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APPENDICES

APPENDIX A

Maximum Allowable Concentration of BaP

The maximum allowable concentration of BaP in soil for difference purposes are shown in TableA-1 and the classified the PAHs-polluting area is shown in Table A-2

Table A-1 Maximum allowable concentration of BaP in soil for difference purposes

The legislation of	Area	Maximum allowable (ng/g)
Catalonia, Spain	Non industrial area	80
	Industrial area	7500
Canada	Agricultural area	100
	Residential area	700
	Commercial area	700
	Industrial area	700

Source: Nadal et al., 2004

The classification criteria of polluted or unpolluted area was counted base on the total amount of three to six ring parent polycyclic aromatic hydrocarbon (m/z 178, phenanthrene/ anthracene), (m/z 202, pyrene/ fluoranthrene), (m/z228 benzo(a)anthracene/ chrysene), (m/z 252, benzo(a) fluoranthrene/ benzo(a)pyrene/ benzo(e) pyrene), (m/z 276, indeno(1,2,3-cd)pyrene/benzo(ghi)perylene).

Table A-2 The classified the PAHs-polluting area

Total concentration per gram dry weight	Indicated as
< 100 ng/g	Low pollution
>1000 ng/n	Chronically polluted industrialized areas and harbors

Source: Baumard et al., 1998

APPENDIX B

The advantage of bioremediation over the other mediation technologies according to the relatively low cost when compared to the physical and chemical processes (Table B-1).

Table B-1 The estimated cost (US dollars) for PAHs treatment in soil

Technologies	**Cost/cubic yard**
Incineration	250-800
Land filling	150-250
Physical-chemical	* 80-120
Bioremediation	40-100

* Facilitate machine not include

Source: Levin&Gealt, 1993

APPENDIX C

The mineral salts medium (MSM)

A mineral salt medium (MSM), liquid medium, used for the isolation and degradation experiments was consisted of the following components per liter.

Table C-1 Composition of MSM used for this study

Element	g/l
KCl	0.25
$Na_2HPO_4.7H_2O$	15.70
$NaH_2PO_4.2H_2O$	6.50
NH_4NO_3	1.0
$MgSO_4.7H_2O$	0.5
$FeSO_4 .7H_2O$	0.1
$MnCl_2.4H_2O$	0.01
$ZnSO_4 7H_2O$	0.1
$CuSO4 .5H_2O$	0.05
$CoCl_2. 6H_2O$	0.01
$Na_2MoO_4. 2H_2O$	0.03

The final pH of medium = 5.5, and 5 mM glucose was used as carbon source supplemented for fungal growth. The MS medium was autoclaved at 121 ^{o}C for 15 min before use.

Malt Extract Solid Agar Media (MEA)

Malt Extract Media (MEA) is consisted of

Malt extracts	20	g/l
Peptone	4	g/l
Glucose	20	g/l
Agar	20	g/l

MEA was autoclaved at 121 ^{o}C for 15 min before use. The 0.5% of streptomycin was added to eliminate the growth of bacteria.

APPENDIX D

The dilution method

The procedure consisted of dilution of the microbial with a series of sterile water. Dilutions were generally prepared in 9 ml of sterile water where 1 gram of sample was added to create a 1:10 dilution. The further dilutions were needed by adding 1 ml from 1:10 dilution in a tube containing total of 9 ml MS medium to precede 1:100 dilution of the original micro-organism, the dilutions as 1:1000 or more dilution could be used if necessary by the same procedures indicated here (Figure D-1). Generally 0.1 ml of the diluted sample was pipette onto the surface of a solidified agar medium combined with 0.5% antibiotic in a Petri dish. The liquid was spreaded over the medium with a sterilized, bent, glass rod. After 48 hours of incubation, the fungal isolates were observed and processed. To improve the accuracy of the methods, at least, the triplicate plates of each dilution were prepared.

