Thesis for the Degree of Master of Engineering

# Characteristics of a novel bacterium, *Bacillus alcalophilus*, for reutilization of red-seaweed waste


by

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The Graduate School

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# 폐 홍조류 재이용을 위한 신균주, 바실러스 알칼로필러스의 분해특성


Advisor : Prof. Joong Kyun Kim

by

Soyean Kang

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# Characteristics of a novel bacterium, *Bacillus alcalophilus*, for reutilization of red-seaweed waste

A Dissertation

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Characteristics of a novel bacterium, *Bacillus alcalophilus*,
for reutilization of red-seaweed waste

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## 요 약

현재, 해조 폐기물의 효율적인 처리가 불가피하게 여겨지고 있다. 이러한 폐 홍조류의 재이용에 있어서, 습지로부터 분리한 *Bacillus alcalophilus* 균주는 잠재적으로 유용하며 이를 이용하여 실험실 단위에서 홍조류의 구성 다당류인 agar 와 carrageenan의 분해 특성을 분석하였다. Porphyra powder, agar, carrageenan에서 37 ℃, 4일 배양 후, 각각 반지름 0.83, 0.87, 1.18 cm의 clear zone을 형성하였고, 100-mL flask 단위에서 1 g/L 농도의 agar 및 carrageenan 만을 탄소원으로 이용하여 실험한 결과, 이 균주의 최적 분해조건인 pH 7.5, 30 ℃에서 각각 0.217 g/L 및 0.250 g/L의 총 환원당을 생성하였다. 본 연구 결과를 통해서 agar와 carrageenan의 분해능을 가지는 *Bacillus alcalophilus*을 이용함으로써, 폐 홍조류를 효과적으로 재이용하고 분해하여 얻어진 환원당은 바이오에탄올 생산을 위한 가치 있는 물질 등으로 재활용하여 환경적인 문제 해결과 더불어 에너지 개발에 효과가 있을 것으로 기대된다.

Characteristics of a novel bacterium, *Bacillus alcalophilus*,
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## Abstract

Efficient treatment of seaweed waste is currently deemed necessary. A strain isolated from a marsh and identified as *Bacillus alcalophilus* is potentially useful in the reutilization of red-seaweed waste. Plate assays indicated that the isolate possessed both agarase and carrageenanase activities. The isolate produced clear zones on *Porphyra* powder, agar and carrageenan with substantial radii of 0.83, 0.87, and 1.18 cm, respectively, after 4 days of incubation at 37℃. In 100-mL flask experiments using agar or carrageenan as the sole carbon sources, the optimal pH and temperature for the degradation of both polysaccharides by the isolate were found to be pH 7.5 and 30℃, respectively. With 5% (v/v) inocula, the isolate produced 0.217 g/L of reducing sugars from 1 g/L of agar and 0.250 g/L of reducing sugars from 1 g/L of carrageenan. This is the first study to directly demonstrate the ability of *B. alcalophilus* to degrade both agar and carrageenan. This bacterium could provide the answer to the problem of red-seaweed waste reutilization, and the reducing sugars produced could be a valuable resource for ethanol production.

# I. INTRODUCTION

Seaweeds are suitable for consumption by human beings and animals and are a favorite food in Asian countries in particular [1]. They are often used in fertilizer, fungicides, herbicides, and phycocolloids such as alginate, carrageenan, and agar [2]. Worldwide consumption of seafood including seaweeds has increased steadily due to its health benefits. In recent years, the amount of seaweed waste has increased due to its culture as an industrial resource and as a depolluting plant for cleaning inland sea areas and eutrophied seawater [3,4]. Accordingly, the disposal and reutilization of seaweed waste has become essential for the preservation of the marine environment and recycling of organic substances [5].

From 2008 to 2010 in Korea, the annual production of seaweed, mostly by aquaculture, averaged approximately 882 thousand tons [6]. The major species were brown seaweed and red seaweed, such as *Porphyra tenera* and *Porphyra yezoensis,* because they are well adapted to Korean weather. Approximately one quarter of the total amount of seaweed produced is assumed to be discarded annually [7]. Unused seaweed waste is customarily discarded via landfill, incineration,

or by dumping into the sea. Therefore, the greater part of seaweed waste has been utilized inefficiently, and its disposal affects the local environment. In addition, the cost for disposal of seaweed waste will markedly increase after 2012 because dumping waste into the sea will become prohibited in Korea according to the London Convention [8]. Due to this circumstance, there is an urgent need to find ecologically acceptable means for the reutilization of seaweed waste.

