Thesis for the Degree of Master of Engineering

Isolation of Nonylphenol-degrading Bacteria and Its Degradation Characteristics

by

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The Graduate School

Pukyong National University

February 2012

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(노닐페놀 분해 미생물 컨소시엄에서 분리된 세균의 노닐페놀 분해 특성)

Advisor : Dr. Yang-Bong Lee

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Dae-Ung Yu

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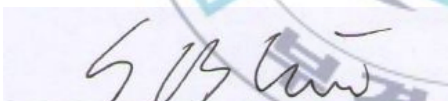

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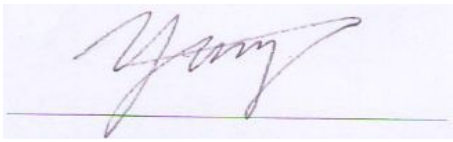

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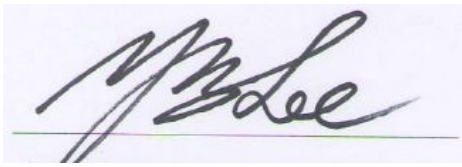

(Member) Dr. Yang-Bong Lee

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노닐페놀 분해 미생물 컨소시엄에서 분리된 세균의 노닐페놀 분해 특성

유 대 웅

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요 약

최근, 환경 중으로 방출되는 난분해성의 생체 유해물질들이 사회적인 문제로 대두되고 있다. 특히, 난분해성이며 미량으로도 수생생물의 내분비계 교란물질로 작용하는 nonylphenol (NP)는 하천폐수나 토양에서 광범위하게 발견된다. NP은 내분비계 장애물질로 지난 50년 이상 동안 산업체와 가정에서 비이온 계면활성제와 세정제로써 전 세계적으로 이용된 alkylphenol polyethoxylates의 분해산물이다. 산업폐수나 도시하수처리공정에서 연안이나 혹은 도시하천으로 직접 유입되며, 자연환경 중에서 분해가 잘 되지 않는 난분해성 물질로 소수성 화합물의 특징으로 인해 수중에서 쉽게 분해되지 않아 환경오염을 가중시키고, 특히 수중생물들에 대한 내분비계 교란물질로서 작용하고 있다.

독성유기화합물에 대한 친환경적인 분해에 대한 필요성 증가에 따라, 수생환경으로부터 NP 분해 미생물을 분리 하기위해 실시하였다. NP 분해 미생물은 NP가 함유된 증균 배지를 이용하여 분리하였으며, 일련의 연구과정에 최종적으로 높은 NP 분해능이 있는 미생물 컨소시엄 SW-03이 분리 되었다. SW-03는 25℃에서 40일 이내에 100 ppm의 NP를 99% 이상 분해하는 것으로 조사 되었다. 또한, NP 분해 단일 세균을 분리하기 위하여 컨소시엄 균주인 SW-03으로부터 NP 분해능이 뛰어난 단일균주를 분리하였다. 분리한 단일 균주(SW-03-A, -B, -C, -D,

-E, -F1, -F2, -G, -H, -I)의 형태학적 특성을 분석하기 위하여 그람 염색을 실시하였다.

NP의 분해능을 동력학적으로 분석(kinetic analysis)한 결과, 대부분의 단일 균주들이 SW-03 보다 높은 NP 분해능을 나타내었으며 이 중 SW-03-F1, -F2, -G, -I의 단일 균주의 분해력이 컨소시엄 균주인 SW-03 및 다른 균들과 비교해 월등히 높았으며 이 중 병원성 균으로 동정된 F2를 제외한 나머지 F1, G, 및 I 세 균주 조합에 따른 NP 분해도 측정 하였다.

분해활성이 높은 4가지 균주는 전자 현미경 사진(SEM)을 찍어 추가적인 형태학적 특성을 파악하였고 16S rDNA 염기서열 분석을 이용하여 균 동정을 실시하였다. 본 연구에서 얻어진 결과들은 추후, NP의 환경친화적인 분해 기술개발에 유용하게 사용될 것으로 기대된다.

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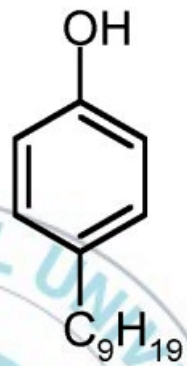
Introduction

Nonylphenol (NP) is a ubiquitous pollutant, which mainly results from the biodegradation of widely used NP polyethoxylate surfactants (Corvini *et al.*, 2004). The NP polyethoxylate is known to be degraded slowly through the aerobic and anaerobic degradation to NP in the sewage disposal plant or water environment. (Giger *et al.*, 1984; Ahel *et al.*, 1994; Fries and Puttmam, 2003; Azevedo *et al.*, 2001). NP is considered to be an endocrine disruptor due to weak ability to mimic estrogen and in turn disrupt the natural balance of hormones in affected organisms (Table 1; Dayue *et al.*, 1999). NP is subsequently discharged into stream or coast from industrial waste water or sewage disposal process and its potential aquatic risks have been extensively studied on aquatic organism as endocrine disruptor (Fairchild *et al.*, 1999; Yadetie and Male, 2002; Karels *et al.*, 2003; Hernadez-Raquet *et al.*, 2007). However, up to date, only less information is only avaliable on the local distribution of NP over worldwide (Björn Wellenius *et al.*, 1994). In Korea, it has been reported that NP was detected in the range of 113 to 3890 ng per g dry weight at the Masan Bay, Gyeongnam (Khim *et al.*, 1999), 6.0 to 119.1 μg per kg at the sediments collected 11 different rivers over the country (Cho *et al.*, 2004), and 3.6 μg per L in the Sihwaho Bay, Gyeongido (Li *et al.*, 2004). These reported revealed that NP was

extensively distributed into the aquatic environment. In particular, it needs not only an investigation into the distribution of NP but also a technology that degrades NP environmentally, because of the trace of NP in the aquatic environment acts to aquatic organisms as an endocrine disruptor.

A biological decomposition method using microorganism, which is called bioremediation,, has often considered an environmentally favorable method to restore the environment contaminated with harmful non-resolvability chemicals (Kim *et al.*, 1997; Kim *et al.*, 2004; Lee *et al.*, 2009). The bioremediation decomposes ultimately organic toxic substances into water and carbon dioxide as mineralization recognized (Ripp *et al.*, 2000; Kim et al., 2007; Kang and Kim, 2007; Lee *et al.*, 2009; Lee *et al.*, 2009). There are several reports related with the bioremediation of NP and most studies are on soil environment not aquatic environment (Tanghe *et al.*, 1999; Fujii *et al*, 2000; Fujii *et al.*, 2001; Corvini *et al.*, 2004; Junghanns *et al.*, 2005; Shi and Bending, 2007). Thus, to date, there have been no unambiguous reports about the bioremediation of NP in aquatic envrionment. Therefore, this study was conducted to isolate a NP-degrading microorganism in aquatic environment and to perform the kinetic analysis of NP degradation by bacterial strains isolated in the current study.

Table 1. Physicochemical properties of NP

Chemical structure	
IUPAC name	4-(2,4-dimethylheptan-3-yl)phenol
Molecular formula	$C_{15}H_{24}O$
Molecular weight	220.35 g/mol
Appearance	White crystals
Density	0.953
pK_a (estimated)	10.28
Vapour pressure	0.3 Pa (25 ℃)
Solubility in water	6 mg/L (pH 7)
Partition coefficient	4.48
Melting point	-8 ~ 2 ℃
Boiling point	293 ~ 297 ℃
Hazards	low level endrocrine disruptor

Materials and Methods

1. Chemicals

NP (Assay >85%) were purchased from Fluka company (Fluka; St.Louis, MO) and other reagents used in the analysis were analytical grade and purchased from commercial source.

2. Medium for strain culture and isolation

YNB (Yeast Nitrogen Base without Amino Acids; Difco, Franklin Lakes, NJ) medium containing 100 ppm NP was used as a basial medium for isolation of NP-degrading bacteria as described by Fujii *et al.* (2000, 2001) and Corvini *et al.* (2004). YNB medium containing 100 ppm NP was used for enrichment culture and YNB agar plate containing 100 ppm NP was used for isolation of NP-degrading single bacterium strain. LB (Luria-Bertani media; Difco, Franklin Lakes, NJ) broth was used for measuring isolated strain of growth (Table 2, 3).

Table 2. Composition of YNB media

Media	Ingredient	Content (per Liter)	
YNB (Yeast Nitrogen Base without Amino Acids)	*Nitrogen Source*		
	Ammonium Sulfate	5.0	g
	Vitamins		
	Biotin	2.0	μg
	Calcium Pantothenate	400.0	μg
	Folic Acid	2.0	μg
	Inositol	2,000.0	μg
	Niacin	400.0	μg
	p-Aminobenzoic Acid	200.0	μg
	Pyridoxine Hydrochloride	400.0	μg
	Riboflavin	200.0	μg
	Thiamine Hydrochloride	400.0	μg
	Compounds Supplying Trace Elements		
	Boric Acid	500.0	μg
	Copper Sulfate	40.0	μg
	Potassium Iodide	100.0	μg
	Ferric Chloride	200.0	μg
	Managamese Sulfate	400.0	μg
	Sodium Molybdate	200.0	μg
	Zinc Sulfate	400.0	μg
	Salts		
	Monopotassium Phosphate	1.0	g
	Magnesium Sulfate	0.5	g
	Sodium Chloride	0.1	g
	Calcium Chloride	0.1	g

Table 3. Composition of LB media

Media	Ingredient	Content (per Liter)
LB (Luria-Bertani media)	Tryptone peptone	10.0 g
	Bacto-yeast extract	5.0 g
	NaCl	5.0 g
	Glucose	1.0 g

3. Isolation and culture of NP-degrading bacteria

In order to isolate NP-degrading bacteria from a degrading microbial consortium, the consortium strain was cultivated in YNB medium containing 100 ppm NP at 25℃ under the aerobic condition. The 100 μl sample was taken with interval and then spreaded on the YNB agar plate containing 100 ppm NP. The agar plate was incubated at 25℃ for 7 days and single colony grown on the plate was collected for further study.

