immediately poured onto a BG-11 soft agar plate. After the agar had solidified, the plates were incubated at 25~28°C with continuous illumination of cool white fluorescent lamps giving an incident light intensity of 35 μmol s^{-1} m^{-2}. Bacterial colonies that produced clear zones on lawns of *A. cylindrica* were picked, purified, and maintained on BG-11 agar plates. Pure cultures were stored at −80°C in BG-11 medium containing 0.1% (v/v) yeast extract supplemented with 20% (v/v) of glycerol.

3. Determination of biochemical and physiological characteristics of bacterial isolate

Purified bacterial isolates were precultured on nutrient agar for following tests. The biochemical and physiological characteristics were determined using the method of Gerhardt *et al.* (1994) and further investigated using carbon utilization, the optimum pH, temperature for growth, a hemolysis test, and gram straining. Oxidase and catalase activity, gelatin liquefaction, arginine dehydrolase, ornithine decarboxylase, etc. were further examined. For investigation of DNA base composition, average G+C value was determined by using the thermal denaturation method (De Lay and Van Muylem, 1963) and was calculated by using the equation of Marmur and Doty (1962), as modified by De Lay (1970).

4. Identification of bacterial isolate with phylogenetic analysis

The chromosomal DNA was isolated using a method described by Yoon *et al.* (1997). The amplification of the 16S rDNA was conducted using two universal primers according to Stackebrandt and Liesack (1993), 5′-GAGTTTGATCCTGGCTCAG-3′ and 5′-AGAAAGGAGGTGATCCAGCC-3′. A PCR was run for 35 cycles in a DNA thermal cycler, Genetic analyzer 377 (Perkin-

Elmer, Boston, USA), employing the thermal profile according to Yoon *et al.* (1997). The 16S rDNA sequence of bacterial isolates AK-07 and AK-13 was aligned using CLUSTAL W software (Nigam *et al.*, 2000). The evolutionary distance matrices were calculated with the DNADIST program within the PHYLIP package (Felsenstein, 1993). The sequences of representative species of the genus *Acinetobacter* and related taxa were cited using the GenBank Database. The values of 16S rDNA similarity were calculated from the alignment, while the evolutionary distances were calculated using a Kimura two-parameter correction. A phylogenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987) based on the calculated distance matrix.

5. Effect of mixed-culture on the alga-lytic activity

To examine alga-lytic activity of AK-07, isolate AK-07 was cultivated in 200 ml BG-11 medium containing 0.1% casitone for 24 hrs and harvested by centrifugation at 8,000 rpm, 20°C for 10 min. The harvested cells were resuspended with fresh BG-11 medium and the initial cell concentration of 1,000 ml adjusted to approximately 1.0 at 660 nm was inoculated.

Two mixed-cultures of *A. cylindrica* and bacterial strain AK-07 were prepared. The cultures were cultivated with agitation by 150 rpm on a rotary shaker. Culture media prepared as above were incubated with about 1×10^6 cells ml^{-1} of *A. cylindrica* as final concentration and cultivated in the algal growth chamber. At intervals during cultivation, the optical densities at 750 nm were measured. As controls, cyanobacterial cultures, which were free from lytic bacteria, were prepared in the BG-11 medium supplemented with 0.05% casitone and cultivated at shaking culture conditions described above.

6. Preparation of cell-free extracts

Cells of isolate AK-07 were harvested by centrifugation at 20,000 rpm and 4°C for 20 min and washed twice with 50 mM phosphate buffer pH 7.2 and resuspended in the same buffer. The cells was homogenized by sonication (MSE 100 watt Ultrasonic Disintegrator, MSE, London, UK) and centrifuged at 20,000 rpm for 20 min under 4°C to remove cell debris. The supernatant was used as the crude enzyme solution. The cell-free extracts were kept at −20°C until used.