Compared with terrestrial plants, seaweeds possess a high water content of approximately 70-90%, a relatively high protein content of approximately 10%, and varying levels of carbohydrates [I9]. The carbohydrate content of red seaweed is 30-60%, consisting mostly of agar and carrageenan [10]. Compared to other seaweeds, red seaweed contains more carbohydrates, which could be advantageous for its use as a resource for bioethanol production [11]. Agar is composed of agarose (70%) and agaropectin (30%). Agarose is a linear polysaccharide composed of alternating residues of 3-*O*-linked β-D-galactopyranose and 4-*O*-linked 3,6-anhydro-α-L-galactopyranose, and carrageenan is a linear sulfated galactopyranose polysaccharide composed of alternating residues of 3-*O*-linked β-D-galactopyranose and 4-*O*-linked 3,6-anhydro-α-D-galactopyranose [12]. This complicated

molecular structure must be hydrolyzed to obtain fermentable sugars for ethanol production (Fig. 1). From an economic point of view, acid hydrolysis is considered to be a promising means of saccharification that is less expensive than enzymatic hydrolysis [11,13]. However, acid hydrolysis produces non-sugar by-products that can inhibit ethanol-producing yeast and bacteria [14-16]. Furthermore, acid hydrolysis poses a problem to the recovery of the reagents and resulting saccharides [17]. For this reason, enzymes that degrade marine algal polysaccharides have also been actively studied using various types of organisms for the past decade [18-20]. Currently, several agarases (mostly β-agarases) have been isolated from several bacterial genera, including *Cytophaga* [21], *Pseudomonas* [22], *Pseudoalteromonas* [23], *Streptomyces* [24], *Alteromonas* [25,26], *Vibrio* [27], *Microscilla* [28], *Microbulbifer* [29], *Zobella* [30], and *Acinetobacter* [31]. Carrageenan-degrading microorganisms are known to have hydrolytic enzymes that are specific to the different types of carrageenan: kappa-carrageenase isolated from *Cytophaga drobachiensis* [32] and *Pseudoalteromonas carrageenovora* [33], iota-carrageenase isolated from *Pseudoalteromonas carrageenovora* [34] and *Alteromonas fortis* [35], and lambda-carrageenase isolated from *Pseudoalteromonas carrageenaovora* [36] and

*Pseudoalteromonas* CL19 [37].

The saccharification of seaweed is an essential operation for ethanol production and has been widely studied in recent years [38]. The advantages of using purified enzymes over intact bacteria are their higher possible levels of catalytic activity and the avoidance of undesirable side-reactions and bacterial reproduction [39]. However, purification of a specific enzyme is likely to be expensive, and the purified enzyme may be more sensitive to inactivation than the enzyme within an intact bacterium. In addition, the pretreatment of algal polysaccharides is required for efficient saccharification when using purified enzyme, which is reported to be expensive when applied to large quantities of biomass [40]. Thus, the isolation of potentially useful microorganisms and using them effectively to degrade seaweed waste is indispensable. Currently, few studies have characterized bacteria for the reutilization of seaweed wastes. This study, therefore, attempted to isolate a novel bacterium capable of degrading agar and carrageenan and to characterize its potential application in the reutilization of red-seaweed waste.

