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APPENDICES

APPENDIX A

CULTURE MEDIA AND REAGENT FOR IDENTIFICATION

All media were dispensed and steriled in autoclave for 15 min at 15 pounds pressure (121 °C) except for acid from carbon sources test which was sterilized at 10 pounds for (110 °C) 10 min.

1. The JCM medium no. 377

yeast extract	5	g
Casamíno acid	5	g
Sodium glutamate	1	g
Tri-sodium citrate	3	g
$MgSO_4.7H_2O$	20	g
KCl	2	g
NaCl	150	g
$FeCl_2.4H_2O$	0.362	g
$MnCl_2.4H_2O$	0.0362	g
Agar	20	g
Distilled water	1	L
Adjust pH 7.2 with NaOH		

2. Marine oxidation-fermentation medium (MOF)

Casitone(Difco)	1	g
Yeast extract	0.1	g
Ammonium sulfate	0.5	g
Tris buffer	0.5	g
Phenol red 0.001% (1.0 ml of0.1%aqueous per 100 ml of medium)		
Artificial sea water	1	L
Adjusted pH to 7.5		

3. Basal medium for utilization test

Utilization of various compounds as sole carbon and energy sources was tested in a mineral liquid medium containing (g/1):

NH_4Cl	1.0	g
K_2HPO_4	0.075	g
$CaCl_2$,	1.45	g
NaCl	30.0	g
$MgCl_2$	6.15	g
KCl	0.75	g
$FeSO_4$	0.028	g

Supplemented with0.2% w/v test substrate.

Growth was determined spectrophotometrically after 2 days cultivation.

4. L-arginine agar medium

Peptone	1.0	g
NaCl	100	g
K_2HPO_4	0.3	g
Phenol red,1.0% aq.solution	1.0	ml
L(+)arginine hydrochloride	10.0	g
Agar	3.0	g
Distilled water	1	L

Dissolve the solids in the water, adjust to pH 7.2 , distribute into tubes or screw-capped (6mm) bottles to a depth of about 16 mm (3.5ml).

5. Aesculin broth

Aesculin	1	g
Ferric citrate	0.5	g
NaCl	100	g
Peptone water	1	L

Adjust pH 7.4

Dissolve the aesculin and iron salt in the peptone water and sterilized at 115 °C for 10 min.

6. Gelatin agar

JCM nO.377 agar medium (omitted casamino acid)	100	ml
Gelatin	1%	(w/v)
Dissolve and adjust pH 7.2.		

7. Starch agar

JCM nO.377 agar medium	100	ml
Starch	1%	(w/v)
Dissolve and adjust pH 7.2.		

8. Tyrosine agar

JCM nO.377 agar medium (omitted casamino acid)	100	ml
L-tyrosine	1%	(w/v)
Dissolve and adjust pH 7.2.		

9. Tween 80 agar medium

JCM nO. 377 agar medium	100	ml
Tween 80	2	ml
Dissolve and adjust pH 7.2.		

10. Deoxyribonuclease (DNase) media

DNase test agar (Difco)	42	g
Distilled water	1	L
Adjust pH 7.3 ± 0.2 and heat to boiling to dissolve completely.		

11. Tryptone water

Tryptone	5%	(w/v)
NaCl	10%	(w/v)
Adjust pH 7.2.		

12. Nitrate broth

Beef extract	10	g
Peptone	10	g
NaCl	5	g
Distilled water	1	L

Dissolve and adjusted pH to 7.2.

13. Flagella staining

Basic fuchisin	0.5	g
Tannic acid	0.2	g
Aluminium sulfate	0.5	g

Solvent was composed of a mixture of 2.0 of 95% ethanol, 0.5 ml of glycerol, and 7.5 ml of tris(hydroxymethyl)aminomethane(tris)buffer.

14. Kovacs'reagent

ρ-dimethylaminobenzaldehyde	5	g
Amyl alcohol	75	g
Conc. HCl	25	ml

Dissolve the aldehyde in the alcohol by gently warming in a water bath (about 50-55 °C). Cool and the acid with care. Protect from light and store at 4 °C.

15. Nitrate test reagent

Solution A: 0.33% sulphanilic acid in 5 N- acetic acid Dissolve by gentle heating

Solution B: 0.6% dimethyl-α-napthylaminein 5 N-acetic acid Dissolve by gentle heating

APPENDIX B

REAGENT FOR CHEMOTAXONOMIC CHARACTERISTIC

1. Cellular fatty acid analysis

1.1 Reagent 1 (Saponification reagent)

Sodium hydroxide	15	g
MeOH (HPLC grade)	50	ml
Mili-Q water	50	ml

Dissolve NaOH pellets in Mili-Q water and add MeOH.