7. Assays of enzyme activities

To determine the activities of chitinase, laminarinase (β-1, 3-D-glucanse), alginase, agarase, carboxymethyl-cellulase (CMCase), a chitin, laminarin, alginate, agar, and CM-cellulose used as substrates were purchased from Sigma-Aldrich Ltd., USA. The most of enzyme activities were measured by colorimetric analysis of the reducing sugar content determined by the procedure of Somogyi (1952) and Nelson (1955). Enzymes and substrate blanks were also included. A unit of enzyme activities is defined as the amount of enzyme catalyzing the release of 1 μmol of correspondent substrate per 1 mg of protein. Protein concentration was measured by the method of Bradford (1976) with bovine serum albumin as the standard. Chitinase and alginase were determined by development of clear zones around the colonies. Congo red was used for detection of *in vivo* CMCase activity as follows: AK-07 colonies on agar plates were lysed by chloroform vapor during 15 min. The plates were overlaid with 5 ml of 50 mM phosphate citric acid buffer, pH 5.2, at 50°C supplemented with agar (0.5% w/v) and CM-cellulose (1% w/v) at 50°C. After 12 hrs of incubation at 30°C, the plates were flooded with Congo red (1% w/v) for 20 min and washed 1 M NaCl. Active clones were surrounded by a yellow halo on a red background. One unit of cellulase activity corresponds to 1 μmol D-glucose equivalent released min^{-1}. Laminarinase activity was determined by measuring the amount of reducing sugar releasing from laminarin. A standard assay mixture (1 ml) was containing enzyme solution properly diluted, 4 mg of laminarin, and 50 mM potassium acetate buffer with pH 5.5. The reactions were run for 30 minutes at 30°C and stopped by boiling for 5 minutes, and reducing-sugar content was determined. A unit of laminarinase activity is defined as the amount of enzyme catalyzing the release of 1 μmol of glucose equivalent per minute. Glycosidase activities were measured with the *p*-nitrophenyl derivatives of relevant monosaccharides (Sigma-Aldrich Ltd., USA) viz., *p*-nitrophenyl-β-D-glucopyranoide, *p*-nitrophenyl-α-D-galatopyranoide, *p*-nitrophenyl-β-D-galatopyranoide, *p*-nitrophenyl-β-N-acetyl-D-gluco-

saminopyranoside, *p*-nitrophenyl-β-N-acetyl-D-galctopyranoside, *p*-nitrophenyl-α-L-fucoside, *p*-nitrophenyl-α-D-manno-pyranoside, and umbellipheryl-β-D-xylopyranoside. Glycosidases activities were determined in 100 mM phosphate buffer with pH 7 at 28°C. 0.05 ml of *p*-nitrophenyl glycoside in 50 mM phosphate buffer with pH 7 and 0.1 ml of cell-free extract were mixed thoroughly and 0.1 ml of 1 M Na_2CO_3 was added to stop the reaction. Specific activities of glycosidase were expressed as amount of enzyme that converted 1 μmol of *p*-nitrophenol per hour.

RESULTS

1. Isolation of alga-lytic bacteria against *Anabaena cylindrica*

A number of bacteria (78 strains from surface waters and 100 from sediments) were isolated from surface waters and sediments. Only nine isolates exhibited lytic abilities against *Anabaena cylindrica* M-1 on the agar plates. A typical plaque of bacterial isolates appeared on the lawns of *A. cylindrica* M-1 cells after 1 week, and then isolate AK-07 was selected as the strongest in lysing the cyanobacterium, *A. cylindrica* M-1.

2. Determination of characteristics of alga-lytic bacterium AK-07

Enrichment cultures were obtained from fresh water sediments with added alga-lytic bacterial strain to favor growth of hydrolytic enzyme-producing microorganisms. Only one bacterial isolate was clearly picked on the plates during the monitoring algal degradation. Strain AK-07 was gram-negative, rod-shaped, catalase positive and oxidase negative. Arginine dihydrolase, lysine and ornithine decarboxylases, lipase, caseinase, alginase, laminarinase (β-1, 3-glucanase), and fucodian hydrolase were positive. Coenzyme Q was ubiquinone Q-7, and G+C contents of the DNA that is similar to the genus *Acinetobacter* (38~47 mol%) were 44.3 mol%. Other phenotypic features are shown in Table 1. Thus, strain AK-07 was found to be very similar to *Acinetobacter* spp. based on its biochemical and physiological characteristics, respectively.

Table 1. Morphological, physiological, and biochemical characteristics of isolate AK-07.