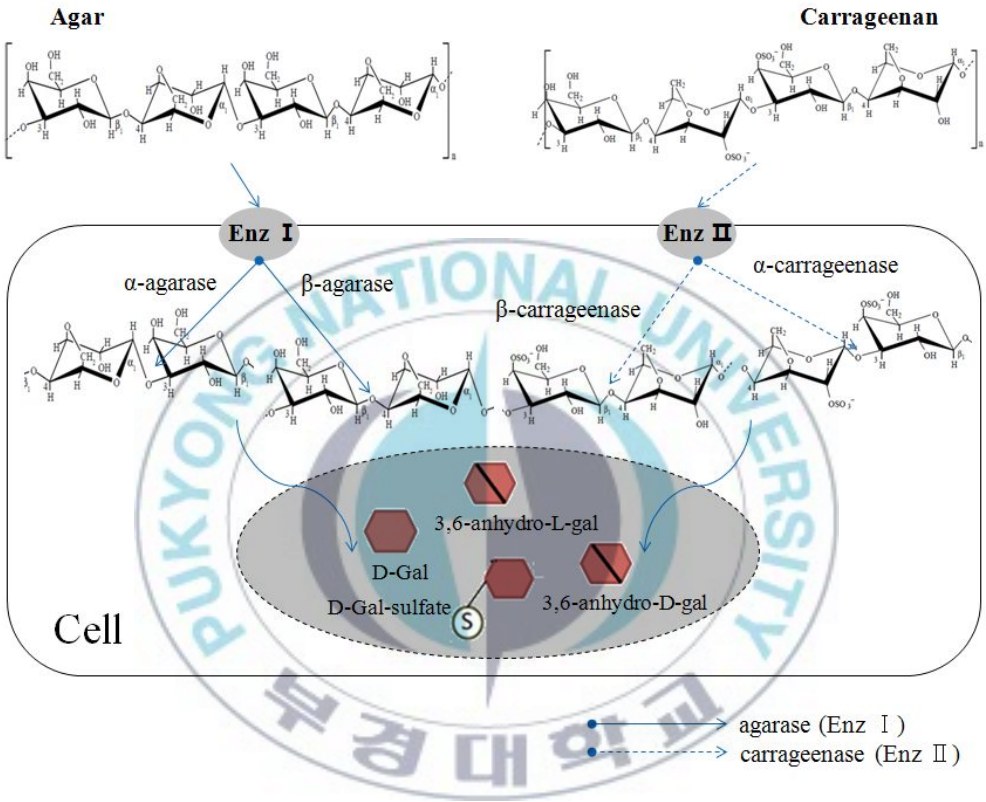


Fig. 1. Schematic representation of agar and carrageenan hydrolytic pathways.

# II. MATERIALS AND METHODS

## 1. *Isolation of potential red-seaweed-waste-degrading microorganisms*

Bacteria with potential applications in the degradation of the main polysaccharides contained in red seaweed, agar and carrageenan, were isolated from marsh, silt and sandbar locations in a coastal area near Busan (Korea), where red seaweed often drift and pile up. One gram of each sample was added to a sterile 250-mL flask that contained (per L): 15 g of *Porphyra* powder as a sole carbon source in seawater (pH 7.4) or 10 g of *Porphyra* powder and 1 g of $NH_4Cl$ in tap water (pH 6.8). Each flask was incubated at 37 ℃ and 180 rpm for 2 weeks. After 2 weeks, 10 mL of each suspended liquid was transferred to fresh liquid medium and incubated under the same conditions. Subsequently, cells in each flask were spread with a platinum loop onto the same media solidified with 1.5% nutrient agar. A purified isolate was obtained by repeated streaking onto fresh agar plates. Each pure strain was maintained on 1.5% nutrient agar plates at 4 ℃ until use and transferred to a fresh agar plate every two weeks.

## *2. Tests for degradation abilities of isolates*

To screen the strains, isolated bacteria were spread first on *Porphyra* powder agar, which contained 10 g/L of *Porphyra* powder, 1 g/L of $NH_4Cl$, 0.05 g/L of bromocresol purple, and 15 g/L agar (pH 6.8). *Porphyra* powder was purchased from a local market and ground using a porcelain mortar and pestle. The final samples were sieved to achieve particle size homogeneity using a 100-mesh sieve. The agar plates were incubated at 37 ℃, and only yellow colonies were chosen after 2 days. Each primarily screened colony was then transferred to a 10-mL tube containing *Porphyra* powder medium and incubated under the same culture conditions. Ten microliters of each 3-day old culture broth was spotted onto *Porphyra* powder agar plates and incubated for 4 days. After 4 days of incubation, 10 mL of Lugol's solution (10 g/L of potassium iodide and 1 g/L of iodine) was poured onto the agar media for staining, according to the method of Saraswathi et al. [41]. After 5 min, the sizes of the clear zones formed around the colonies were measured. For the strain showing the largest clear zone, the degradation ability was tested further, both on solid agar medium (10 g/L of agar, 2 g/L of $NaNO_3$, 0.5 g/L of $K_2HPO_4$, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.5 g/L of KCl, 0.1 g/L of

$CaCl_2 \cdot 2H_2O$, 0.02 g/L of $FeSO_4 \cdot 7H_2O$, 0.5 g/L of yeast extract, and tap water, pH 6.8) and on solid carrageenan (mostly κ-carrageenan) medium (10 g/L of carrageenan, 1 g/L of $NH_4Cl$, 0.3 g/L of $K_2HPO_4$, 0.1 g/L of $KH_2PO_4$, 0.4 g/L of yeast extract, and tap water, pH 6.8) using the same method. The sizes of the clear zones were measured after 2, 4 and 6 days of incubation. Agar and carrageenan were purchased from the Sigma-Aldrich Company (St. Louis, MO, USA).

### *3. Identification of the isolate*

The strain with the highest potential utility was selected by means of a test of its degradation abilities, and it was identified primarily on the basis of colony morphology, Gram reaction of a bacterial smear, and microscopic examination.

Specific identification of the isolate was carried out using 16S rDNA sequence analysis. DNA was extracted using an AccuPrep® Genomic DNA extraction kit (Bioneer, Korea) according to the manufacturer's instructions. PCR amplification of the DNA using the 158F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3') were performed with a

PCR thermal cycler (DICE model TP600, Takara, Japan). The reaction mixture contained primers (10 pmoles /㎕), 2.5 mM dNTPs, 10x reaction buffer, 2.5 U Taq polymerase (Takara, Japan), 1 ㎍ DNA template and sterilized water to achieve a final volume of 50 ㎕. PCR was performed under the following conditions: initial denaturation at 95 ℃ for 5 min; 30 cycles of 95 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 30 s; and a final extension step at 72 ℃ for 10 min. Five microliters of amplification products was separated by electrophoresis on a 1% agarose gel in 0.5% TAE buffer at 100 V for 10 min. Gels were stained with ethidium bromide and photographed under UV light. Gel images were recorded using a digital camera.