1.2 Reagent 2 (Methylation reagent)

6 N HCl	65	ml
MeOH (HPLC grade)	55	ml

pH must be below 1.5.

1.3 Reagent 3 (Extraction solvent)

n-Hexane (HPLC grade or n-Hexane 1000)	50	ml
Methyl-tert-Butyl Ether (HPLC grade)	50	ml

1.4 Reagent 4 (base wash reagent)

Sodium hydroxide	1.2	g
Mili-Q water	100	ml

1.5 Reagent 5 (Saturated sodium chloride)

2. Polar lipids

2.1 Ninhydrin solution

Ninhydrin	0.5	g
1-Butanol saturated in water	100	ml

2.2 Dittmer&Lester reagent

Solution A

MoO_3	4.011	g
25 N H_2SO_4	100	mL

Dissolve 4.011 g of MoO3 in 100 mL of 25N H2SO4 by heating.

Solution B

Molybdenum powder	0.178	g
Solution A	50	mL

Add 0.178 g of molybdenum powder to 50 mL of solution A, and boil it for 15 minutes. After cooling, remove the precipitate by decantation. Before spraying, mix solution A (50 mL) plus solution B (50 mL) plus water (100 mL). Added 0.178 g of molybdenum powder to 50 ml of solution A and boiled it for 15 minutes. Cooled and removed the precipitate by decantation.

2.3 Anisaldehyde reagent

Ethanol	90	ml
H_2SO_4	5	ml
p-Anisaldehyde	5.0	ml
Acetic acid	1.0	ml

APPENDIX C

REAGENT FOR DNA EXTRACTION AND PURIFICATION DNA BASE COMPOSITION; DNA-DNA HYBRIDIZATION AND 16S rRNA SEQUENCING

1. DNA extraction and DNA base composition

1.1 Saline –EDTA(0.15m NaCl + 0.1 M EDTA)

NaCl	8.76	g
EDTA	37.22	g

NaCl and EDTA were dissolved in 1 L ultra pure water and adjusted the pH 8.0 by adding N HCl and then steriled by autoclaving at 121 °C, 15 pounds/inch pressure,for 15 min.

1.2 10% (W/V) SDS

Sodium dodecyl sulphate	10	g
Distilled water	90	ml

Dissolved and made up to 100 ml with distilled water.

1.3 Phenol: Chloroform (1:1, v/v)

Crystalline phenol was liquidified in water bath at 65°C and mixed with chloroform in the ratio of 1:1 (v/v). The solution was stored in a light tight bottle.

1.4 20 x SSC (20 x standard saline citrate)

NaCl	17.5	g
Sodium citrate	8.8	g
Distilled water	1	L

Adjusted pH to 7.0 and steriled by autoclaveing at 121 °C 15 pounds / inch2 pressure, for 15 minutes. Note: To prepare 0.1× SSC and 0.2× SSC, the 20× SSC were diluted at 200 and 100 times, respectively before used.

1.5 RNase A solution

RNase A	20	mg
0.15MNaCl	10	ml

Dissolved 20 mg of RNase A in 10 ml 0.15 M NaCl and heated at 95°C for 5-10 min. Kept in -20°C.

1.6 0.1 M Tris-HCl (pH 7.5)

Tris(hydroxymethyl)aminomethane	1.21	g
Distilled water	90	ml

Dissolved and adjusted to pH 7.5 by adding 0.1 N HCl. Made to 100 ml with distilled water.

1.7 RNase T1 solution

RNase T1	80	ul
0.1 M Tris-HCl (pH 7.5)	10	ml

Mixed 80 µl of RNase T1 in 10 ml of 0.1 M Tris-HCl (pH 7.5) and heated at 95°C for 5 min. Kept in -20°C.

1.8 40 mM CH3COONa + 12 mM ZnSO4 (pH 5.3)

CH_3COONa	3.28	g
$ZnSO_4$	1.94	g
Distilled water	90	ml

Dissolved and adjusted to pH 5.3 by adding 0.1 N HCl or 0.1 N NaOH. Made to 100 ml with distilled water.

1.9 Nuclease P1 solution

Nuclease P1	0.1	mg
40 mM CH_3COONa + 12 mM $ZnSO_4$ (pH 5.3)	1	ml

Dissolved and stored at 4°C.