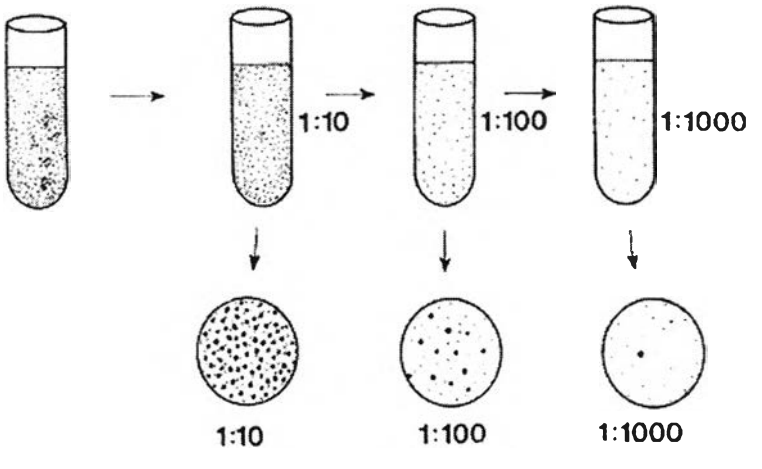

Figure D-1 The scheme of dilution method used for producing pure colony.
Source: http://www.apsnet.org/education/IllustratedGlossary

APPENDIX E

HPLC calibration curve of benzo(a)pyrene

A calibration curve was developed for determining the BaP concentration extracted from liquid cultures. The stock solution of Benzo(a)pyrene standard in acetonitrile was diluted to obtain the desirable concentration (triplicate per each). The calibration standards were analyzed similar to the sample procedures by passing through the Hewlette Packard Hyposil C18 reverse phase column (250mm x 4mm) equipped with UV 254 nm detector with the flow rate of 1.0 ml/min. Calibration curve of BaP was shown in Figure G-1