Agarose pieces containing amplified DNA were excised from the gel, and the DNA was recovered using the AccuPrep® SV Gel and PCR Clean-up System (Promega, USA). The purified products were ligated into the pGEM T-easy vector (Invitrogen) and then transformed into *E. coli* DH5α MCR Competent Cells according to the manufacturer's instructions (Promega). Colonies were blue/white screened on LB agar with ampicillin (Sigma), X-gal (Promega) and isopropyl-β-D-thiogalactopyranoside (IPTG) (Promega). White colonies were randomly chosen, cultivated and stored in freezing medium (5 g/L of yeast extract, 10 g/L of NaCl, 10 g/L of Bacto

tryptone, and 0.1 g/L of ampicillin) at -80 ℃. Plasmid DNA was extracted using the AccuPrep® Plasmid Extraction Kit (Bioneer, Korea) and sequencing was performed by Macrogen, Ltd. (Seoul, Korea). The 5′- and 3′-ends of the constructs were sequenced using M13 primers flanking the cloning sites. These sequences were compared with entries in GenBank (National Center for Biotechnology Information, Rockville Pike, Bethesda, MD) using the Advanced Basic-Local-Alignment-Search-Tool (BLAST) similarity search option [42] accessible from the homepage at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The BioEdit Sequence Alignment Editor version 5.0.9 [43] was used to check alignment and remove all positions with gaps before calculating distances using the DNAdist program in PHYLIP (version 3.5c; distributed by J. Felsenstein, University of Washington, Seattle).

### *4. Characteristics of degradation by the isolate*

To ascertain the optimal pH and temperature for the degradation of agar and carrageenan by the isolate, each experiment was executed in a 100 mL flask (50 mL working

volume). In a shaking incubator at 150 rpm, each flask was incubated in parallel at various pHs (5.5, 6.5, 7.5, and 8.5), and the experiment was repeated at different temperatures (25, 30, 37, and 45 ℃). For the agar degradation experiments, the isolate was cultivated in culture medium containing the following: 1 g/L of agar, 2 g/L of $NaNO_3$, 0.5 g/L of $K_2HPO_4$, 0.5 g/L of $MgSO_4 \cdot 7H_2O$, 0.5 g/L of KCl, 0.1 g/L of $CaCl_2 \cdot 2H_2O$, 0.02 g/L of $FeSO_4 \cdot 7H_2O$, 0.5 g/L of yeast extract, and tap water. The culture medium for the carrageenan biodegradation experiments contained: 1 g/L of carrageenan, 1 g/L of $NH_4Cl$, 0.3 g/L of $K_2HPO_4$, 0.1 g/L of $KH_2PO_4$, 0.4 g/L of yeast extract, and tap water. The pH of the culture media was adjusted to 7.5 before autoclaving, and the culture media were sterilized at 121 ℃ for 15 min. During the experiment, samples were taken periodically from the flasks to measure pH, optical density (OD) of cells and the concentrations of total reducing sugars. The characteristic of degradation by the isolate was investigated using the same agar and carrageenan culture media under the optimal conditions.

### *5. Analytical methods*

Cell growth of the isolate was measured using a VIS/UV spectrophotometer at 400nm. The characterization of the isolate's degradation ability on *Porphyra* powder, agar or carrageenan was deduced from the quantities of total reducing sugars produced. The total reducing sugars were quantified using the 3,5-dinitrosalicylic acid (DNS) method [44]. The DNS regent was prepared by mixing 5.3 g of 3,5-dinitrosalicyclic acid and 9.9 g NaOH into 708 mL of distilled water (DW) in a stirred beaker. Subsequently, 153 g of Rochelle salts (potassium sodium tartrate), 4.05 g of phenol and 4.15 g of sodium sulfite were added. To create the standard curve, 2 mL of different concentrations of glucose were added to 8 mL of the DNS reagent, mixed well and then heated in boiling water for 5 min. The samples were cooled to room temperature and their absorbances at 550 nm were determined using a VIS/UV spectrophotometer. A blank solution consisting of 2 ml DW and 8 ml DNS regent was used to zero the spectrophotometer before analysis. The concentrations of reducing sugars in subsequent withdrawn culture broth samples were obtained using the glucose standard curve.