1.10 Alkaline phosphatase solution

Alkaline phosphatase	2.4	units
0.1 M Tris-HCl (pH 8.1)	1	ml

2. DNA-DNA hybridization

2.1 Phosphate-buffer saline (PBS)

NaCl	8.00	g
KCl	0.20	g
KH_2PO_4	0.12	g
Na_2HPO_4(anhydrous)	0.91	g
Distilled water	1	L

Steriled by autoclaveing at 121 °C, 15pounds/inch 2 pressure, for 15 minutes

2.2 20 x SSC (20 x standard saline citrate)

NaCl	17.5	g
Sodium citrate	8.8	g
Distilled water	1	L

Adjusted pH to 7.0 and steriled by autoclaveing at 121 °C 15 pounds / inch2 pressure, for 15 minutes

2.3 100 x Denhardt solution

Bovine serum albmin (Fraction V)	2	g
Polyvinylpyrrolidone	2	g
Ficoll400	2	ml

Dissolve in 100 ml ultra pure water and was stored at 4 °C until used.

2.4 Salmon sperm

Salmon sperm DNA	10	mg per ml

Salmon sperm DNA 10 mg was dissolved in 10 Mm Tris + EDTA buffer pH 7.6 volume 1 ml, boiled for 10 min and then immediately cooled in ice. Sonicated salmon sperm DNA solution foe 3 min and was store at 4 °C until used.

2.5 Prehybridization solution

100x Denhardt solution	2	ml
10 mg/ml Salmon sperm DNA	1	ml
20x SSC	10	ml
Formamide	50	ml
Distilled water	34	ml

All of ingredients were dissolved in ultra pure water steriled and kept at 4 °C

2.6 Hybridization solution

Prehybridization	100	ml
Dextran sulfate	5	g

Dissolved dextran sulfate in Prehybridization solution and keep at 4 °C

2.7 Solution 1

Bovine serum albumin (Fraction V)	0.25	g
Triton X – 100	50	µl
PBS	50	ml

All of ingredients were mixed and keep at 4 °C

2.8 Solution 2

Strepavidin –POD conjugate	1	µl
Solution1	4	ml

DissolvednStrepavidin- POD conjugate in solution 1 before used. The solution 2 was freshly prepared.

2.9 Solution 3

3,3',5,5' Tetramenthylbenzidine (TMB)		
(10 mg/ml in DMFO)	100	ml
0.3% H_2O_2	100	ml

0.1 M citric + 0.2 M Na_2HPO_4 buffer pH 6.2 in 10% DMFO 5 ml

All of ingredients were mixed and used. The solution 3 was freshly

3. 16S rRNA analysis

3.1 Primers for 16S rRNA amplification and Sequencing

Forward primer

EB-10F 5'-AGTTTGATCCTGGCTC-3'

EB-530F 5'-GTGCCAGCAGCCGCGG-3'

EB-1110F 5'-GCAACGAGCGCAACCC-3'

9F 5'-GAGTTTGATCCTGGCTCAG-3'

339F 5'-CTCCTACGGGAGGCAGCAG-3'

785F 5'-GGATTAGATACCCTGGTAGTC-3'

1099F 5'-GCAACGAGCGCAACCC-3'

Reverse primer

EB-1530R 5'-AAGGAGGTGATCCAGCC-3'

EB-520R 5'-ACCGCGGCTGCTGGC-3'

EB-1100R 5'-AGGGTTGCGCTCGTTG-3'

1541R 5'-AAGGAGGTGATCCAGCC-3'

357R 5'-CTGCTGCCTCCCGTAG-3'

802R 5'-TACCAGGGTATCTAATCCC-3'

1100R 5'-AGGGTTGCGCTCGTTG-3'

APPENDIX D

STANDARD ASSAY METHODS

1. Determination of protein and soluble peptide

The protein and soluble peptide content was measured by the method of Lowry et al. (1951) with bovine serum albumin and tyrosine as standard, respectively.

1.1 Reagents

A: 2% sodium carbonate in 0.1N NaOH

B: 0.5% $CuSO_4$.$5H_2O$ in 1% sodium citrate

C: 1 N Folin-Ciocalteu's phenol reagent (2N Folin Phenol was diluted with distilled water to the final concentration in 1N, the solution should be freshly prepared before use.)

D: 1 ml Reagent B + 50 ml Reagent A (or similar ratio) Make up immediately before use.