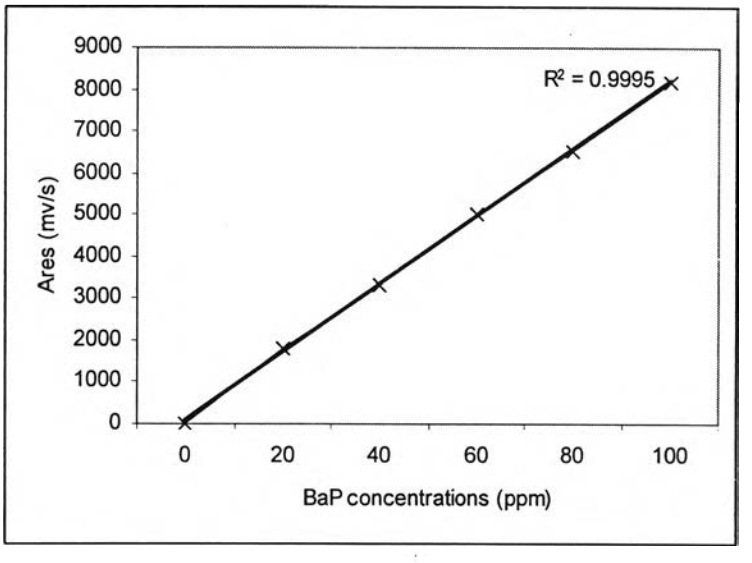

Figure E-1 Calibration curve of BaP

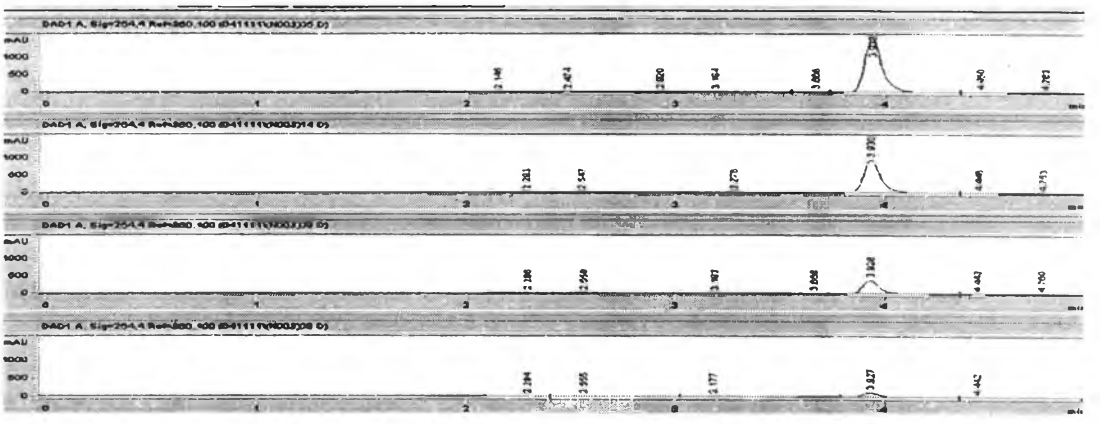

Figure E-2 The HPLC chromatogram of remaining BaP obtained at time 0, 10, 20, and 30 days of incubation (from top to down) from the *Aspergillus niger* N003

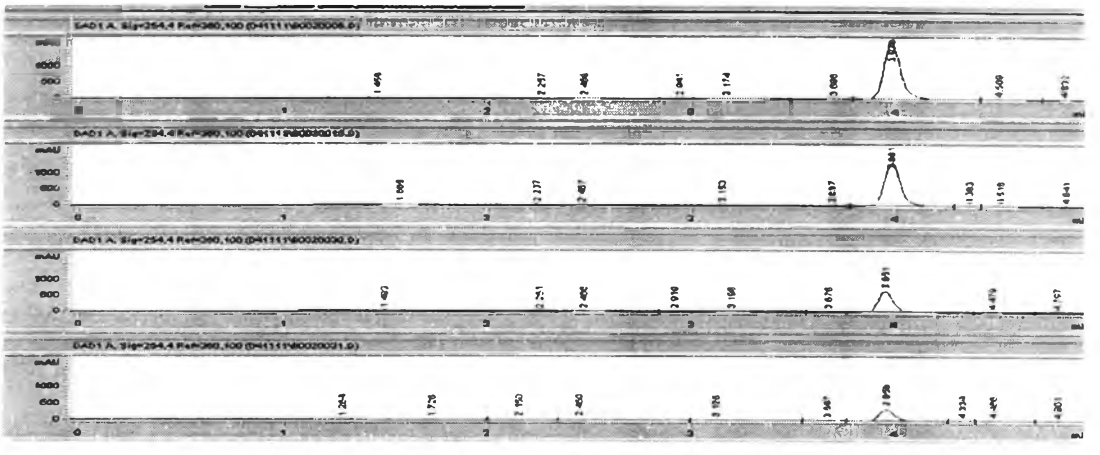

Figure E-3 The HPLC chromatogram of remaining BaP obtained at time 0, 10, 20, and 30 days of incubation (from top to down) from the *Aspergillus niger* B002