# III. RESULTS AND DISCUSSION

## 1. *Screening of potential red-seaweed-waste-degrading bacteria*

By repeated streaking on agar plates containing bromocresol purple, six strains were purified. The six isolates were given the names SYR1 to SYR6. Among these strains, only the SYR4 strain demonstrated degradation abilities for all of the *Porphyra* powder, agar and carrageenan media tested (Fig. 2). In the study by Yanagisawa et al. [45], Meicelase, which consisted of a mixture of hydrolyzing enzymes, showed diverse activities on different types of glucans. They reported that the rate and the pattern of biodegradation of the algal polysaccharides strongly depended upon the structure; however, the reason for this dependence has not yet been clarified. In this study, the isolate SYR4, as a single strain, demonstrated its ability to degrade agar and carrageenan. Although there have been many reports on agar- or carrageenan-degrading bacteria, few bacteria degrade both: Erasmus et al. [11] reported a few enteric bacteria from the digestive system of *Halioteis midae* capable of degrading both agar and carrageenan.

Ziayoddin et al. [46] also reported that the agar-degrading bacterium *Pseudomonas aeruginosa* ZSL-2 had the ability to degrade carrageenan.

For ethanol production from red-seaweed polysaccharides, reducing sugars must be obtained by microbial degradation. Hence, the degradation ability of the SYR4 strain was examined on different types of red-seaweed polysaccharides. As shown in Table 1, substantial radii of clear zones of 0.83, 0.87, and 1.18 cm, excluding colony radii, were observed on *Porphyra* powder, agar and carrageenan, respectively, after 4 days of incubation and radii of clear zones of 1.5, 1.38 and 1.53 cm, respectively, were observed after 6 days of incubation, with the clear zones enlarging as incubation time increased. Thus, the degradation ability of the SYR4 strain appeared to be higher for carrageenan. This result indicates that the SYR4 strain would be a good candidate to reutilize red-seaweed waste in which agar and carrageenan are present at 50-70% [9]. For this reason, the SYR4 strain was identified and characterized in later experiments.

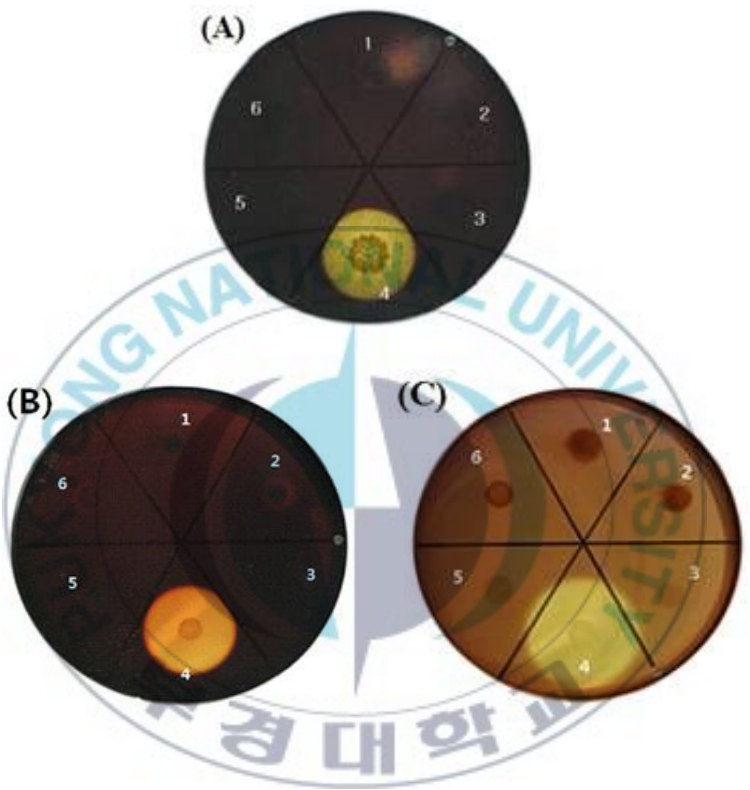


Fig. 2. Clear zones formed by the isolate on *Porphyra* powder (A), agar (B), and carrageenan (C) solid media.

Table 1. Sizes of clear zones formed on *Porphyra* powder, agar and carrageenan solid media[a]

| Solid medium | Incubation time (day) | Radius of clear zone (cm) | Radius of colony (cm) |
|---|---|---|---|
| *Porphyra* powder | 0 | 0 | 0 |
| | 2 | 1.20±0.01 | 0.50±0.01 |
| | 4 | 1.43±0.12 | 0.60±0.02 |
| | 6 | 2.20±0.01 | 0.70±0.02 |
| Agar | 0 | 0 | 0 |
| | 2 | 0.95±0.13 | 0.30±0.01 |
| | 4 | 1.22±0.04 | 0.35±0.01 |
| | 6 | 1.73±0.13 | 0.35±0.02 |
| Carrageenan | 0 | 0 | 0 |
| | 2 | 1.10±0.10 | 0.35±0.01 |
| | 4 | 1.58±0.07 | 0.40±0.01 |
| | 6 | 1.93±0.03 | 0.40±0.02 |

[a]Each culture medium was adjusted to pH 6.8 and incubated at 37 ℃. Values represent mean±S.D. of three replicates.