Characteristics	AK-07
Cell shape	Short rod
Cell diameter	0.8~1.4 μm
Gram staining	−
Optimum temperature	28°C
Growth on 42°C	−
Optimum pH	6-8
Motility	+
Catalase	+
Oxidase	−
Arginine	+
Ornithine	+
Lysine	+
Citrate utilization	+
Hemolysis	−
Production of	
Amylase	−
Caseinase	+
Gelatinase	−
Agarase	−
Carboxymethyl-cellulase (CMCase)	−
Lipase	+
Chitinase	−
Alginase	+
Laminarinase	+
Fucodian hydrolase	+
Carbohydrate utilization of	
D-Lactate	+
D-Mannose	−
Lactose	−
D-Arabinose	−
D-Galactose	−
Sorbitol	+
D-Fucos	-
D-Ribose	+
D-Xylose	+
Glycerol	−
Mannitol	−
Cellobiose	−
Trehalose	−
Sucrose	−
Coenzyme Q	Q-7
G+C contents (mol %)	44.3%

3. Phylogenic analysis

The 16S rDNA sequence was analyzed to determine which species matched strain AK-07 with the highest homology among the *Acinetobacter* species cited in the GenBank. The phylogenetic tree constructed using the neighbor-joining method is shown in Fig. 1. The sequencing data was aligned to construct a phylogenetic tree. The

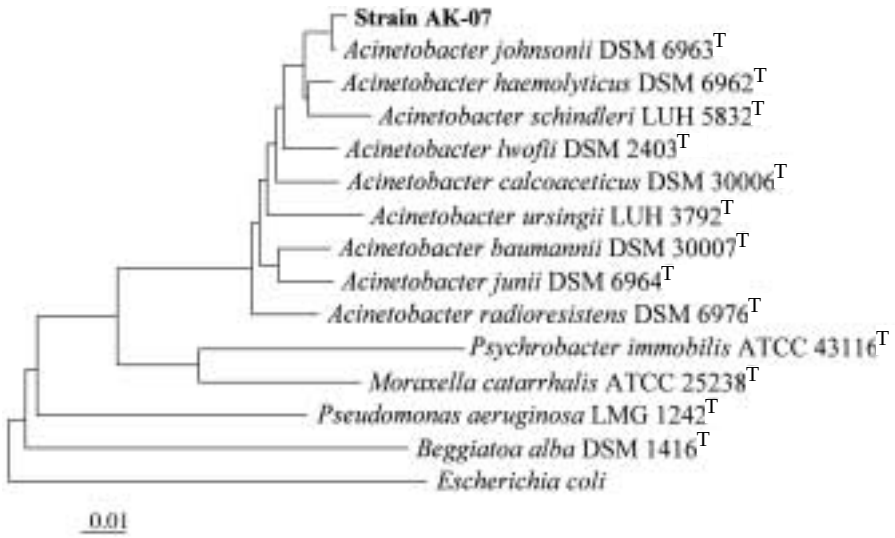

Fig. 1. Phylogenetic tree based on 16S rDNA sequences showing the positions of strain AK-07, the type strains of *Acinetobacter* species and the representatives of some other related taxa. The scale bar represents 0.01 substitutions per nucleotide position.

phylogenetic position of strain AK-07 was then compared with certain *Acinetobacter* species and related taxa in a dendrogram. In the phylogenetic tree, strain AK-07 was closest to *Acinetobacter johnsonii* DSM 6963^T and part of a robust monophyletic cluster with *Acinetobacter haemolyticus* DSM 6962^T, *Acinetobacter schindler* LUH 5832^T, *Acinetobacter lwofii* DSM 2403^T, and *Acinetobacter calcoaceticus* DSM 30006^T. The level of sequence similarity of strain AK-07 within the monophyletic cluster was greater than 97% (Fig. 1). The sequence of strain AK-07 was almost identical to that of *Acinetobacter johnsonii* DSM 6963^T with a 99.5% similarity. Hence, this appears to be the first report that *A. johnsonii* AK-07 has ability of algal lytic against *Anabaena cylindrica*.