4. Identification of NP-degrading bacteria

Isolated strains from NP-degrading microbial consortium was identified by morphological, biochemical and genetical characteristics. A light microscopy (Motic 300, Motic, Richmond, Canada) and a scanning electron microscope (SEM; Hitachi S-2400 scanning electron microscope; Hitachi Ltd., Tokyo, Japan) were used for the morphological analysis, VITEK Gram Negative Identification card (GNI-) or VITEK Gram Positive Identification card (GNI+) (Biomerieux Inc, Missouri, USA) was used for physiochemical analysis. Also, the identification of each single strain was determined through 16S rDNA sequences. G-spin™ Genomic DNA Extraction Kit (iNtRON, Korea) was used for chromosomal DNA extraction, 27F

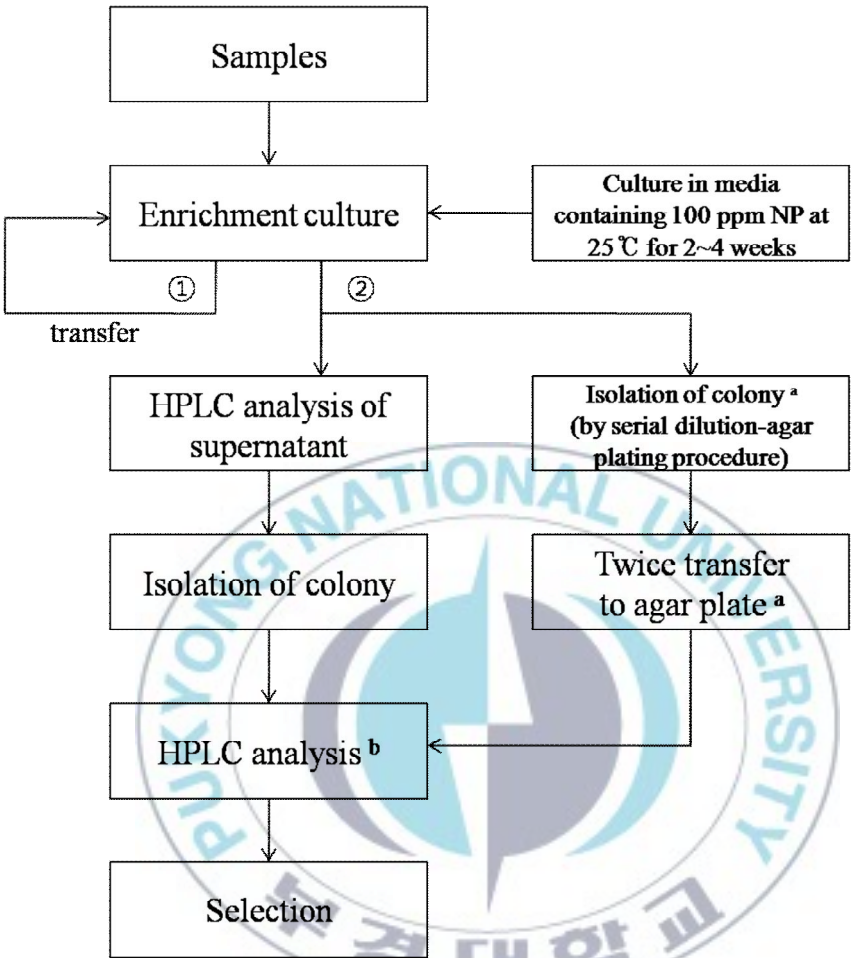
(5′-GTTTGGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGGGATCCAGCC-3′) primer (Takara, Shiga, Japan) were used for Polymerase Chain Reaction (PCR) to amplify 16S rDNA. PCR was conducted as the following conditions. The mixture, which was added 2 μL of 20 pmole each primer, 25 ng DNA template, 0.5 μL Taq polymerase (2.5 U), 5 μL of 10X Taq polymerase buffer, 1 μL of 10 mM dNTP, and 39 μL of dH_2O, was denatured for 2 min at 94℃. After denaturation, PCR was cycled 25 times at 94℃ for 1 min, at 52℃ for 1 min, at 72℃ for 2 min, followed by incubation at 72℃ for 5 min.

The amplified PCR products was sequenced by SolGent (Daejeon, Korea). Homology search of sequence was conducted through Ribosomal database (http://www.ncbi.nim.nih.gov/BLAST/).

5. NP Extraction and HPLC analysis

The ability of NP-biodegradation by single isolated bacteria was determined to analysis the remaining NP content in medium using a high performance liquid chromatography (HPLC) (Fujii *et al.*, 2000). NP was extracted for HPLC analysis as followed a method. After the cultivation, 1 ml of medium was taken into a tube, and then added 4 ml of deionized water and 15 ml of acetonitrile. The mixture was mixed for 3 min using vortex mixer to extract a remaining NP. After extraction, a sample of upper layer was carefully taken, filtrated

with 0.2 μm filter (DISMIC-25AS, ADVANTEC, Japan), and then analyzed by a HPLC (Flexar HPLC System, PerkinElmer, Waltham, MA) equiped with equipped with Shiseido C_{18} reverse-phase column (250mm×4.6mm, I.D.5μm; Shiseido Co., Tokyo, Japan). For the detection of NP, a elution of 75% acetonitrile with 25% of water was used at a flow rate of 1.0 ml per min and elute were monitored at 277 nm. Amount of remaining NP was indicated as a percentage value of the reduced NP peak. HPLC analysis conditions are listed in (Table 4).



[a]Bacteria was cultured on YNB medium agar plate containing 100 ppm NP.
[b]Bacteria was cultured on YNB media containing 100 ppm NP and the remaining NP was determined by HPLC.

Fig. 1. Experiment procedures for isolation of NP-degrading bacteria.

Table 4. Analysis conditions of NP using HPLC

HPLC Analysis System	
Product	Flexar HPLC System
Manufacturer	PerkinElmer (USA)
HPLC Condition	
Column	CAPCELL PAK C18 Paticle size : 5 μm Size : 4.6 mmø × 250 mm SHISEIDO (Japan)
Detection	UV 277 nm
Flow rate	1.0 ml per min
Mobile phase	(A) Water (B) Acetonitrile
Isocratic	(A) 25% H_2O (B) 75% CH_3CN
Running time	15 min

6. Kinetic analysis of NP degradation

Degradation rate constant (k_1) and half-life time of NP degradation ($t_{1/2}$) by NP-degrading bacteria were calculated by the following formula;

$$k_1 = -\{(\ln S/S_0)/t\}$$

$$t_{1/2} = \ln 2/k_1$$

S_0, Initial substrate concentration;

S, Substrate concentration;

t, Time;

k_1, Degradation ratio

A significant difference was tested by ANOVA method (Chang *et al.*, 2007).

Results and Discussion

1. Isolation of NP-degrading bacteria from NP-degrading consortium SW-03

NP-degrading single strains were isolated from NP-degrading microbial consortium SW-03 strain. The consortium strain was cultured in YNB medium containing 100 ppm NP and then 100 ul of culture was spreaded on YNB agar plate containing 100 ppm NP to obtain a single colony utilizing NP as a single carbon source. After cultivation at 25℃ for 7 days, colonies grown on YNB agar plate containing 100 ppm NP were taken for further study. The morphology of each colony was confirmed by Gran staining methods using a light microscope. Finally, ten colonies, which exhibited different cell morphology, were obtained through single colony isolation. The strains were named as SW-03-A, -B, -C, -D, -E, -F1, -F2, -G, -H, and -I, respectively. All strains isolated from NP-degrading microbial consortium SW-03 were Gram (-) bacteria except SW-03-F2 (Fig. 2). The ten colonies were cultured in 1/10 folds LB medium and stocked at -70℃ in 25% glycerol solution for reservation.

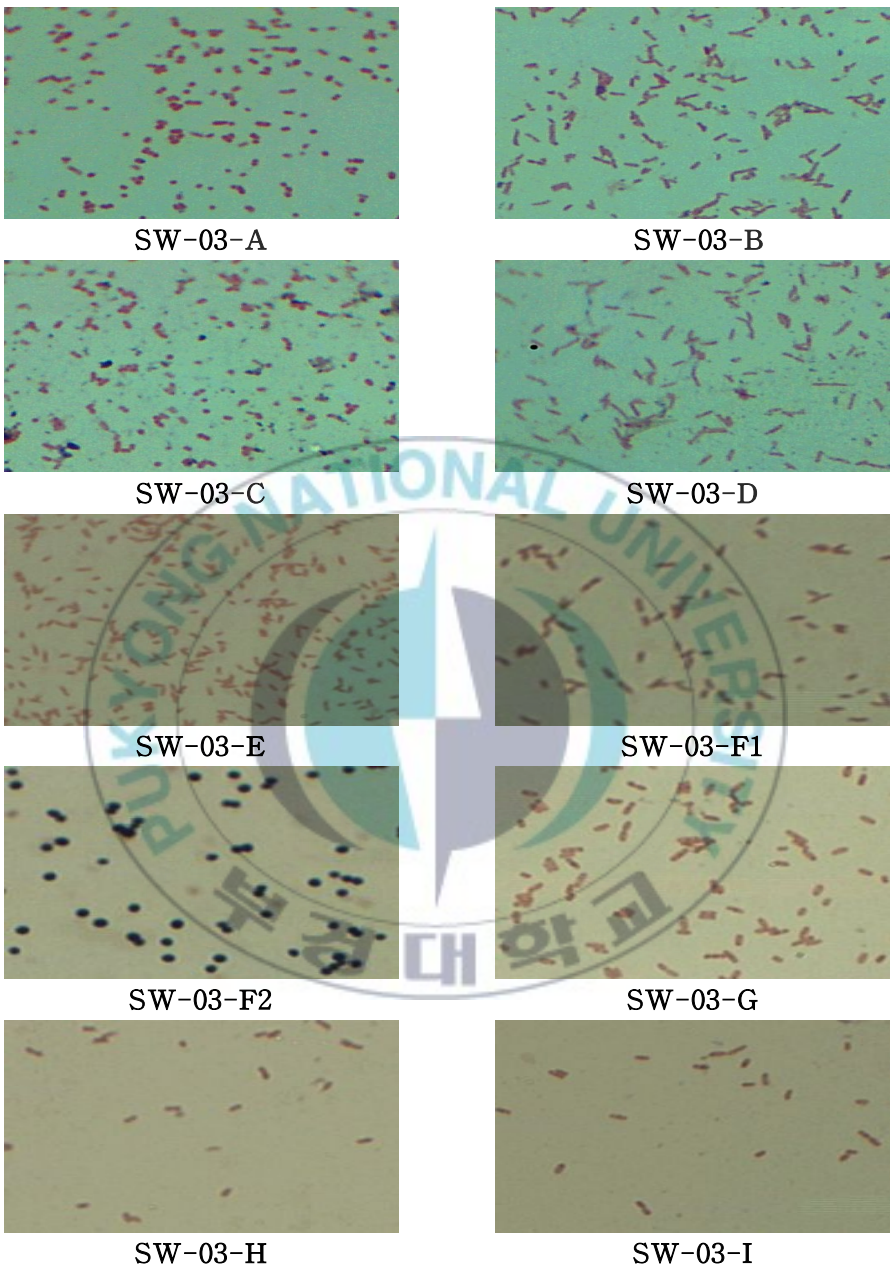


Fig. 2. Light microscopic images of strains isolated from NP-degrading microbial consortium SW-03.

2. NP degradation and bacterial growth by NP-degrading bacteria

In order to evaluate the efficiency of NP degradation activity by the isolated bacteria from NP-degrading microbial consortium SW-03 strain, the NP remaining ratio was calculated by comparing a HPLC peak area of NP remained in medium with the peak area of control (non-inoculated sample). The ratio was expressed as a percental concentration. Also, the bacterial growth was measured to monitor a change of optical density at 600 nm.

After inoculation of 1% pre-culture, cells were aerobically cultivated at 25℃ and samples were taken with 5 days of interval. NP was extracted and analyzed as described in Materials and Methods.

The growth of isolated bacteria was observed in YNB medium containing 100 ppm NP, suggesting the isolates are capable of degrading NP as a single carbon source (Fig. 3-12). Thus, the isolates will be a NP-degrading bacteria. As progressed the bacterial growth, the NP concentration gradually decreased over 40 days of cultivation. After 40 day of cultivation, no NP was observed in all of isolates. However, there is differences of NP-degrading patterns between the isolated bacteria.

In case of SW-03-A and -C strains, NP was degraded over 85% within 10 days of incubation and completely degraded around about 40 days (Fig. 3 and 5). By the growth of SW-03-B, over 85% of NP

was degrade within 15 days (Fig. 4). SW-03-D strain showed the degradation activity of over 70% NP within 10 days (Fig. 6). In SW-03-E strain, NP was degraded over 80% within 15 days (Fig. 7). SW-03-F1 strain was capable of degrading over 70% NP within 5 days and of degrading completely NP about at 35 days (Fig. 8). SW-03-F2 strain also degraded over 90% NP within 15 days and completely degraded NP about at 30 days (Fig. 9). SW-03-G strain exhibited the degradation activity of over 85% NP within 15 days and and then took 35 days incubation for the complete NP degradation (Fig. 10). In SW-03-H strain, NP was degraded over 75% within 10 days and NP was not completely degraded over the incubation periods (Fig. 11). By the growth of SW-03-I strain, over 90% NP was degraded within 15 days and NP was completely degraded about 35 days (Fig. 12).