1.2 Procedure

1.2.1. Place 0.1 ml of proper dilution of culture broth (for protein determination) or clear supernatant of reaction mixture (for soluble peptide determination)

1.2.2. Add 1 ml of Reagent D into the tube and vortex immediately. Incubate at room temperature for 10 min3. After the 10 min incubation, add 0.1 ml of Reagent C to sample and vortex immediately. Incubate 30 min at room temperature.

1.2.3 Absorbance (OD) of samples was measured at 750 nm. Concentrations of the samples were compared to the standard curve for determination of values. Distilled water was used instead of sample as a blank.

1.3 Preparation of standard curve of tyrosine

Standards of 0, 0.1, 0.2, 0.3, 0.5, 0.7 and 1.0 mM were prepared from tyrosine.

2. Equipment and Reagent

2.1 Polyacrylamide gel electrophoresis (PAGE) reagents

Monomer solution

Acrylamide	30%	(w/v)
Bisacrylamide	0.8%	(w/v)

Made up to 100 ml with deionized water.

Note: Acrylamide is a neurotoxin observes extreme caution to minimize skin contact and inhalation. The solution can be store up to 3 months at 4°C in the dark.

2.2 4× Resolving gel buffer

Tris(hydroxymethyl)aminomethane	18.15	g
Deionized water	90	ml

Dissolved and adjusted the pH to 8.8 by using 0.1 N HCl. Made up to 100 ml with deionized water. Note: The solution can be store up to 3 months at 4°C in the dark.

2.3 4× Stacking gel buffer

Tris(hydroxymethyl)aminomethane	6	g
Deionized water	90	ml

Dissolved and adjusted the pH to 6.8 by using 0.1 N HCl. Made up to 100 ml with deionized water. Note: The solution can be store up to 3 months at 4°C in the dark.

2.4 10× Tank buffer for SDS-PAGE

Tris(hydroxymethyl)aminomethane	30.28	g
Glycine	144.13	g
Sodium dodecyl sulfate	10	g
Distilled water	900	ml

Dissolve and made up to 1 litter with distilled water.

Note: Diluted 10 times before use. The solution can be store up to 1

month at room temperature. 10× Tank buffer for Native-PAGE was prepared in a similar manner, except that the addition of SDS was omitted.

2.5 10% Sodium dodecyl sulphate (SDS)

Sodium dodecyl sulfate	10	g
Deionized water	90	ml

Dissolved and made to 100 ml with deionized water.

2.6 2× Sample buffer for SDS-PAGE

4× Stacking gel buffer	2.5	ml
Glycerol	2	ml
10% (w/v) SDS	4	ml
Broomphenol blue (2 mg/ml)	1	ml
β-mercaptoethanol	0.2	ml

Dissolved and made up to 10 ml with deionized water.

Note: The reagent should be filtered before use. 2× Sample buffer for Native-PAGE was prepared in a similar manner, except that the addition of SDS was omitted.

2.7 12.5% Running gel for SDS-PAGE

Deionized water	4.1314	ml
4× Running gel buffer	3.25	ml
Monomer solution	5.4171	ml
10% (w/v) SDS	130	µl
10% (w/v) Ammonium persulfate	65	µl
TEMED	6.5	µl

Note: 12.5% Running gel for Native-PAGE was prepared in a similar manner, except that the addition of SDS was omitted.

2.8 4% Stacking gel for SDS-PAGE

Deionized water	3.053	ml
4× Stacking gel buffer	1.25	ml
Monomer solution	667	µl
10% (w/v) SDS	50	µl
10% (w/v) Ammonium persulfate	25	µl
TEMED	5	µl

Note: 4% Stacking gel for Native-PAGE was prepared in a similar manner, except that the addition of SDS was omitted.

2.9 Staining solution

Coomassie brilliant blue (R-250)	1.25	g
Ethanol	450	ml
Acetic acid	100	ml

Dissolve and made up to 1 litter with distilled water.

Note: The reagent should be filtered before use. Store the solution in the dark.

2.10 Destaining solution

Methanol	300	ml
Acetic acid	100	ml

Dissolve and made up to 1 litter with distilled water.