4. Algicidal effect of *Acinetobacter johnsonii* AK-07

Algal lytic activities of *Acinetobacter johnsonii* AK-07 against *A. cylindrica* when the mixed-culture was agitated by 150 rpm, cyanobacterial vegetative cells disappeared completely 12 days after inoculation of alga-lytic bacteria (Fig. 2). Rapid lyses of *A. cylindrica* M-1 occurred in the presence of *A. johnsonii* AK-07. *A. cylindrica* M-1 and cells of AK-07 were growth together, the algal population quickly declined by AK-07

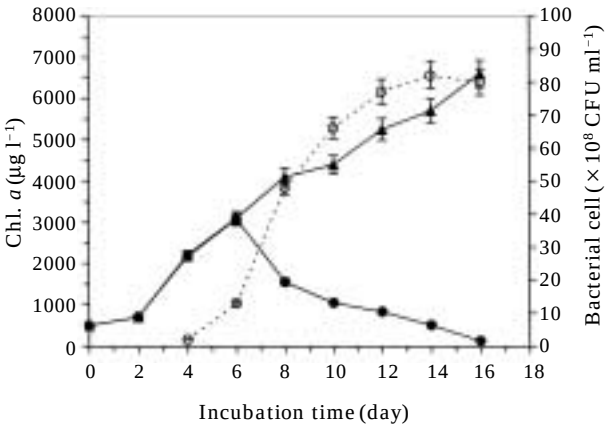

Fig. 2. Influence of *Acinetobacter johnsonii* AK-02 on the growth of *Anabaena cylindrica* M-1. *A. cylindrica* M-1 in BG-11 medium ether in the presence of AK-07 (right line, ●) or in the absence of bacteria strain (right line, ▲). In the mixed culture, bacterial cells of AK-07 (dotted line, ○) was added to the *A. cylindrica* M-1 culture 4 days after the start of cultivation, as indicated by the arrow.

around 6 days after the start of cultivation. In contrast, bacterial cells were able to grow in the mixed culture and the population of AK-07 increased up to 8×10^8 cfu ml^{-1} within 16 days after the start of cultivation. In Fig. 2, the cell growth of *A. johnsonii* AK-07 in mixed culture

with alga followed the batch growth curve of a lag, an exponential, and stationary phase. In bacteria-free extracellular filtrate, however, the alga-lytic activity was not found.

5. Enzyme activities of *Acinetobacter johnsonii* AK-07

In this study, we analyzed hydrolytic enzyme profiles of *A. johnsonii* AK-07. The two species, free-living bacteria, expressed proteases (caseinase and/or gelatinase), lipase, and prominent laminarinases (β-1, 3-glucanase), while the majority of *A. johnsonii* AK-07 also exhibited caseinase, alginase, fucodian hydrolase, β-galctosidase, β-glucosidase, β-glucosaminidase, and β-xylosidase that hydrolyzed mainly β-glycosidic bonds (Fig. 3). Other glycosidase such as α-galctosidase, α-fucosidase and α-mannosidase, which degraded α-glycosidic bonds were not detected. Nevertheless, *A. johnsonii* AK-07 has complex enzyme systems for the hydrolysis of cyanobacteria or algal polysaccharides.

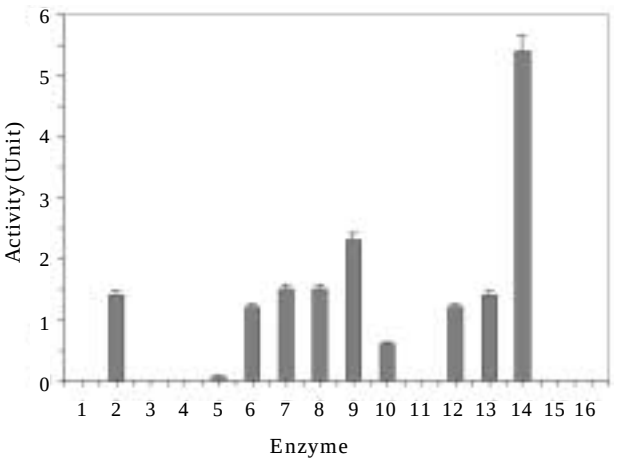

Fig. 3. Comparative enzyme activities and enzyme profiles of *Acinetobacter johnsonii* AK-07 (■). Values are given as an enzyme activity. One unit of gylcanase or glycosidase activity of enzyme that liberated 1 μmol from reducing ends of corresponding monosaccharides or 1 μmol from *p*-nitrophenol of corresponding substrates per 1 hour, respectively. 1. Agarase, 2. Alginase, 3. Amylase, 4. Chitinase, 5. CMCase, 6. Fucodian hydrolase, 7. Laminarinase, 8. Lipase, 9. Protease, 10. β-Glucosaminidase, 11. α-Galatosidase, 12. β-Galactosidase, 13. β-Glucosidase, 14. β-Xylosidase, 15. α-Fucosidase, 16. α-Mannosidase.