It has previously been reported that *Sphingomonas xenophaga* Bayr strain was capable of degrading over 90% NP after 2 weeks of incubation (Gabriel *et al.*, 2005) and that *Sphingomonas* sp. TTNP3 strain degraded over 80% NP within 2 weeks (Corvini *et al.*, 2004). Also, Fujii *et al.* (2000) reported that a microbial consortium isolated from aqueous environment exhibited a NP-degrading activity to metabolize about 70% NP after 45 days incubation. Considering these results, some of isolated strains considered to harbor superior NP-degrading activity compared to the other NP-degrading bacteria.

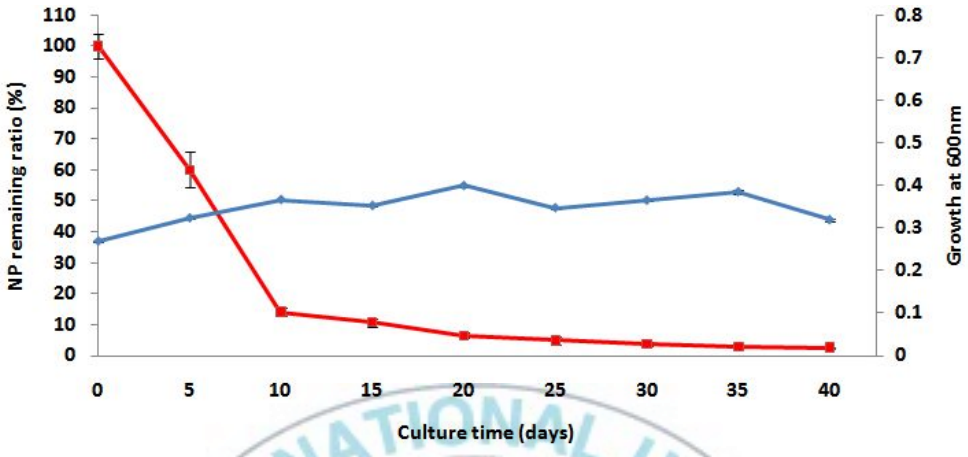


Fig. 3. Nonylphenol degradation by the growth of SW-03-A strain.

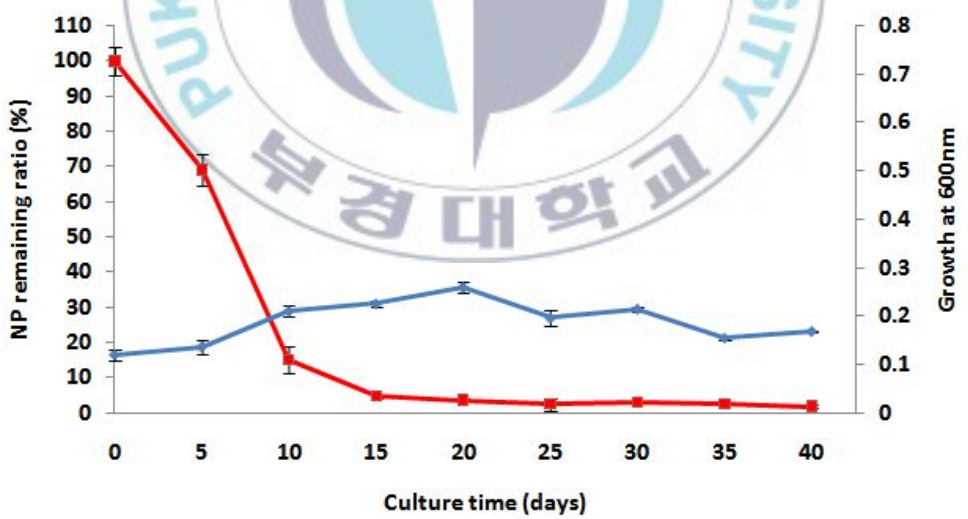


Fig. 4. Nonylphenol degradation by the growth of SW-03-B strain.

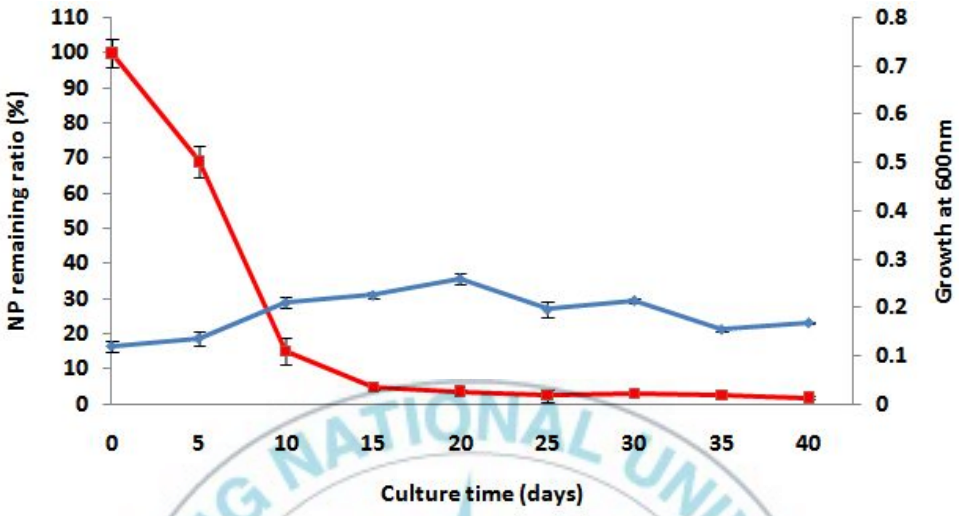


Fig. 5. Nonylphenol degradation by the growth of SW-03-C strain.

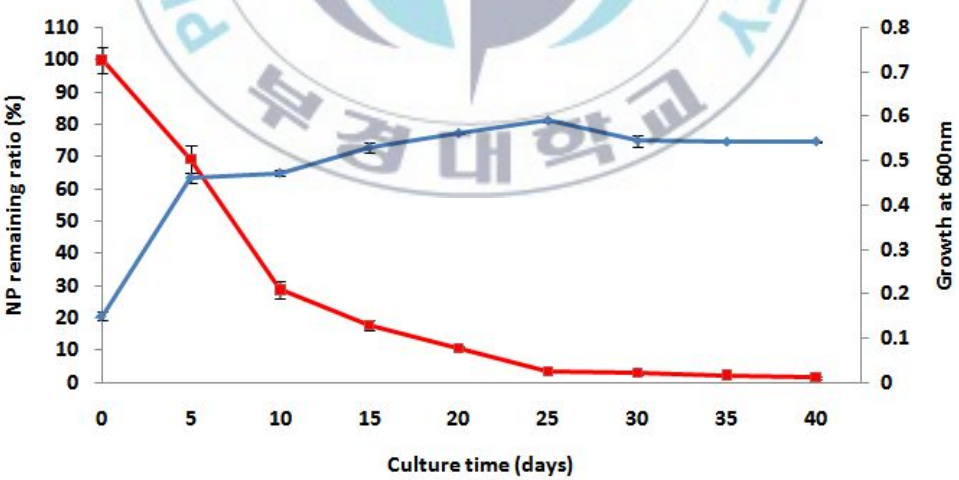


Fig. 6. Nonylphenol degradation by the growth of SW-03-D strain.

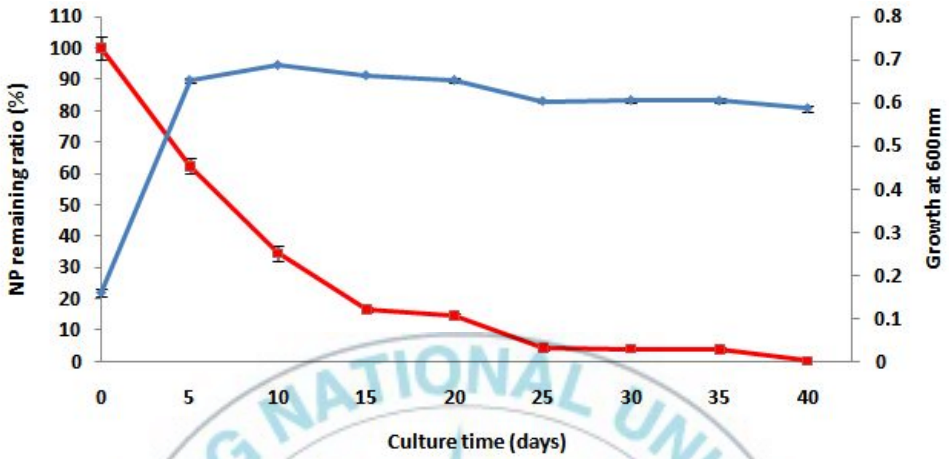


Fig. 7. Nonylphenol degradation by the growth of SW-03-E strain.

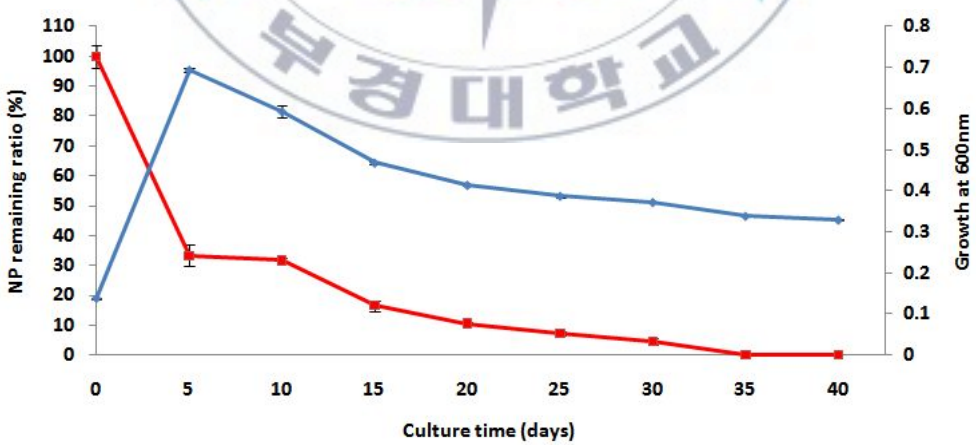


Fig. 8. Nonylphenol degradation by the growth of SW-03-F1 strain.

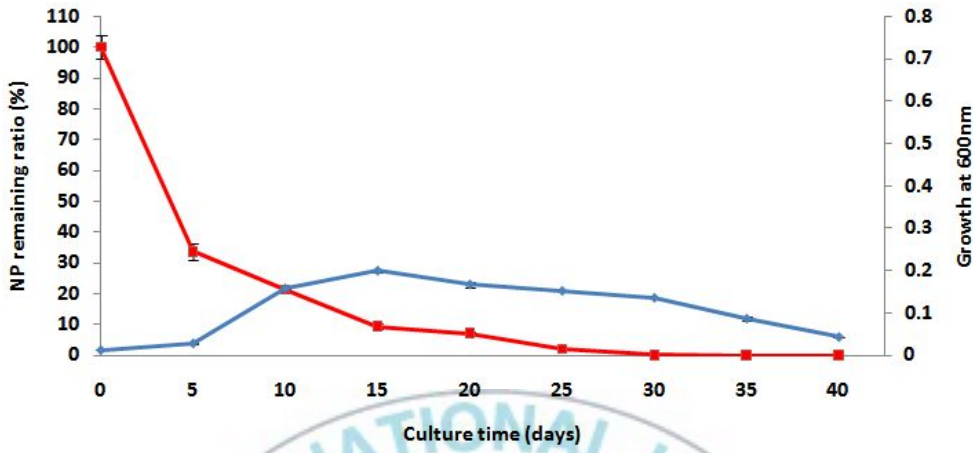


Fig. 9. Nonylphenol degradation by the growth of SW-03-F2 strain.