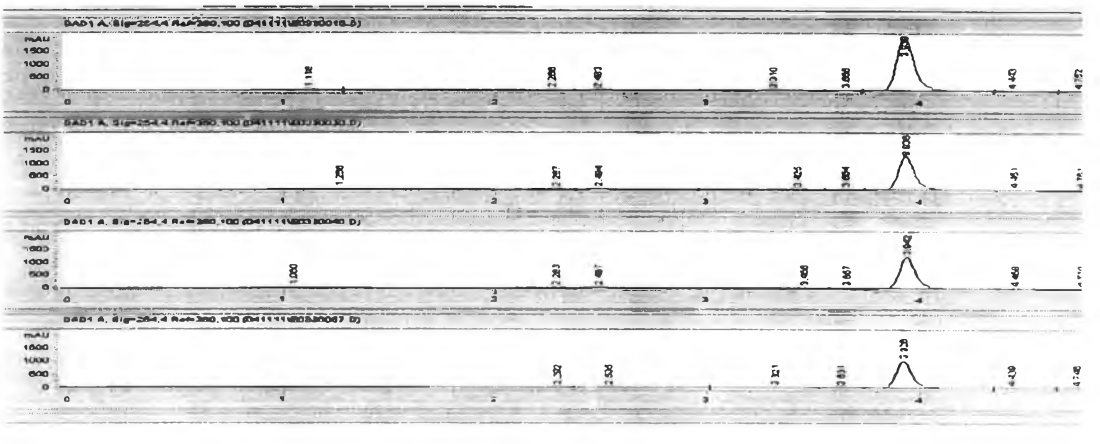

Figure E-4 The HPLC chromatogram of remaining BaP obtained at time 0, 10, 20, and 30 days of incubation (from top to down) from the *Fusarium oxysporum* E033

APPENDIX F

Characterization of fungal isolates

Table F-1 Characteristic of fungal isolates from primary and secondary screening

Isolates	Morphological classification (color, shape, etc.)	Primary screening on solid agar at 14 days (growth rate in cm.)		Degradation efficiency in liquid media with 100 ppm BaP
		control	with 100 ppm BaP	
E001	white, round shape	6.50	Death	NP
E002	fade yellow	8.00	Death	NP
E003	deep green	5.50	Death	NP
E004	green+	4.50	2.00	NP
E005	white	8.50	Death	NP
E006	green++	6.20	Death	NP
E007	greenish blue	1.75	Death	NP
E008	white mycelium	3.00	0.80	NP
E009	brown++	5.75	6.50	20.00%
E010	yellow, white border	1.90	Death	NP
E011	white, thin mycelium	1.50	Death	NP
E012	yellow green	4.30	1.20	NP
E013	brown+	2.50	Death	NP
E014	orange+	8.50	Death	NP
E015	grey, thick mycelium	5.50	6.30	21.80%
E016	grey, round shape	4.50	5.20	18.54%
E017	green, yellow border	4.50	1.55	NP
E018	white	8.50	Death	NP
E019	bright green	3.00	Death	NP
E020	brown +++	4.30	3.00	NP
E021	bright yellow	6.80	4.15	NP
E022	green, white border	5.60	3.20	NP
E023	white mycelium, brown spore	2.50	Death	NP
E024	orange, thick mycelium	4.10	Death	NP
E025	greenish yellow	3.80	0.80	NP
E026	fade orange, undefined	6.10	3.50	NP
E027	white mycelium, grey spore	4.15	6.00	32.25%
E028	orange++	7.20	Death	NP
E029	white, thin mycelium, flower shape	7.30	Death	NP
E030	black spore	2.00	Death	NP
E031	white mycelium, yellow spore	1.90	Death	NP

Isolates	Morphological classification (color, shape, etc.)	Primary screening on solid agar at 14 days (growth rate in cm.)		Degradation efficiency in liquid media with 100 ppm BaP
		control	with 100 ppm BaP	
E032	deep grey	2.40	Death	NP
E033	white, thick mycelium	3.00	6.55	65.40%
E034	white mycelium, flower shape	6.40	3.60	31.15%
E035	yellow mycelium, brown spore	2.00	Death	NP
E036	deep green, flower shape	3.00	1.00	NP
E037	fade orange	4.80	3.50	NP
E038	yellow thin mycelium	8.10	2.20	NP
E039	greenish blue	8.00	Death	NP
E040	pink	4.00	Death	NP
N001	white mycelium, black spore	2.80	2.20+clear zone	12.15%
N002	white mycelium, black spore in middle	2.50	2.10+clear zone	23.32%
N003	white mycelium, black spore in middle	4.50	8.00	79.85%
N004	fade pink	3.55	3.60	21.25%
N005	fade yellow mycelium	5.10	3.20+clear zone	22.45%
N006	black spore flower shape	4.55	Death	NP
N007	white mycelium	5.50	5.00+clear zone	
B001	white mycelium	2.20	Death	NP
B002	white mycelium, black spore	5.50	8.20	69.95%
B003	white, thick mycelium	7.50	2.30	NP
B004	white, thick round shape	3.55	Death	NP
B005	white, black spore in border	6.00	2.50	NP
B006	white thin mycelium	3.10	2.50	NP