DISCUSSION

Monitoring of bacteria to control cyanobaceria causing water blooms, numerous samples were collected from lakes or reservoirs that have suffering from algal blooming by *Anabaena* spp. Our identification of nine algicidal strains from 178 isolates screened suggests that such activity may be common among freshwater bacteria. Daft and Stewart (1971) isolated only four strains of Myxobacteriales, which caused lyses of 40 strains of cyanobacteria. A recent report by Yamamoto *et al.* (1998), who isolated 83 actinomycete strains lethal to toxin-producing cyanobacterium *Microcystis aeruginosa*, provides further evidence for the compound of algicidal microorganism involved in lyses. The low densities of alga-lytic bacteria encountered in the environment may be due to insufficient inorganic fertility. In this work, algicidal bacterium AK-07 isolated from sediments in eutrophic lake is associated with a bloom population of the target algal species. Bacterial strain AK-07 was isolated from sediments of Lake of Sukchon to degrade the cyanobacterium *Anabaena cylindrica* and identified *Acinetobacter johnsonii* based on its 16S rDNA sequencing analysis (Fig. 1). The death of *A. cylindrica* M-1 was detected when AK-07 was added to the algal culture (Fig. 2) but not when only the culture filtrates was added. This is supporting AK-07 did not release extracellular products to suppress to *A. cylindrica*, and that the bacterial strains killed the algal cells by direct contact. Therefore, the enzymes on the surfaces of the bacteria might be effective alga-lytic agents to cause lyses of cells (Manage *et al.*, 2000). It produces several particular enzymes catalyzing hydrolysis of complex polysaccharides or peptidoglycans of cyanobacteria. Some of the corresponding glycanases are detected in cell free extracts of *Acinetobacter johnsonii* AK-07, viz. alginase, fucodian hydrolase, and glycanase including β-galctosidase, β-glucosidase, β-glucosaminidase, and β-xylosidase that hydrolyzed mainly β-glycosidic bonds (Table 1 and Fig. 3). Recently, two species of *Flexibacter* that lysed the cyanobacterium *Oscillatoria williamsii* produce lysozyme as one of cell inhibition compounds (Sallal, 1994). Burnham *et al.* (1981) provided *Myxococcus xanthus*, which degraded the cyanobacterium *Phormidium luridum* var.

olivacea, lyses the cells by the release of a lysozyme−like enzyme. Kim *et al.* (1997) reported *Moraxalla* sp. CK−1, which has been known to inhibit the growth of *Anabaena cylindrica*, produces aminodase, or endopeptidase. Carotenoids, peptidoglycan−associated proteins, and lipopolysaccharide, laminarinase and cellulose are one of the major constituents of cyanobacteria including *Microcystis* and *Anabaena* (Warren, 1996). The peptidoglycan is to be covalently linked to a wall polysaccharide. In our results, highly active amylase and fucodian hydrolase are not active, and the highest activity of β−glucosidase and laminarinase also exhibited in to the strain AK−07 studied herein. Laminarins, alginic acids, cellulose, and fucodians are the major constituents of algal cell walls up to 50∼80% of defatted algal mass (Warren, 1996). However, mainly laminarinase are often detected in microorganisms. This is most likely due to the important defense mechanisms by digesting fungal cell walls, etc., and releasing oligosaccharide that switch the production of antifungal compounds (Harmova and Fincher, 2001), and their ability to hydrolyze the reserved β−1, 3−glucan. In Fig. 3, glycanse and lipase in *A. johnsonii* AK−07 are showed high alga−lytic activities. Proteins such as lysozyme, protease, and lipase extracted from microorganisms caused cell lyses (Burnham, 1976; Yamamoto and Suzuki, 1990; Mitsutani *et al.*, 1988).