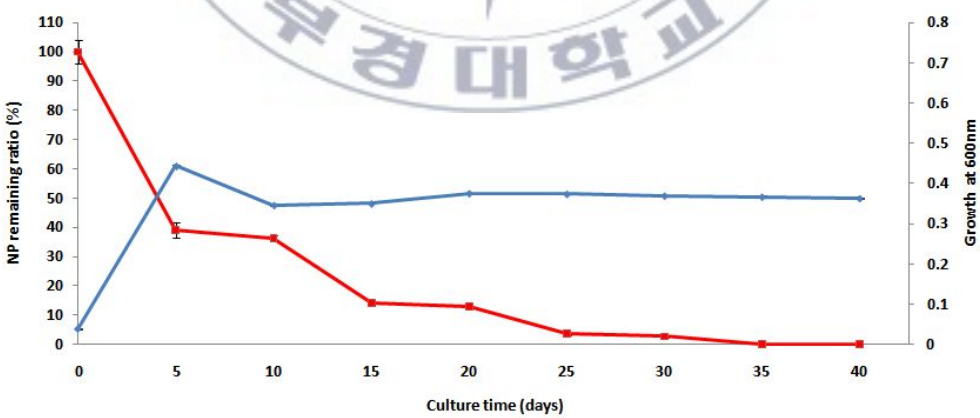


Fig. 10. Nonylphenol degradation by the growth of SW-03-G strain.

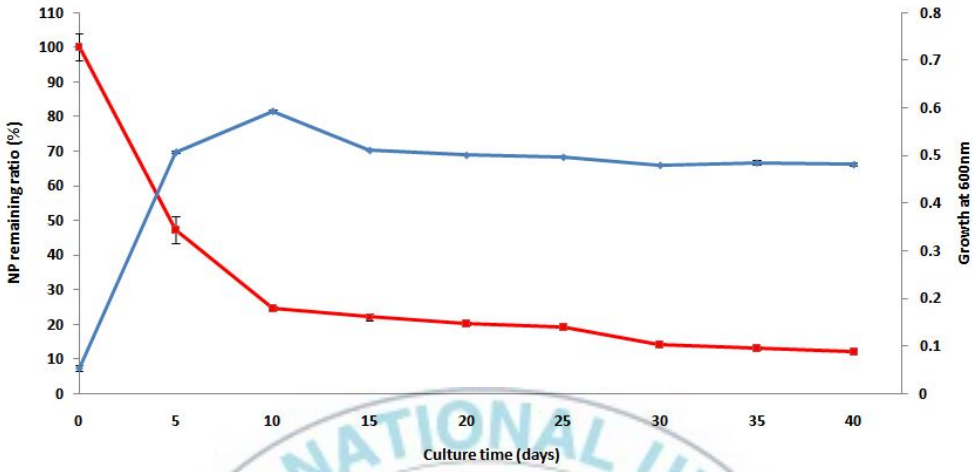


Fig. 11. Nonylphenol degradation by the growth of SW-03-H strain.

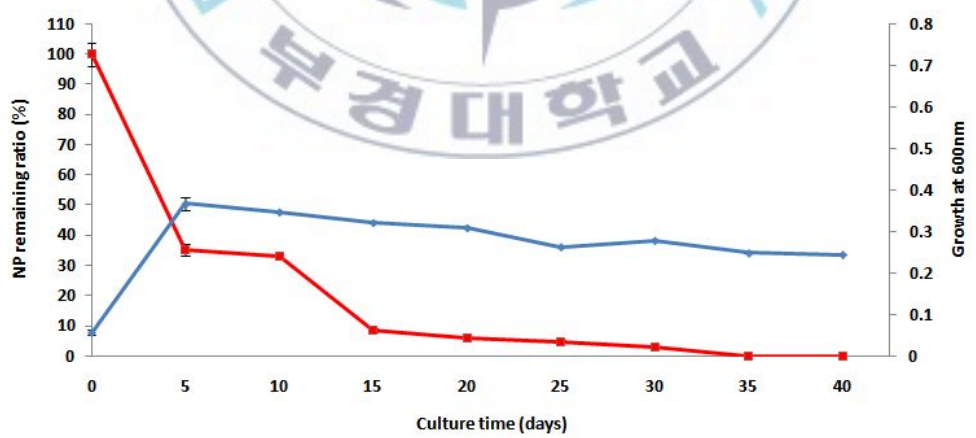


Fig. 12. Nonylphenol degradation by the growth of SW-03-I strain.

3. Kinetic analysis of NP degradation by bacteria isolated from NP-degrading microbial consortium SW-03

As shown in above results, the NP-degrading patterns were different according to the isolates. In order to evaluate the NP-degrading activity between the isolates, a kinetic analysis was performed as described in Materials and Methods.

As summarized in Table 5, most strains isolated from the NP-degrading consortium exhibited the reduced NP degradation rate constant (k_1) and half-life time of NP degradation ($t_{1/2}$). Thus, the NP-degrading activity of the isolates was superior than that of NP-degrading microbial consortium SW-03. Among them, SW-03-F1, -F2, -G, and -I strains were showed higher NP-degrading activity. compared to other isolates and the consortium strain. The k_1 values of SW-03-F1, -F2, -G, and -I strains ranging from 0.340 to 0.456 were about 6 folds higher than that of the consortium strain. The half-life time of NP degradation by the four strains was dramatically decreased in the range of 1.5 to 1.7 days. The $t_{1/2}$ times were about 1/6 folds lower than that of the consortium strain. (Chang *et al.* 2007) also peformed a kinetic analysis of NP degradation in soil. They reported the k_1 value of 0.054 and $t_{1/2}$ value of 12.8 under the condition of pH 7.0 at 20℃. These results suggested that the isolates will be useful to develop a starter strain for biodegradation of NP.

Table 5. Kinetic analysis of nonylphenol degradation by bacteria isolated from nonylphenol-degrading consortium SW-03

Strain	k_1 (1/day)	$t_{1/2}$ (day)	R (coefficient of correlation)
Control	0.011	61.8	0.99
SW-03	0.074	9.3	0.99
SW-03-A	0.092	7.5	0.99
SW-03-B	0.102	6.8	0.98
SW-03-C	0.100	6.9	0.99
SW-03-D	0.103	6.7	0.99
SW-03-E	0.159	4.4	0.99
SW-03-F1	0.340	1.7	0.99
SW-03-F2	0.456	1.5	0.99
SW-03-G	0.399	1.7	0.99
SW-03-H	0.054	12.8	0.99
SW-03-I	0.399	1.7	0.99

4. Morphological analysis of isolates from NP-degrading microbial consortium SW-03 using SEM

Considering the kinetic analysis of NP degradation, four strains (SW-03-F1, -F2, -G, and -I) exhibiting superior NP-degrading activity were selected for further study. In order to investigate cell surface structure of the isolates, morphological feature of SW-03-F1, -F2, -G, and -I was identified by a SEM analysis. As shown in Fig. 13, SW-03-F1, -G and I were a bacillus and SW-03-F3 was a cocci. Considering the results obtained from SEM profiles and Gram staining, SW-03-F1, -G and I were identified to be Gram (-) bacilli and SW-03-F2 was to be a Gram (+) cocci.

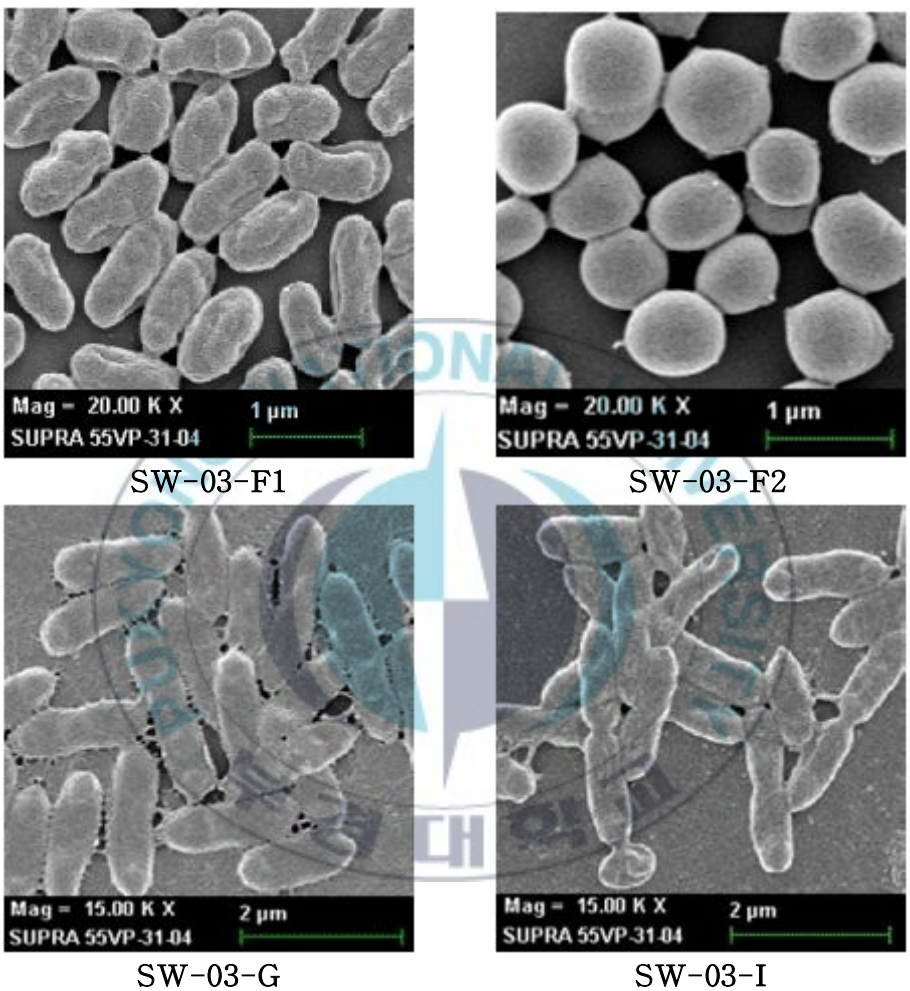


Fig. 13. Scanning electron microscope (SEM) result of SW-03-F1, -F2, -G, -I.

5. Biochemical characteristics of bacteria isolated from NP-degrading microbial consortium SW-03

In order to verify the biological characteristics of bacteria isolated from NP-degrading microbial consortium SW-03, it was used a VITEK Gram Negative Identification card (GNI-) or VITEK Gram Positive Identification card (GNI+) (Biomerieux Inc, Durham, NC) depend on the results of the Gram staining. The results of biochemical reaction were listed in Table 6 to 9.

The SW-03-F1 and SW-03-G strains exhibited the same biological characteristics among strains tested in this study, suggesting that two strains will be allied species. Others showed different features in biological characteristics.