## *2. Identification of the isolate*

Based on microscopic observation, the isolate SYR4 was very motile in the vegetative state and possessed Gram-positive rods measuring 0.5-1.2 $\mu$m in width and 2-4 $\mu$m in length, and formed endospores. The colors of the colonies formed on *Porphyra* powder, agar and carrageenan media were orange, pink and white, respectively. The species-specific identification of the isolate was performed using 16S rDNA sequence analysis. The 1,560 bp-sized fragment of the 16S rDNA gene of the isolate was amplified and sequenced. Sequence analysis of the 16S rDNA gene and BLAST sequence comparison confirmed that the isolate was *Bacillus alcalophilus* (GenBank Accession No.: EU231621.1) with a similarity of 97%. Because this strain demonstrated distinct degradation ability for both agar and carrageenan, we have applied for a Korean patent (No.: 10-2012-0024992) after depositing it into the Korean Agricultural Culture Collection (KACC) as KACC91705P. Among *Bacillus* species, only two strains have been reported to have agar-degrading ability: *B. cereus* as a marine bacterium [47] and *B. agarexedens* as a non-marine bacterium isolated from soil [48].

### 3. *Characteristics of the isolate for reutilization of red-seaweed waste*

To find the optimal pH and temperature for cell growth of the isolate and for its production of reducing sugars, an experiment was executed in 100-mL flasks. The highest optical density of cells (1.50) and production of reducing sugars (0.22 g/L) were obtained from the isolate's cultivation on 1 g/L of agar at pH 7.5 (Fig. 3A). Maximal values obtained from cultivation on carrageenan were also achieved at pH 7.5, with a cell density of 1.12 and a concentration of reducing sugars of 0.25 g/L (Fig. 3B). The isolate showed better cell growth on agar, but it produced a higher concentration of reducing sugars on carrageenan. As shown in Fig. 4, the highest cell densities and reducing sugar concentrations were achieved at 30 °C, 1.49 and 0.22 g/L on agar, and 1.13 and 0.25 g/L on carrageenan, respectively.

Under the optimal conditions, the degradation characteristics of the isolate were examined during growth in 100-mL flasks using agar or carrageenan as sole carbon sources. A time course for the degradation of agar by the isolate revealed that cell growth was accompanied by a slight drop in the pH (Fig. 5). Cell density reached its maximum

(1.53) after 12 h of cultivation and then decreased slowly. The maximum concentration (0.22 g/L) of reducing sugars was achieved at 24 h, together with cellular motility. The production persisted after 48 h, although the cell mass was decreased slightly at that time. The same trend was found in cultivation using carrageenan as the carbon source (Fig. 6). The cell density reached its maximum (1.11) after 12 h, with a slight drop in pH. The total reducing sugars were produced gradually, and their maximum concentration (0.25 g/L) was achieved after 24 h. In this study, the cells reached stationary phase within 12 h, although cellular motility was high. In these experiments, 1 g/L of agar or carrageenan was used as a sole carbon source. When a higher concentration of agar or carrageenan was used, the cell density significantly decreased with a concomitant decrease in cellular motility due to the high viscosity of the culture medium (data not shown). Therefore, fed-batch operation would be appropriate for the efficient production of fermentable sugars [49]. The yields of reducing sugar from agar and carrageenan were estimated to be 22 and 25%, respectively.

The reducing sugars produced may be utilized in many fields because red seaweeds have been reported to contain biologically active substances that can be used as anticoagulant,

antithrombotic, anti-inflammatory, anti-tumor, contraceptive, and anti-viral agents [2,50]. Recently, this usage was extended to alternative energy because red seaweeds have great potential for use as a feedstock for future bioethanol production [9,51]. Due to limited natural resources, bioethanol is being considered as a potential liquid fuel [52], and its production from seaweed is promising and ecofriendly. Recently, the red algae *Kappaphycus alvarezii* [53,54], *Eucheuma* and *Hypnea* [55] have been reported as sources of bioethanol. In the present study, the strain SYR4 produced 0.22 g/L of reducing sugars from 1 g $L^{-1}$ of agar and 0.25 g $L^{-1}$ of reducing sugars from 1 g $L^{-1}$ of carrageenan. In previous studies [56], the conversion rates of reducing sugars to ethanol by fermentation were reported to be in the range of 30-40%. According to this report, 7-10 wt % of ethanol can be produced by the isolate from the red-seaweed polysaccharides. This ethanol yield is almost threefold lower compared with those (23.5-29 wt %) obtained via various means of pretreatment prior to fermentation, including enzyme [57], alkaline [58] or high-temperature acid pretreatments [59,60]. However, the direct application of a microorganism itself precludes the cost incurred for enzymatic or chemical saccharification of the polysaccharides, which will result in a considerable reduction to the product cost [40]. As a result,

biodegradation using a novel bacterium, *B. alcalophilus,* seems to be cost-effective for recycling red-seaweed waste. For better degradation, fed-batch fermentation would be required and is expected to yield higher productivity. Further study may provide the answer to the impending problem concerning the prohibition of dumping seaweed waste into the sea.