In conclusion, the results obtained in this study suggest that strain AK−07 of *A. johnsonii* most likely plays to degrade polysaccharides or peptidoglycans of algal cell walls in initial stages. The present work, hence, enhance our understanding of the functional interaction between algal cell structures and enzymes of alga−lytic bacterium during degradation of the cyanobacterium *A. cylindrica*. Identification and purification of the most active enzyme is now in progress. Elucidation of the mechanism of the selective alga−lytic enzyme against harmful blue−green algae is necessary to control water blooms. Nevertheless, algicidal bacterial strains AK−07 might be used as a biocontrol agent for management of cyanobacterial blooms in eutrophic lakes.

ABSTRACT

To investigate bacteria with algal lytic activities against *Anabaena cylindrica* when water blooming occurs and to study enzyme profiles of alga−lytic bacteria, various bacterial strains were isolated from surface waters and sediments in eutrophic lakes or reservoirs in Korea. A bacterial strain AK−07 was characterized and identified as *Acinetobacter johnsonii* based on its 16S rDNA base sequence. When AK−07 was co−cultivated with *A. cylindrica*, bacterial cells propagated to 8×10^8 cfu ml^{-1} and lyses algal cells. However, culture filtrates of AK−07 did not exhibit algal lytic activities. That suggesting the enzymes on the surfaces of the bacterium might be effective algal lytic agents to cause lyses of cells. *Acinetobacter johnsonii* AK−07 exhibited high degradation activities against *A. cylindrica*, and formed alginase, caseinase, lipase, fucodian hydrolase, and laminarinase. Moreover, glycosidases for example β−galatosidase, β−glucosidase, β−glucosaminidase, and β−xylosidase, which hydrolyzed β−*O*−glycosidic bonds, were found in cell−free extracts of *A. johnsonii* AK−07. Other glycosidase such as α−galactosidases, α−*N*−Ac−galctosidases, α−mannosidases, and α−*L*−fucosidases, which cleavage α−*O*−glycosidic bonds are not detected. In the results, enzyme systems of *A. johnsonii* AK−07 were very complex to degrade cell walls of cyanobacteria. The polysaccharides or peptidoglycans of *A. cylindrica* may be hydrolyzed and metabolized to a range of easily utilizable monosaccharides or other low molecular weight organic substances by strain AK−07 of *A. johnsonii*.

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REFERENCES

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein−dye binding. *Anal. Biochem*. **72**: 248−254.

Burnham, J.C., S.A. Collart and B.W. Highison. 1981. Entrapment and lysis of the cyanobacterium *Phormidium luridum* by aqueous colonies of

Myxococcus xanthus PCO_2. *Arch. Microbiol.* **129**: 285–294.

Burnham. J.C., T. Stecak, and G. Locher. 1976. Extracellularsis of the blue–green algae *Phormidium luridum* by *Bdellovibrio bacteriovorus*. *J. Phycol.* **12**: 306–313.

Carmichael, W.W. 1994. The toxins of cyanobacteria. *Sci. Am.* **270**: 64–72.

Castenholz, R.W. 1988. Culturing methods for cyanobacteria. *Methods Enzymol.* **167**: 68–92.

Daft, M.J. and W.D. Stewart. 1971. Bacterial pathogens of freshwater blue–green algae. *New Phytol.* **70**: 819–829.

De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* **101**: 738–754.

De Ley, J. and J. Van Muylem. 1963. Some applications of deoxyribonucleic acid base composition in bacterial taxonomy. *Antonic van Leeuwenhoek J. Microb. Serol.* **29**: 344–358.

Fay, P. 1992. Oxygen relations of nitrogen fixation in cyanobacteria. *Microbiol. Rev.* **56**: 340–373.

Felsenstein, J. 1993. PHYLIP: Phylogenetic Inference Package. Version 3.5. Seattle, University of Washington, Washington, USA.

Gerhardt, P., R.G.F. Murray, W.A. Wood, and N.R. Krieg. 1994. Methods for general and molecular bacteriology. American Society for Microbiology Press, Washington D.C., USA.

Gonzalez. J.M., W.B. Whitman, R.E. Hodson, and M.A. Moran. 1996. Identifying numerically abundant culturable bacteria from complex communities: an example from a lignin enrichment culture. *Appl. Environ. Microbiol.* **62**: 4433–4440.

Harada, K.–I. 1996. Chemistry and detection of *microcystins* pp. 103–148. *In*: Toxic *Microcystis* (M.F. Watanabe, K.–I. Harada, W.W. Carmichael and H. Fujiki, eds.). CRC Press, London.