Table 6. Biochemical characteristics of isolated strain SW-03-F1

Mnemonic	Biochemicaltest(Substratename)	Result
APPA	α-phe-proarylamidase	-
ADO	Adonitol	+
PyrA	L-pyrrolydonyl-arylamidase	+
IARL	L-Arabitol	+
dCEL	D-Cellobiose	+
BGAL	β-galactopyranosidase	-
H_2S	H_2Sproduction	-
BNAG	β-N-Acetylglucosaminidase	-
AGLTp	Glutamyl-arylamidasepNA	-
dGLU	D-glucose	+
GGT	ɣ-glutamyltransferase	-
OFF	Fermentativeglucose	-
BGLU	β-glucosidase	-
dMAL	D-maltose	+
dMAN	D-mannitol	+
dMNE	D-mannose	+
BXYL	β-xylosidase	-
BAlap	β-alaninearylamidase	-
ProA	L-prolinearylamidasepNA	+
LIP	Lipase	-
PLE	Palatinose	+
TyrA	Tyrosinearylamidase	-
URE	Urease	-
dSOR	D-sorbitol	+
SAC	Sucrose	+
dTAG	D-Tagatose	+
dTRE	D-Trehalose	+
CIT	Citrate(sodium)	-
MNT	Malonate	-
5KG	5-Keto-D-gluconate	-
ILATk	L-Lactatealkalinisation	-
AGLU	α-glucosidase	-
SUCT	Succinatealkalinisation	-
NAGA	β-N-acetyl-galactosaminidase	-
AGAL	α-Galactosidase	-
PHOS	Phosphatase	-
GlyA	Glycine-arylamidase	+
ODC	omithinedecarboxylase	-
LDC	lysinedecarboxylase	-
IHISa	L-Histidineassimilation	-
CMT	Courmarate	-
BGUR	β-glucuronidase	-
O129R	O/129Resistance	-
GGAA	Glu-gly-arg-arylamidase	-
IMLTa	L-malateassimilation	-
ELLM	Ellman	+
ILATa	L-lactateassimilation	-

Table 7. Biochemical characteristics of isolated strain SW-03-F2

Mnemonic	Biochemicaltest(Substratename)	Result
APPA	α-phe-proarylamidase	-
ADO	Adonitol	-
PyrA	L-pyrrolydonyl-arylamidase	(-)
IARL	L-Arabitol	-
dCEL	D-Cellobiose	-
BGAL	β-galactopyranosidase	+
H_2S	H_2Sprodustion	-
BNAG	β-N-Acetylglucosaminidase	-
AGLTp	Glutamyl-arylamidasepNA	-
dGLU	D-glucose	+
GGT	ɣ-glutamyltransferase	-
OFF	Fermentativeglucose	-
BGLU	β-glucosidase	-
dMAL	D-maltose	+
dMAN	D-mannitol	+
dMNE	D-mannose	-
BXYL	β-xylosidase	-
BAlap	β-alaninearylamidase	-
ProA	L-prolinearylamidasepNA	-
LIP	Lipase	-
PLE	Palatinose	-
TyrA	Tyrosinearylamidase	-
URE	Urease	+
dSOR	D-sorbitol	-
SAC	Sucrose	+
dTAG	D-Tagatose	-
dTRE	D-Trehalose	+
CIT	Citrate(sodium)	-
MNT	Malonate	-
5KG	5-Keto-D-gluconate	-
ILATk	L-Lactatealkalinisation	+
AGLU	α-glucosidase	-
SUCT	Succinatealkalinisation	-
NAGA	β-N-acetyl-galactosaminidase	-
AGAL	α-Galactosidase	-
PHOS	Phosphatase	-
GlyA	Glycine-arylamidase	-
ODC	omithinedecarboxylase	-
LDC	lysinedecarboxylase	-
IHISa	L-Histidineassimilation	-
CMT	Courmarate	+
BGUR	β-glucuronidase	-
O129R	O/129Resistance	-
GGAA	Glu-gly-arg-arylamidase	-
IMLTa	L-malateassimilation	-
ELLM	Ellman	-
ILATa	L-lactateassimilation	-

Table 8. Biochemical characteristics of isolated strain SW-03-G

Mnemonic	Biochemicaltest(Substratename)	Result
APPA	α-phe-proarylamidase	-
ADO	Adonitol	+
PyrA	L-pyrrolydonyl-arylamidase	-
IARL	L-Arabitol	+
dCEL	D-Cellobiose	+
BGAL	β-galactopyranosidase	-
H_2S	H_2Sprodustion	-
BNAG	β-N-Acetylglucosaminidase	-
AGLTp	Glutamyl-arylamidasepNA	-
dGLU	D-glucose	+
GGT	ɣ-glutamyltransferase	-
OFF	Fermentativeglucose	-
BGLU	β-glucosidase	-
dMAL	D-maltose	+
dMAN	D-mannitol	+
dMNE	D-mannose	-
BXYL	β-xylosidase	-
BAlap	β-alaninearylamidase	-
ProA	L-prolinearylamidasepNA	-
LIP	Lipase	-
PLE	Palatinose	+
TyrA	Tyrosinearylamidase	-
URE	Urease	-
dSOR	D-sorbitol	+
SAC	Sucrose	+
dTAG	D-Tagatose	+
dTRE	D-Trehalose	+
CIT	Citrate(sodium)	-
MNT	Malonate	-
5KG	5-Keto-D-gluconate	-
ILATk	L-Lactatealkalinisation	-
AGLU	α-glucosidase	-
SUCT	Succinatealkalinisation	-
NAGA	β-N-acetyl-galactosaminidase	-
AGAL	α-Galactosidase	-
PHOS	Phosphatase	-
GlyA	Glycine-arylamidase	-
ODC	omithinedecarboxylase	-
LDC	lysinedecarboxylase	-
IHISa	L-Histidineassimilation	-
CMT	Courmarate	-
BGUR	β-glucuronidase	-
O129R	O/129Resistance	-
GGAA	Glu-gly-arg-arylamidase	-
IMLTa	L-malateassimilation	-
ELLM	Ellman	-
ILATa	L-lactateassimilation	-

Table 9. Biochemical characteristics of isolated strain SW-03-I

Mnemonic	Biochemicaltest(Substratename)	Result
APPA	α-phe-proarylamidase	-
ADO	Adonitol	+
PyrA	L-pyrrolydonyl-arylamidase	-
IARL	L-Arabitol	+
dCEL	D-Cellobiose	+
BGAL	β-galactopyranosidase	-
H_2S	H_2Sprodustion	-
BNAG	β-N-Acetylglucosaminidase	-
AGLTp	Glutamyl-arylamidasepNA	-
dGLU	D-glucose	+
GGT	ɣ-glutamyltransferase	-
OFF	Fermentativeglucose	-
BGLU	β-glucosidase	-
dMAL	D-maltose	+
dMAN	D-mannitol	+
dMNE	D-mannose	-
BXYL	β-xylosidase	-
BAlap	β-alaninearylamidase	-
ProA	L-prolinearylamidasepNA	-
LIP	Lipase	+
PLE	Palatinose	+
TyrA	Tyrosinearylamidase	-
URE	Urease	-
dSOR	D-sorbitol	+
SAC	Sucrose	+
dTAG	D-Tagatose	+
dTRE	D-Trehalose	+
CIT	Citrate(sodium)	-
MNT	Malonate	-
5KG	5-Keto-D-gluconate	-
ILATk	L-Lactatealkalinisation	-
AGLU	α-glucosidase	-
SUCT	Succinatealkalinisation	-
NAGA	β-N-acetyl-galactosaminidase	-
AGAL	α-Galactosidase	-
PHOS	Phosphatase	-
GlyA	Glycine-arylamidase	-
ODC	omithinedecarboxylase	-
LDC	lysinedecarboxylase	-
IHISa	L-Histidineassimilation	-
CMT	Courmarate	-
BGUR	β-glucuronidase	-
O129R	O/129Resistance	-
GGAA	Glu-gly-arg-arylamidase	-
IMLTa	L-malateassimilation	-
ELLM	Ellman	-
ILATa	L-lactateassimilation	-

6. Identification of isolates from NP-degrading microbial consortium SW-03 using genetical analysis

The analysis of biological characteristics suggested that the two strains, SW-03-F1 and SW-03-G, will be allied species. However, the analysis of biological characteristics only provides a limit information in the identification of bacteria. Therefore, in order to perform more detail investigation, a genetical analysis using bacterial 16S rDNA was conducted. The 16S rDNA was amplified and sequenced by a commercial company (Solgent Tech., Daejeon, Korea).

PCR products of 16S rDNA about 1.3 to 1.5 kb were obtained. The 16S rDNA sequences were analyzed with other 16S rDNA sequences in GeneBank databases through the BLAST search. The homology research indicated that the isolated strain SW-03-F1, -F2, -G, and I exhibited 99% identity with other *Ochrobactrum* sp., *Staphylococcus* sp., *Achromobacter* sp., and *Achromobacter* sp., respectively (Table 10 to 13). Among them, *Ochrobactrum* sp. has been known as a constantly dominant bacteria in NP-degrading microbial community of soil (Chang *et al*., 2007). The community analysis of NP-degrading bacterial consortium obtained from a textile wastewater pretreatment plant revealed that the presence of those typical of *Achromobacter* sp. (Di Gioia *et al*., 2008). The genus *Staphylococcus* has never been reported to be able to degrade NP. However, the genera *Sphingomonas,* which already reported for their biodegradation activity toward 4-NP, were not isolated in the current study (Thanghe *et al.,* 1999; Fujii *et al*., 2001; Corvini *et al*., 2004; Gabriel *et al*., 2005, b).

Table 10. 16S rDNA sequences (1314bp) of strain SW-3-F1 and homology search based on 16SrDNA sequences

```
   1 gaacggcagc acggacttcg gtctggtggc gagtggcgaa cgggtgagta atgtatcgga
  61 acgtgcccag tagcggggga taactacgcg aaagcgtagc taataccgca tacgccctac
 121 gggggaaagc aggggatcgc aagaccttgc actattggag cggccgatat cggattagct
 181 agttggtggg gtaacggctc accaaggcga cgatccgtag ctggtttgag aggacgacca
 241 gccacactgg gactgagaca cggcccagac tcctacggga ggcagcagtg gggaattttg
 301 gacaatgggg gaaaccctga tccagccatc ccgcgtgtgc gatgaaggcc ttcgggttgt
 361 aaagcacttt tggcaggaaa gaaacgtcat gggttaatac cccgtgaaac tgacggtacc
 421 tgcagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta gggtgcaagc
 481 gttaatcgga attactgggc gtaaagcgtg cgcaggcggt tcggaaagaa agatgtgaaa
 541 tcccagagct taactttgga actgcatttt taactaccgg gctagagtgt gtcagaggga
 601 ggtggaattc cgcgtgtagc agtgaaatgc gtagatatgc ggaggaacac cgatggcgaa
 661 ggcagcctcc tgggataaca ctgacgctca tgcacgaaag cgtggggagc aaacaggatt
 721 agataccctg gtagtccacg ccctaaacga tgtcaactag ctgttggggc cttcgggcct
 781 tggtagcgca gctaacgcgt gaagttgacc gcctggggag tacggtcgca agattaaaac
 841 tcaaaggaat tgacggggac ccgcacaagc ggtggatgat gtggattaat tcgatgcaac
 901 gcgaaaaacc ttacctaccc ttgacatgtc tggaatgccg aagagatttg gcagtgctcg
 961 caagagaacc ggaacacagg tgctgcatgg ctgtcgtcag ctcgtgtcgt gagatgttgg
1021 gttaagtccc gcaacgagcg caacccttgt cattagttgc tacgaaaggg cactctaatg
1081 agactgccgg tgacaaaccg gaggaaggtg gggatgacgt caagtcctca tggcccttat
1141 gggtagggct tcacacgtca tacaatggtc gggacagagg gtcgccaacc cgcgaggggg
1201 agccaatccc agaaacccga tcgtagtccg gatcgcagtc tgcaactcga ctgcgtgaag
1261 tcggaatcgc tagtaatcgc ggatcagcat gtcgcggtga atacgttccc gg
```

Reference (accession no.)	Identity (%)
Ochrobactrum anthropi CCUG 1821(AM114404)	99.0
Ochrobactrum sp. CA01 (HQ670703)	99.0
Ochrobactrum sp. lsd01 (GQ180164)	99.0
Ochrobactrum tritici SCII24 (AM114402)	99.0