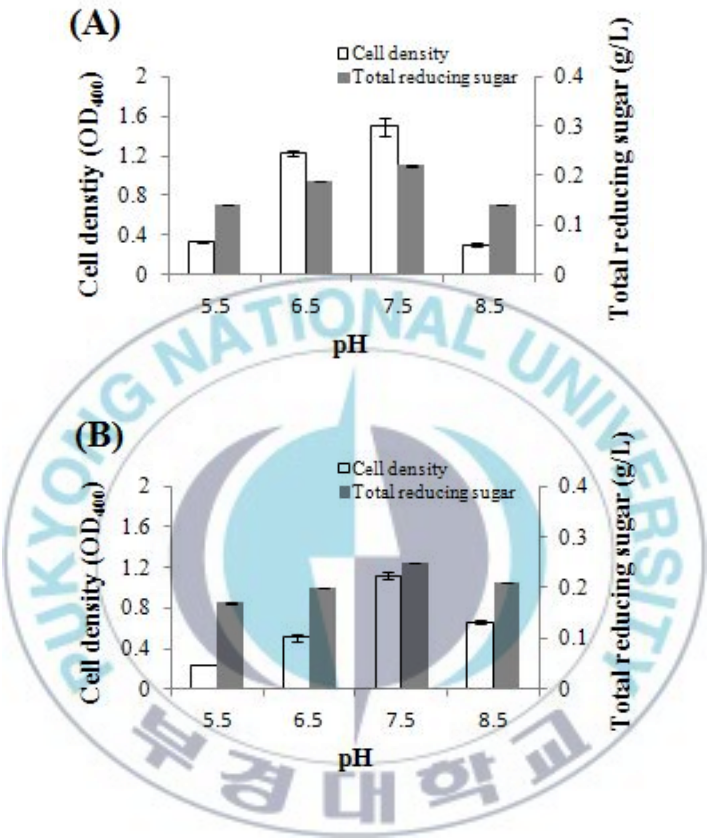


Fig. 3. Cell growth and the production of reducing sugars on agar (A) and carrageenan (B) by the isolate at various pHs. Error bars: mean±S.D. of three replicates.

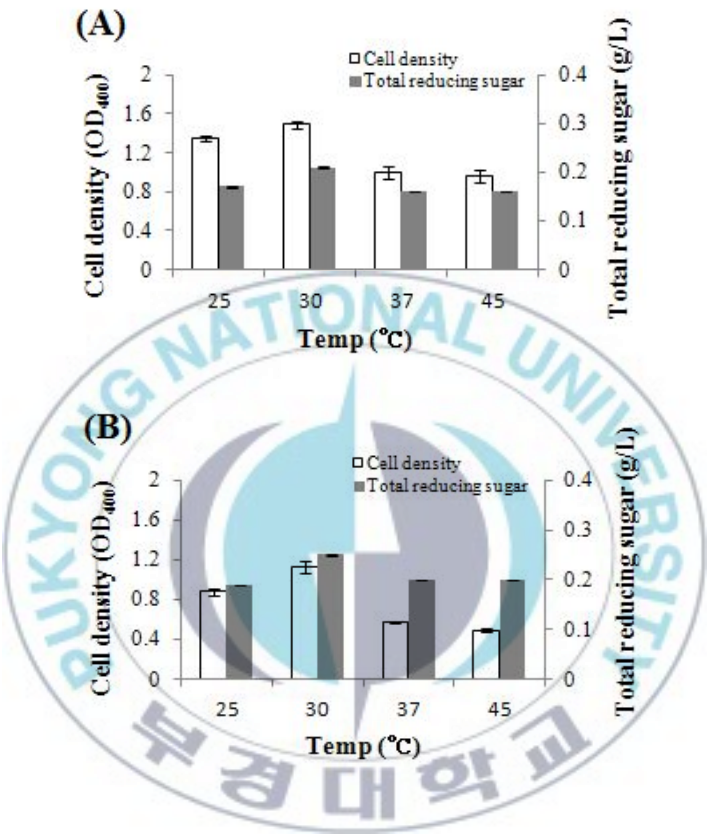


Fig. 4. Cell growth and the production of reducing sugars on agar (A) and carrageenan (B) by the isolate at various temperatures. Error bars: mean±S.D. of three replicates.