Harmova, M. and G.B. Fincher. 2001. Structure–function relationships of beta–D–glucan, endo– and exohydrolases from higher plants. *Pl. Mol. Biol.* **47**: 73–228.

Khan, Z.U.M., Z.U.T. Begum, R. Mandal, and M.Z. Hossain. 1994. Cyanobacteria in rice soils. *World J. Microbiol. Biotechol.* **10**: 296–298.

Kim, C.–H., Y.–K. Choi, and B.–R. Min. 1997. Lysis of *Anabaena cylindrica* (cyanobacterium) cell wall by extracellular enzyme of *Moraxella* sp. CK–1. *Kor. J. Environ. Biol.* **15**: 89–97.

Kim, M.–C. 2001. Planktonic community structure in microbial loop from Kyougan Stream and Sukchon Reservior. Master Thesis, Hanyang Uiversity, 88p.

Manage, P.M., Z. Kawqbata, and S. Nakano. 2000. Algicidal effect of the bacterium *Alcaligenes denitrificans* on *Microcystis* spp. *Aquat. Microb. Ecol.* **22**: 111–117.

Marmur, J. and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**: 109–118.

McGuire, R.M., J.M. Jones, E.G. Means, G. Lzaguire, and A.E. Reston. 1984. Controlling attached blue–green algae with copper sulfate. *Res. Technol.* **27**: 60–65.

Mitsutani, A., A. Uchida, and Y. Ishida. 1988. Occurrence of blue–green algae and algal lytic bacteria in Lake Biwa. *Bull. Jap. Soc. Microbiol. Ecol.* **2**: 21–28.

Nelson, N.J. 1955. Colorimetric analysis of sugars. *Methods Enzymol.* **3**: 85–86.

Nigam, P., G. Armour, I.M. Banat, D. Singh, and R. Marchant. 2000. Physical removal of textile dyes and solid–state fermentation of dye–adsorbed agricultural residues. *Bioresour. Technol.* **72**: 219–226.

Reyssac, S.J. and M. Pletikosic. 1990. Cyanobacteria in fishponds. *Aquaculture* **88**: 1–20.

Rippka, R. 1988. Isolation and purification of cyanobacteria. *Methods Enzymol.* **167**: 3–27

Saitou, N. and M. Nei. 1987. The neighbor–joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425.

Sallal, A.K. 1994. Lysis of cyanobacteria with *Flexibacter* spp. isolated from domestic sewage. *Microbios* **77**: 57–67.

Shilo, M. 1970. Lysis of blue–green algae by myxobacter. *J. Bacteriol.* **104**: 453–461.

Somogyi, M. 1952. Notes in sugar determination. *J. Biol. Chem.* **195**: 19–23.

Stackebrandt, E. and W. Liesack. 1993. Nucleic acids and classification. pp. 152–189. *In*: Handbook of New bacterial Systematic (M. Goodfellow and A.G.O. Donnell, eds.). Academic Press, New York.

Tsuchiya, Y., M.F. Watanabe, and M. Watanabe. 1992. Volatile organic sulfur compounds associated with blue–green algae from inland waters of Japan. *Wat. Sci. Technol.* **25**: 123–130.

Warren, J. 1996. Microbial hydrolysis of polysaccharides. *Ann. Rev. Microbiol.* **50**: 183–212.

Yamamoto, Y., T. Kouchiwa, Y. Hogoki, K. Hotta, H. Uchida, and K.–I. Harada. 1998. Distribution and identification of actinomycetes lysing cyanobacteria in a eutrophic lake. *J. Appl. Phycol.* **10**: 391–397.

Yamamoto, Y. and K. Suzuki. 1990. Distribution and algal–lysing activity of fruiting myxobacteria in Lake Suwa. *J. Phycol.* **26**: 457–462.

Yoon, J.–H., S.–T. Lee, S.–B. Kim, W.–Y. Kim, M.

Goodfellow, and Y.-H. Park. 1997. Restriction fragment length polymorphisms analysis of PCR-amplified 16S ribosomal DNA for rapid identification of *Saccharomonospora* strains. *Int. J. Syst. Bacteriol.* **47**: 111-114.

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