Table 11. 16S rDNA sequences (1515bp) of strain SW-3-F2 and homology search based on 16SrDNA sequences

```
   1 gctcaggatg aacgctggcg gcgtgcctaa tacatgcaag tcgagcgaac agataaggag
  61 cttgctcctt tgacgttagc ggcggacggg tgagtaacac gtgggtaacc tacctataag
 121 actgggataa cttcgggaaa ccggagctaa taccggataa catttggaac cgcatggttc
 181 taaagtgaaa gatggttttg ctatcactta tagatggacc cgcgccgtat tagctagttg
 241 gtaaggtaac ggcttaccaa ggcgacgata cgtagccgac ctgagagggt gatcggccac
 301 actggaactg agacacggtc cagactccta cgggaggcag cagtagggaa tcttccgcaa
 361 tgggcgaaag cctgacggag caacgccgcg tgagtgatga agggtttcgg ctcgtaaaac
 421 tctgttatta gggaagaaca aacgtgtaag taactgtgca cgtcttgacg gtacctaatc
 481 agaaagccac ggctaactac gtgccagcag ccgcggtaat acgtaggtgg caagcgttat
 541 ccggaattat tgggcgtaaa gcgcgcgtag gcggtttctt aagtctgatg tgaaagccca
 601 cggctcaacc gtggagggtc attggaaact gggaaacttg agtgcagaag aggaaagtgg
 661 aattccatgt gtagcggtga aatgcgcaga gatatggagg aacaccagtg gcgaaggcga
 721 ctttctggtc tgtaactgac gctgatgtgc gaaagcgtgg ggatcaaaca ggattagata
 781 ccctggtagt ccacgccgta aacgatgagt gctaagtgtt agggggtttc cgcccccttag
 841 tgctgcagct aacgcattaa gcactccgcc tggggagtac gaccgcaagg ttgaaactca
 901 aaggaattga cggggacccg cacaagcggt ggagcatgtg gtttaattcg aagcaacgcg
 961 aagaacctta ccaaatcttg acatcctttg aaaactctag agatagagcc ttccccttcg
1021 ggggacaaag tgacaggtgg tgcatggttg tcgtcagctc gtgtcgtgag atgttgggtt
1081 aagtcccgca acgagcgcaa cccttaagct tagttgccat cattaagttg ggcactctag
1141 gttgactgcc ggtgacaaac cggaggaagg tggggatgac gtcaaatcat catgcccctt
1201 atgatttggg ctacacacgt gctacaatgg acaatacaaa gggcagctaa accgcgaggt
1261 catgcaaatc ccataaagtt gttctcagtt cggattgtag tctgcaactc gactacatga
1321 agctggaatc gctagtaatc gtagatcagc atgctacggt gaatacgttc ccgggtcttg
1381 tacacaccgc ccgtcacacc acgagagttt gtaacacccg aagccggtgg agtaaccatt
1441 tatggagcta gccgtcgaag gtgggacaaa tgattggggt gaagtcgtaa caaggtagcc
1501 gtatcggaag gtgc
```

Reference (accession no.)	Identity (%)
Staphylococcus sp. BQN4T-04 (FJ380997)	99.0
Staphylococcus sp. 2qH-9 (EU489561)	99.0
Staphylococcus saprophyticus subsp. saprophyticus BQN1T-01d (FJ380970)	99.0
Staphylococcus saprophyticus subsp. saprophyticus BAC2101 (HM355690)	99.0

Table 12. 16S rDNA sequences (1382bp) of strain SW-3-G and homology search based on 16SrDNA sequences

1	aagtcgaacg gcagcacgga cttcggtctg gtggcgagtg gcgaacgggt gagtaatgta
61	tcggaacgtg cccagtagcg ggggataact acgcgaaagc gtagctaata ccgcatacgc
121	cctacggggg aaagcagggg atcgcaagac cttgcactat tggagcggcc gatatcggat
181	tagctagttg gtggggtaac ggctcaccaa ggcgacgatc cgtagctggt ttgagaggac
241	gaccagccac actgggactg agacacggcc cagactccta cgggaggcag cagtggggaa
301	ttttggacaa tgggggaaac cctgatccag ccatcccgcg tgtgcgatga aggccttcgg
361	gttgtaaagc acttttggca ggaaagaaac gtcgygggtt aataccccgc ggaactgacg
421	gtacctgcag aataagcacc ggctaactac gtgccagcag ccgcggtaat acgtagggtg
481	caagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttcgga aagaaagatg
541	tgaaatccca gagcttaact ttggaactgc atttttaact accgggctag agtgtgtcag
601	agggaggtgg aattccgcgt gtagcagtga aatgcgtaga tatgcggagg aacaccgatg
661	gcgaaggcag cctcctggga taacactgac gctcatgcac gaaagcgtgg ggagcaaaca
721	ggattagata ccctggtagt ccacgcccta aacgatgtca actagctgtt ggggccttcg
781	ggccttggta gcgcagctaa cgcgtgaagt tgaccgcctg gggagtacgg tcgcaagatt
841	aaaactcaaa ggaattgacg gggacccgca caagcggtgg atgatgtgga ttaattcgat
901	gcaacgcgaa aaaccttacc tacccttgac atgtctggaa tcctgaagag atttaggagt
961	gctcgcaaga gaaccggaac acaggtgctg catggctgtc gtcagctcgt gtcgtgagat
1021	gttgggttaa gtcccgcaac gagcgcaacc cttgtcatta gttgctacga aagggcactc
1081	taatgagact gccggtgaca aaccggagga aggtggggat gacgtcaagt cctcatggcc
1141	cttatgggta gggcttcaca cgtcatacaa tggtcgggac agagggtcgc caacccgcga
1201	gggggagcca atcccagaaa cccgatcgta gtccggatcg cagtctgcaa ctcgactgcg
1261	tgaagtcgga atcgctagta atcgcggatc agcatgtcgc ggtgaatacg ttcccgggtc
1321	ttgtacacac cgcccgtcac accatgggag tgggttttac cagaagtagt tagcctaacc
1381	g

Reference (accession no.)	Identity (%)
Achromobacter sp. DG (HQ437668)	99.0
Achromobacter sp. P3 (FJ556879)	99.0
Achromobacter insolitus CCM7182 (FM999733)	99.0
Alcaligenaceae bacterium a001-61 (HM468067)	99.0

Table 13. 16S rDNA sequences (1382bp) of strain SW-3-I and homology search based on 16SrDNA sequences

```
   1 agagttgatc ctggctcaga ttgaacgcta gcgggatgcc ttacacatgc aagtcgaacg
  61 gcagcacgga cttcggtctg gtggcgagtg gcgaacgggt gagtaatgta tcggaacgtg
 121 cccagtagcg ggggataact acgcgaaagc gtagctaata ccgcatacgc cctacggggg
 181 aaagcagggg atcgcaagac cttgcactat tggagcggcc gatatcggat tagctagttg
 241 gtggggtaac ggctcaccaa ggcgacgatc cgtagctggt ttgagaggac gaccagccac
 301 actgggactg agacacggcc cagactccta cgggaggcag cagtggggaa ttttggacaa
 361 tgggggaaac cctgatccag ccatcccgcg tgtgcgatga aggccttcgg gttgtaaagc
 421 acttttggca ggaaagaaac gtcgtgggtt aataccccgc gaaactgacg gtacctgcag
 481 aataagcacc ggctaactac gtgccagcag ccgcggtaat acgtagggtg caagcgttaa
 541 tcggaattac tgggcgtaaa gcgtgcgcag gcggttcgga aagaaagatg tgaaatccca
 601 gagcttaact ttggaactgc atttttaact accgagctag agtgtgtcag agggaggtgg
 661 aattccgcgt gtagcagtga aatgcgtaga tatgcggagg aacaccgatg gcgaaggcag
 721 cctcctggga taacactgac gctcatgcac gaaagcgtgg ggagcaaaca ggattagata
 781 ccctggtagt ccacgcccta aacgatgtca actagctgtt ggggccttcg ggccttggta
 841 gcgcagctaa cgcgtgaagt tgaccgcctg gggagtacgg tcgcaagatt aaaactcaaa
 901 ggaattgacg gggacccgca caagcggtgg atgatgtgga ttaattcgat gcaacgcgaa
 961 aaaccttacc tacccttgac atgtctggaa tcctgaagag atttaggagt gctcgcaaga
1021 gaaccggaac acaggtgctg catggctgtc gtcagctcgt gtcgtgagat gttgggttaa
1081 gtcccgcaac gagcgcaacc cttgtcatta gttgctacga aagggcactc taatgagact
1141 gccggtgaca aaccggagga aggtggggat gacgtcaagt cctcatggcc cttatgggta
1201 gggcttcaca cgtcatacaa tggtcgggac agagggtcgc caacccgcga gggggagcca
1261 atcccagaaa cccgatcgta gtccggatcg cagtctgcaa ctcgactgcg tgaagtcgga
1321 atcgctagta atcgcggatc agcatgtcgc ggtgaatacg ttcccgggtc ttgtacacac
1381 cgcccgtcac accatgggag tgggttttac cagaagtagt tagcctaacc gtaaggggcg
1441 cgattaccac ggtaggattc atgactgggg tgaagtcgta acaaggtagc cgtatcggaa
1501 ggtgcggctg gatcacctcc tt
```

Reference (accession no.)	Identity (%)
Alcaligenes sp. MH112 (FJ626643)	99.0
Achromobacter sp. P3 (FJ556879)	99.0
Alcaligenes sp. cxh-4(EF059708)	99.0
Achromobacter insolitus CCM7182 (FM999733)	99.0

7. NP degradation by the co-culture using NP-degrading bacteria

The kinetic analysis of NP degrading by the bacterial strains isolated from SW-03 revealed that four strains (SW-03-F1, -F2, -G, and -I) were capable of degrading efficiently NP. However, SW-03-F2, which was tentatively identified to belong the genus *Staphylococcus*, exhibited high homology with *Staphylococcus saprophyticus* that is often implicated in urinary tract infections (Kuroda *et al*., 2005). Therefore, SW-03-F1, -G, and -I strains were chosen to investigate the co-culture effect on NP degradation and each strain was mixed with the following number of cases; SW-03-F1/-G, SW-03-F1/-I, SW-03-G/-I, and SW-03-F1/-G/-I.

The NP remaining ratio was calculated as mentioned above and the bacterial growth was also monitored during the periods of co-culture. After the inoculation of each of 1% pre-culture, cells were aerobically cultivated at 25℃ and samples were taken with 5 days of interval as done above. NP remained in the medium was extracted and analyzed as described in Materials and Methods. As the bacterial growth progressed, the concentration of NP gradually decreased over 40 days of cultivation. In case of SW-03-G/-I, SW-03-F1/-G, and SW-03-F1/-G/-I, NP was degraded over 85% within 15 days of incubation (Fig. 14, 16 and 17). By the co-culture of SW-03-F1/-I, over 85% NP was degrade within 20 days (Fig. 15). However, NP

was still detected in all of co-culture after 40 days of incubation, suggesting the co-culture of isolates resulted in disturbing the NP degradation compared to the NP degradation by single isolate.

As shown in above results, the NP-degrading patterns by the co-culture of isolates were different according to the types of mixed strains. In order to evaluate more detail the difference of NP-degrading activity between the co-cultures of isolates, a kinetic analysis was conducted as described in Materials and Methods. The NP degradation rate constant (k_1) values by co-cultures of isolates (SW-03-G/-I, SW-03-F1/-G, SW-03-F1/-I and SW-03-F1/-G/-I) were in ranging from 0.081 to 0.092 that were about 1.3 folds higher than that of the consortium SW-O3 strain. The half-life time of NP degradation ($t_{1/2}$) was steadily decreased in the range of 7.5 to 8.6 days that were about 4/5 folds lower than that of the consortium strain. However, the NP-degrading activities by the co-cultures of the isolates were inferior than those of most isolates (Table 14). These results suggested that the three strains (SW-03-F1, -G and -I) will be antagonistic each other in NP degradation under the conditions performed in this study.

It has been previously reported that NP was not detected in the three-membered (BCaL1, BCaL2 and VA 160 strains) co-culture during the experiment around about 25 days (Diana Di *et al.*, 2004). Indeed, the BCaL1/BCaL2 co-culture potential was further enhanced by co-culturing them with the non-degrading *Bacillus* VA 160 strain.