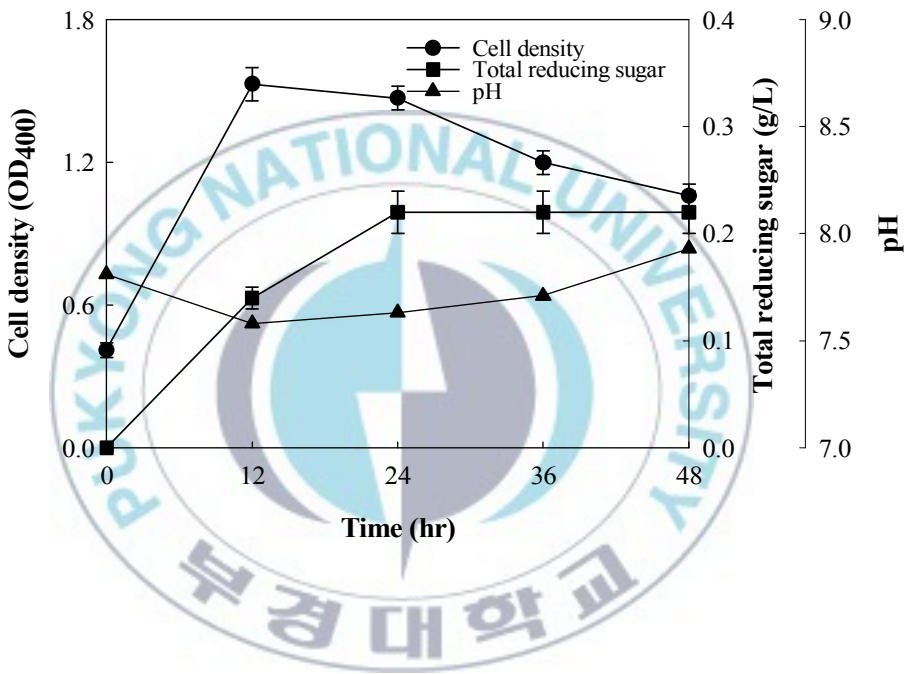


Fig. 5. Degradation of agar by the isolate under optimal pH and temperature. Error bars: mean±S.D. of three replicates.

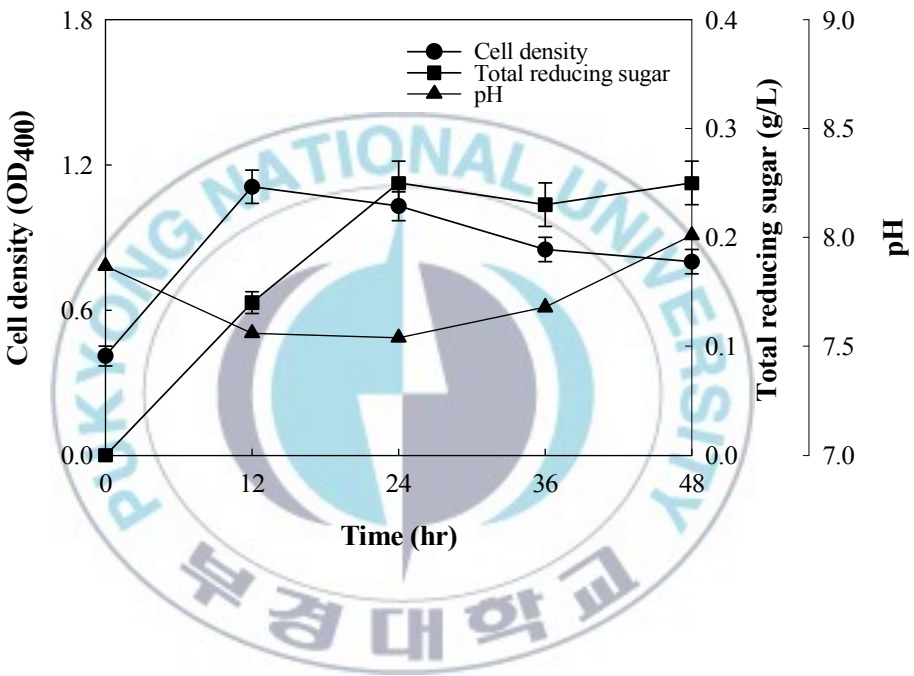


Fig. 6. Degradation of carrageenan by the isolate under optimal pH and temperature. Error bars: mean±S.D. of three replicates.

# IV. CONCLUSIONS

A *B. alcalophilus* strain was newly isolated from a marsh and characterized for reutilization of red-seaweed waste. The isolate showed high degradative abilities for both agar and carrageenan. The yields of reducing sugars from agar and carrageenan were 22 and 25%, respectively. Ecofriendly treatment of red-seaweed by this isolate is feasible, and the reducing sugars produced would be a valuable resource for bioethanol production. Thus, this may be a good method to turn waste into valuable resources with low energy consumption. To achieve higher productivity, fed-batch fermentation should be investigated.

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