NP= Not performed

APPENDIX G

Sequence of base of primers, promoter and sequence alignment of the three promising fungi

Table G-1 The sequences of primers and promoter (as a sequencing primer) used in the process of identification of fungi

Primers or promoters	**Base sequences (5'–>3')**
ITS 1 primer	TCCGTAGGTGAACCTGCGC
ITS 1F primer	CTTGGTCATTTAGAGGAAGTAA
ITS 4 primer	TCCTCCGCTTATTGATATGC
T7 promoter	TAATACGACTCACTATAGGGCGAATTGGGTACCGCCC/GGGCTAGA

For blasting of DNA achieved to gene bank go to

URL-**http://fungalgenomics.concordia.ca/fungi/** **or at** NCBI or at DDBJ

URL-**http://www.ddbj.nig.ac.jp**

Table G-2 The sequences of ITS of the 3 promising fungi

Fungal strains	**Base sequence**
Aspergillus niger strain N003	CTTGGTCATTTAGAGGAAGTAAAAGCGTAACAAGGTTTCCGTAGGTGA ACCTGCGGAAGGATCATTACCGAGTGCGGGCTGCCTCCGGCGCCCAA CCTCCCACCCTTGAATACTAAACACTGTTGCTTCGGCGGGGAGCCCCT TCCGGGGGGCAAGCCGCCGGGGACCACTGAACTTCATGCCTGAGAGT GATGCAGTCTGAGTCTGAATTATAAATCAGTCAAAACTTTCAACAATG GATCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAACTGCGATAA GTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCAC ATTGCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTG CTGCCCTTCAAGCCCGGCTTGTGTGTTGGGTCGTCGTCCCCCCCGGGG GACGGGCCCGAAAGGCAGCGGCGGCACCGTGTCCGGTCCTCGAGCGT ATGGGGCTTTGTCACCCGCTCGATTAGGGCCGGCCGGGCGCCAGCCG GCGTCATCAATCTATTTTACCAGGTTGACCTCGGATCAGGTAGGGATA CCCGCTGAACTTAAGCATATCAATAAGCGGAGGA
Fusarium oxysporum strain E033	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTG AACCAGCGGAGGGATCATTACCGAGTTATACAACTCATCAACCCTGTG AACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCGTAACA CGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTT CTTCTGTGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTC TTGGCTCTGGCATCGAGTAAGAACGCAGCGAAACGCGATAAGTAATG TGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG CCCGCCAGTATTCTGGCGGGCTGCCTGTTCGAGCGTCATTACAACCCT CAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCCCTGCGGG CACAACGCCGTCCCCCAAATACAGTGGCGGTCCCGCCGCAGCTTCCAT TGCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGCGCGGCCACGCC GTAAAACACCCAACTTCTGAATGTTGACCTCGAATCAGGTAGGAATAC CCGCTGAACTTAAGCATATCAATAAGCGGAGGA
Aspergillus niger strain B002	CTTGGTCATTTAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTG AACCTGCGGAAGGATCATTACCGAGTGCGGGTCCTTTGGGCCCAACCT CCCATCCGTGTCTATTATACCCTGTTGCTTCGGCGGGCCCGCCGCTTGT CGGCCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGA GACCCCAACACGAACACTGTCTGAAAGCGTGCAGTCTGAGTTGATTGA ATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGGTTCCGGCATC GATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATT CAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCC GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTG TGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGAAATTCA GCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATG CTCTGTAGGATTGGCCGGCGCCTGCCGACGTTTTCCAACCATTTTTTCC AGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATATC AATAAGCGGAGGA

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