Considering these results, it was concluded that the NP-degrading bacteria isolated in the current study is not suitable strains to degrade NP by the co-culture methods.

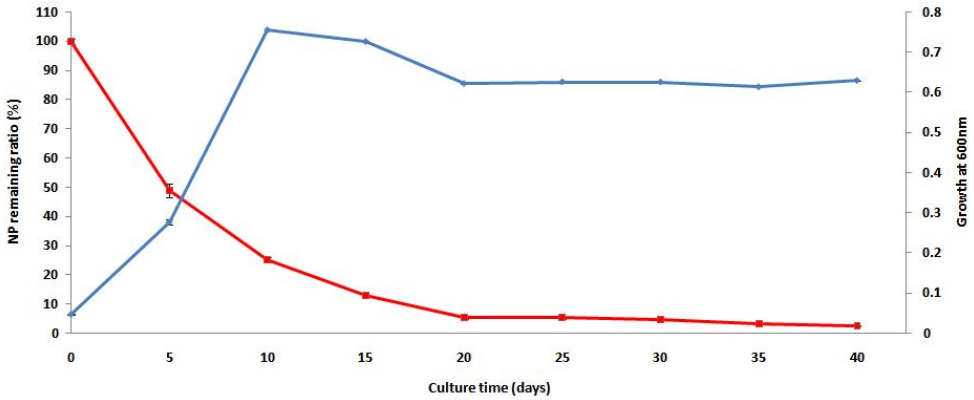


Fig. 14. Nonylphenol degradation and growth by the co-culture of SW-03-G mixed with -I strain.

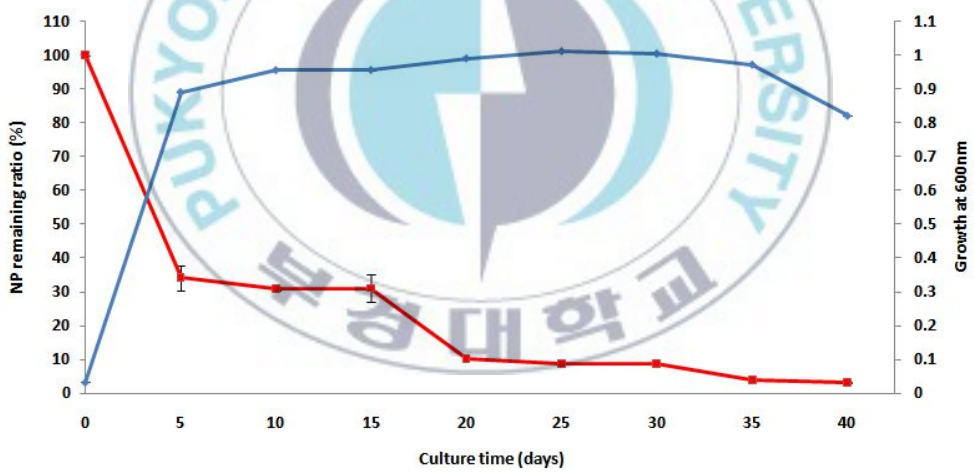


Fig. 15. Nonylphenol degradation and growth by the co-culture of SW-03-F1 mixed with -I strain.

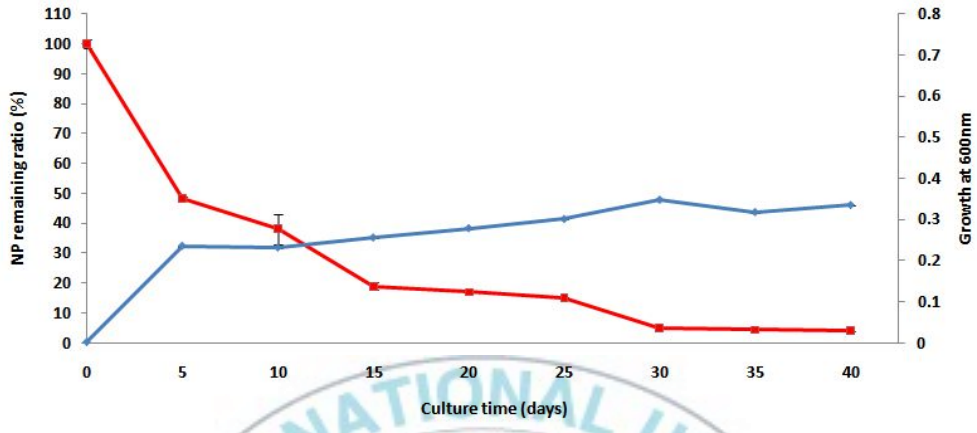


Fig. 16. Nonylphenol degradation and growth by the co-culture of SW-03-F1 mixed with -G strain.

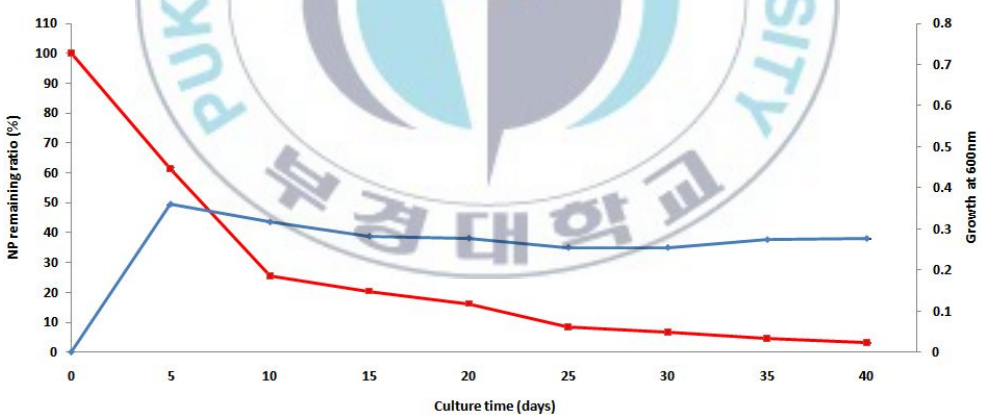


Fig. 17. Nonylphenol degradation and growth by the co-culture of SW-03-F1 mixed with -G and -I strain.

Table 14. Kinetic analysis of nonylphenol degrading by the co-cultures

Strain	k_1 (1/day)	$t_{1/2}$ (day)	R (coefficient of correlation)
Control	0.011	61.8	0.99
SW-03	0.074	9.3	0.99
SW-03-G/-I	0.081	8.6	0.99
SW-03-F1/-I	0.092	7.5	0.99
SW-03-F1/-G	0.086	8.0	0.99
SW-03-F1/-G/-I	0.086	8.0	0.99

Summary

This study was conducted to isolate a NP-degrading microorganism in aquatic environment and to perform the kinetic analysis of NP degradation by bacterial strains isolated in the current study.

NP-degrading single strains were isolated from NP-degrading microbial consortium SW-03 strain. Ten colonies, which exhibited different cell morphology, were obtained through single colony isolation. The strains were named as SW-03-A, -B, -C, -D, -E, -F1, -F2, -G, -H, and -I, respectively. All strains were Gram (-) bacteria except SW-03-F2.

SW-03-F1, -G, and -I strains were chosen to investigate the co-culture effect on NP degradation. As the bacterial growth progressed, the concentration of NP gradually decreased over 40 days of cultivation. In case of SW-03-G/-I, SW-03-G/-F1, and SW-03-G/-F1/-I, NP was degraded over 85% within 15 days of incubation. By the co-culture of SW-03-F1/-I, over 85% NP was degrade within 20 days. However, NP was still detected in all of co-culture after 40 days of incubation, suggesting the co-culture of isolates resulted in disturbing the NP degradation compared to the NP degradation by single isolate.

The NP degradation rate constant (k_1) values by co-cultures of isolates (SW-03-G/-I, SW-03-F1/-G, SW-03-F1/-I and SW-03-F1/-G/-I) were in ranging from 0.081 to 0.092 that were about 1.3 folds higher than that of the consortium SW-O3 strain. The

half-life time of NP degradation ($t_{1/2}$) was steadily decreased in the range of 7.5 to 8.6 days that were about 4/5 folds lower than that of the consortium strain. However, the NP-degrading activities by the co-cultures of the isolates were inferior than those of most isolates. These results suggested that the three strains (SW-03-F1, -G and -I) will be antagonistic each other in NP degradation under the conditions performed in this study.

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References

Ahel M., Giger W. & Koch M. 1994. Behaviour of alkylphenol polyethoxylate surfactants in the aquatic environment-I. Occurrence and transformation in sewage treatment. *Water Research*, 28, 1131-1142.

Ahn M.Y. & Kim J.E. 1998. Transformation of nitroaromatics and their reduced metabolites by oxidative coupling reaction. *Journal of the Korean Society for Applied Biological Chemistry*, 17, 239-245.

Azevedo D.A., Lacorte S., Viana P. & Barceló D. 2001. Occurrence of nonylphenol and bisphenol-A in surface waters from Portugal. *Journal of the Brazilian Chemical Society*, 2001, 12, 532-537.

Bennie D.T., Sullivan C.A., Lee H.B., Peart T.E., & Maguire R.J. 1997. Occurrence of alkylphenols and alkylphenol mono- and diethoxylates in natural waters of the Laurentian great lakes basin and the upper St. Lawrence river. *Science of The Total Environment*, 193, 263-275.

Berns K.I. & Thomas C.A. 1965. Isolation of the high molecular DNA from *Haemophilus influenzae*. *Journal of Molecular Biology*, 11, 476-490.

Bromwich P., Cohen J., Stewart I. & Walker A. 1994. Decline in sperm counts: an artifact of changed reference range of "normal"?. *British Medical Journal*, 309, 19-22.

Calafat A.M., Kuklenyik Z., Reidy J.A., Caudill S.P., Ekong J. & Needham L.L. 2004. Urinary concentrations of bisphenol A and 4-nonylphenol in a human reference population. *Environmental. Health Perspectives*, 113, 391-395.

Cho H.S., Kim Y.O., Seol S.W. & Horiguchi T. 2004. A Study on the pollution of nonylphenol in surface sediment in Gwangyang bay

and Yeosu sound. *The Korean Environmental Sciences Society*, 13, 567-570.

Colborn T., Saal F.S. & Soto A.M. 1993. Developmental effects of endocrine disrupting chemicals in wildlife and humans. *Environmental Health Perspectives*, 101, 378-384.

Corvini P.F., Meesters R.J., Schäffer A., Schröder H.F., Vinken R., Hollender J. 2004. Degradation of a nonylphenol single isomer by *Sphingomonas* sp. strain TTNP3 leads to a hydroxylation-induced migration product. *Applied and Environmental Microbiology*, 70, 6897 - 6900.

Daidoji T., Ozawa M., Sakamoto H., Sako T., Inoue H., Kurihara R., Hashimoto S. & Yokota H. 2006. Slow elimination of nonylphenol from rat intestine. *Drug Metabolism and Disposition,* 34, 184-190.

Di Gioia D, Salvadori L, Zanaroli G, Coppini E, Fava F, and Barberio C. 2008. Characterization of 4-nonylphenol-degradingbacterial consortium obtained from a textile wastewater pretreatment plant. Arch Microbiol. 190, 673-683.

Dunbar J., Ticknor L.O. & Kuske C.R. 2000. Assessment of microbial diversity in four southwestern united states soils by 16S rRNA gene terminal restriction fragment analysis. *Applied and Environmental Microbiology*, 66, 2943-2950.

Fairchild W.L., Swansburg E.O., Arenault J.T. & Brown S.B. 1999. Does an association between pesticide use and subsequent declines in catch of Atlantic salmon (*Salmo salar*) represent a case of endocrine disruption. *Environmental Health Perspectives*, 107, 349-357.

Ferrara F., Fabietti F., Delise M., Bocca A.P. & Funari E. 2001. Alkylphenolic compounds in edible molluscs of the Adriatic sea (Italy). *Environmental Science and Technology*, 35, 3109 - 3112.

Fries E. & Puttmam W. 2003. Occurrence and behaviour of 4-nonylphenol in river water of Germany. *Journal of Environmental Monitoring*, 5, 598-603.

Fujii K., Urano N., Kimura S., Nomura Y. & Karube I. 2000. Microbial degradation of nonylphenol in some aquatic enviroments. *Fisheries Science*, 66, 44-48.

Fujii K., Urano N., Ushio H., Satomi M. & Kimura S. 2001. *Sphingomonas cloacae* sp. nov., a nonylphenol-degrading bacterium isolated from wastewater of a sewage-treatment plant in Tokyo. *International journal of Systematic and Evolutionary Microbiology*, 51, 603-610.

Gabriel FLP, Giger W, Guenther K, Kohler H-PE (2005a) DiVerential degradation of nonylphenol isomers by *Sphingomonas xenophaga* Bayram. Appl Environ Microbiol 71:1123 - -1129

Gabriel FLP, Heidelberg A, Rentsch D, Giger W, Guenther K, Kohler H-PE (2005b) A novel metabolic pathway for degradation of 4-nonylphenol environmental contaminants by *Sphingomonas xenophaga* Bayram. J Biol Chem 280:15526 - -15533

Giger W., Brunner P.H. & Schaffner C. 1984. 4-nonylphenol in sewage sludge: Accumulation of toxic metabolites from nonionic surfactants. *Science, New Series*, 225, 623 - 625.

Gronen S., Denslow N., Manning S., Barnes S., Barnes D. & Brouwer M. 1999. Serum vitellogenin levels and reproductive impairment of male Japanese medaka (*Oryzias latipes*) exposed to 4-tertoctylphenol. *Environmental Health Perspectives*, 107, 385 - 390.

Hernadez-Raquet G., Soef A., Delgenes N. & Balaguer P. 2007.

Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. *Water Research*, 41, 2643-2651.

Hesselsoe M., Jensen D., Skals K., Olesen T., Moldrup P., Roslev P., Mortensen G.K. & Henriksen K. 2001. Degradation of 4-nonylphenol in homogeneous and nonhomogeneous mixtures of soil and sewage sludge. *Environmental Science and Technology*, 35, 3695-3700.

Isobe T., Nishiyama H., Nakashima A. & Takada H. 2001. Distribution and behavior of nonylphenol, octylphenol, and nonylphenol monoethoxylate in Tokyo metropolitan area: their association with aquatic particles and sedimentary distribution. *Environmental Science and Technology*, 35, 1041 - 1049.

Won S., Lim K.S., Yu D.U., Park M.E., Jeong E.T., Kim D.M., Chung Y.H., Kim Y.M. 2011. Isolation of a Nonylphenol-degrading Microbial Consortium. The Korean Society of fisheries and Aquatic Science, 44-4.

Jobling S. & Sumpter J.P. 1993. Detergent components in sewage effluent are weakly estrogenic to fish: an in vitro study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquatic Toxicology*, 27, 361 - 372.

John D.M. & White G.F. 1998. Mechanism for biotransformation of nonylphenol polyethoxylates to xenoestrogens in *Pseudomonas putida*. *Journal of Bacteriology*, 180, 4332-4338.

Junghanns C., Moeder M., Krauss G., Martin C. & Schlosser D. 2005. Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. *Microbiology*, 151, 45-57.

Karels A.A., Manning S. & Brouwer T.H. 2003. Reproductive effects of estrogenic and antiestrogenic chemicals on sheepshead minnows (*Cyprinodon variegatus*). *Environmental Toxicology and Chemistry*,

22, 855-865.

Khim J.S., Kannan K., Villeneuve D.L., Koh C.H. & Giesy J.P. 1999, Characterization and distribution of trace organic contaminants in sediment from Masan bay, Korea: 1. Instrumental analysis. *Environmental Science and Technology*, 33, 4199-4205.

Kiceniuk J.W. & Ray S. 1994. Analysis of contaminants in edible aquatic resources: General considerations, metals, organometallics, tainting, and organics (food science and tech). *VCH Publishers*, 429-452.

Kim D.M. & Shiraishi H. 2005, Ecological modeling for toxic substances-I. Numerical simulation of transport and fate of nonylphenol in Tokyo bay. *The Korean Environmental Sciences Society*, 14, 827-833.

Korach K.S. 1993. Surprising places of estrogenic activity. *Endocrinology*, 132, 2277-2278.

Kuroda M, Yamashita A, Hirakawa H, Kumano M, Morikawa K, Higashide M, Maruyama A, Inose Y, Matoba K, Toh H, Kuhara S, Hattori M, and Ohta T. 2005. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. Proc. Natl. Acad. Sci. U.S.A. 102, 13272 - 13277.

Kwon T.D. & Kim J.E. 1998. Oxidative coupling of herbicide propanil and its metabolite, DCA(3,4-dichloroaniline) to humic monomers. *Journal of the Korean Society for Applied Biological Chemistry*, 41, 384-389.

Lee W.S. & Kim J.E. 1998. Effects of soil organic matter and oxidoreductase on adsorption and desorption of herbicide oxadiazon in soils. *The Korean Socirty of Pesticide Science*, 2, 70-78.

Lee Y.K., Eom S.H., Hwang H.J., Lim K.S., Yang J.Y., Chung Y.H.,

Kim D.-M., Lee M.S., Rhee I.K., Kim Y.M. 2009. Cloning and Mutational Analysis of Catechol 2,3-dioxygenase from 3,4-Dichloroaniline Degrading Bacterium Pseudomonas sp. KB35B. Journal of the Korean Society for Applied Biological Chemistry 52(3) 258-263 ISSN 1225-9675 KCI

Li D., Dong M., Shim W.J., Hong S.H., Oh J.R., Yim U.H., Jeung J.H., Kannan N., Kim E.S. & Cho S.R. 2005. Seasonal and spartial distribution of nonylphenol and IBP in Saemangeum bay, Korea. *Marine Pollution Bulletin*, 51, 966-974.

Li D., Dong M., Shim W.J., Yim U.H., Hong S.H. & Kannan N. 2008. Distribution characteristics of nonylphenolic chemicals in Masan bay environments, Korea. *Chemosphere*, 71, 1162-1172.

Li D.H., Kim M.S., Shim W.J., Yim U.H., Hong S.H. & Oh J.R. 2004. Distribution of nonylphenol in Gwangyang bay and the surrounding streams. *Korea Socirty of Environmental Biology*, 22, 71-77.

Li D.H., Kim M.S., Shim W.J., Yim U.H., Oh J.R. & Kwon Y.J. 2004. Seasonal flux of nonylphenol in Han river, Korea. *Chemosphere*, 56, 1-6.

Li D.H., Kim M.S., Oh J.R. & Park J.M. 2004. Distribution characters of nonylphenol in the artificial lake Shihwa, Korea and surrounding creeks in Korea. *Chemosphere*, 56, 783-790.

Li Z., Li D., Oh J.R. & Je J.G. 2004. Seasonal and spatial distribution of nonylphenol in Shihwa lake, Korea. *Chemosphere*, 56, 611-618.

Nimrod A.C. & Benson W.H. 1996. Environmental estrogenic effects of alkylphenol ethoxylates. *Critical Reviews in Toxicology*, 26, 335-364.

Park J.W., Dec J., Kim J.E. & Bollag J.M. 1999. Effect of humic constituents on the transformation of chlorinated phenols and anilines in the presence of oxidoreductive enzymes or birnessite.

Environmental Science and Technology, 33, 2028-2034.

Park J.W., Kim J.E. & Lee Y.K. 1999. Transformation of pentachlorophenol by oxidoreductive catalysts. *Journal of the Korean Society for Applied Biological Chemistry*, 42, 330-335.

Renner R. 1997. European bans on surfactant trigger transatlantic debate. *Environmental Science and Technology*, 31, 316 - 320.

Ripp S., Nivens D.E., Ahn Y., Werner C., Jarrell I.V., Easter J.P., Cox C.D., Burlage R.S. & Sayler G.S. 2000. Controlled field release of a bioluminescent genetically engineered microorganism for bioremediation process monitoring and control. *Environmental Science and Technology*, 34, 846-853.

Sabik H., Gagne F., Blaise C., Marcogliese D.J. & Jeannot R. 2003. Occurrence of alkylphenol polyethoxylates in the St. Lawrence river and their bioconcentration by mussels (*Elliptio complanata*). *Chemosphere*, 51, 349-356.

Safe S.H. 2000. Endocrine disruptors and human health-Is there a problem? An update. *Environmental Health Perspectives*, 108, 487-493.

Shao B., Hu J. & Yang M. 2003. Nonylphenol ethoxylates and their biodegradation intermediates in water and sludge of a sewage treatment plant. *Bulletin of Environmental Contamination and Toxicology*, 70, 527 - 532.

Shao B., Hu J., Yang M., An W. & Tao S. 2007. Nonylphenol and nonylphenol ethoxylates in river water, drinking water, and fish tissues in the area of Chongqing, China. *Archives of Environmental Contamination and Toxicology*, 48, 467-473.

Sharpe R.M. 2001. Hormones and testis development and the possible adverse effects of environmental chemicals. *Toxicology Letters*, 120, 221-232.

Shi S. & Bending G.D. 2007. Changes to the structure of *Sphingomonas* spp. communities associated with biodegradation of the herbicide isoproturon in soil. *FEMS Microbiology Letters*, 269, 110-116.

Soares A., Guieysse B., Delgado O. & Mattiasson B. 2003. Aerobic biodegradation of nonylphenol by cold-adapted bacteria. *Biotechnology Letters*, 25, 731-738.

Soares A., Guieysse B., Jefferson B., Cartmell E. & Lester J.N. 2008. Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environment International*, 34, 1033-1049.

Tanghe T., Dhooge W. & Verstraete W. 1999. Isolation of a bacterial strain able to degrade branched nonylphenol. *Applied and Environmental Microbiology*, 65, 746-751.

Toomey B.H., Monteverdi G.H. & Di Giulio R.T. 1999. Octylphenol induces vitellogenin production and cell death in fish hepatocytes. *Environmental Toxicology and Chemistry*, 18, 734-739.

U.S. EPA. 1998. Research plan for endocrine disruptors.

Welshons W.V., Thayer K.A., Judy B.M., Taylor J.A., Curran E.M. & vom Saal F.S. 2003. Large effects from small exposures-I. Mechanisms for endocrine-disrupting chemicals with estrogenic activity. *Environmental Health Perspectives*, 111, 994-1006.

Yadetie F. & Male R. 2002. Effects of 4-nonylphenol on gene expression of pituitary hormones in juvenile Atlantic salmon (*Salmo salar*). *Aquatic Toxicology*, 58, 113-129.

Yoshimura K. 1986. Biodegradation and fish toxicity of nonionic surfactants. *Journal of the American Oil Chemists' Society*, 63, 1590-1596.

국립환경연구원. 1998. 내분비계장애물질이란?.

국립환경연구원. 1999. 내분비계장애물질의 이해와 대응.

식품의약품안전청. 2007. 식품 중 알킬페놀류란?.

해양수산부. 2002. 내분비계 장애물질의 해양생태계 영향과 거동 연구.

환경부. 2007. 환경백서, 627.

환경부 국립환경과학원. 2007. 제9차 내분비계장애물질 조사·연구사업 결과보고.

환경부 국립환경과학원. 2008. 제10차 내분비계장애물질 조사·연구사업 결과보고.

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http://www.clu-in.org (Clean-Up Information)

http://www.env.go.jp (Ministry of the Environment Government of Japan)