APPENDIX E

16S rDNA SEQUENCE OF REPRESENTATIVE STRAIN

1. The 16S rDNA nucleotide sequence of MSK2-1

GATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCG
AGCGCGGGAAGCAAGCAGATCTCCTTCGGGAGTGACGCTTGTGGAACGA
GCGGCGGACGGGTGAGTAACACGTGGGCAACCTACCTGTAAGACTGGGA
TAACTCCGGGAAACCGGGGCTAATACCGGATGAAACAAAGCGTCGCATG
ACGCAATGTTAAAAGGCGGCATATGCTGTCACTTACAGATGGGCCCGCGG
CGCATTAGCTAGTTGGTGAGGTAAAGGCTCACCAAGGCAACGATGCGTAG
CCGACTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCAGATCT
ACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGT
TAGGGAAGAACAAGTGCCATTCGAATAGGTTGGCACCTTGACGGTACCTA
ACCAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGG
GGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCC
TTTAAGTCTGATGTGAAAGCCCACGGCTTAACCGTGGAGGGCCATTGGAA
ACTGGAGGACTTGAGTACAGAAGAGGAGAGTGGAATTCCACGTGTAGCG
GTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTC
TGGTCTGTAACTGACGCTGAGGTGCGAAAGCGTGGGTAGCGAACAGGATT
AGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGGG
GTTTCCGCCCCTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGA
GTACGGCCGCAAGGCTGAAACTCAAAAGAATTGACGGGGACCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGG
TCTTGACATCCTCTGACACCCCTAGAGATAGGGCATTCCCTTCGGGGACA
GAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGT
TGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAA
TGGATGGAACAAAGGGCAGCGAAGCCGCGAGGCCAAGCAAATCCCATAA
AACCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGG
AATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGT
CTTGTACACACCGCCCGTCACACCACGAGAGTTGGTAACACCCGAAGTCG
GTGAGGTAACCTTTTGGAGCCAGCCGCCGAAGGTGGGACCAATGATTG

2. The 16S rDNA nucleotide sequence of CHM1-4

CAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCGGGAA
GCAAGCTGCATCCTCTTCGGAGGTGACGCTTGTGGAACGAGCGGCGGACG
GGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACCCCGGGA
AACCGGGGCTAATACCGGATAATACTTTTCATCACCTGATGGAAAGTTGAA
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TGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGG
GTGATCGGCCACACTGGGACTGAGACACGGCCCAAACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAaC
AAGTGCCGTTCGAATAGGGCGGCACcTTGACGGTACctAACCAGAAAGCCCc
GGCTAACTACGTGCCAGCAGCCGcGGTAATACGTAGGGGGCAAGCGTTGTC
CGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTG
AAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAGGACTTGAG
TACAGAAGAGGAGAGTGGAATTCCCACGTGTAGCGGTGAAATGCGTAGAG
ATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACG
GTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTC
CACGCCGTAAACGATGAGTGCTAGGTGTTAGGGGGTTTCCGCCCTTTAGTG
CTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGTACGGCCGCAAGGCT
GAAACTCAAAAGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT
TTAATTCGACGCAACGCGAAGATCCTTACCAGGTCTTGACATCCTCTGCAA
TCGGTAGAGATACCGAGTTCCCTTCGGGGACAGAGTGACAGGTGGTGCAT
GGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGATCTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGTGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCC
CTTATGACCTGGGCTACACACGTGCTACAATGGATGGAACAAAGGGAAGC
AAAACCGCGAGGTCAAGCAAATCCCATAAAACCATTCTCAGTTCGGATTGC
AGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTAGTAATCGCGGATCA
GCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC
CACGAGAGTTGGTAACACCCGAAGTTCGGTGAGGTAACCTTTG

3. The 16S rDNA nucleotide sequence of TP3-3

TCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCGGG
AAGCAGGCAGATCCTCTTCGGAGGTGACGCCTGTGGAACGAGCGGCGGA
CGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGATTGGGATAACCCCGG
GAAACCGGGGCTAATACCGGATAATACTTTTCGTTGCATAACGAGAAGTT
GAAAGGCGGCTTTTAGCTGTCACTTACAGATGGGCCCGCGGCGCATTAGC
TAGTTGGTAAGGTAACGGCTTACCAAGGCGACGATGCGTAGCCGACCTGA
GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCA
ACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTCAGG
GAAGAACAAGTGCCGTTCGAATAGGGCGGCACCTGACGGTACCTGACCA
GAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGG
CAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTT
AAGTCTGATGTGAAAGCCCACGGCTTAACCGTGGAGGGCCATTGGAAACT
GGAGGACTTGAGTACAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGG
TCTGTAACTGACGCTGAGGTGCGAAAGCGTGGGGAGCGAACAGGATTAG
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TTCCGCCCCTTAGTGCTGAAGTTAACGCATTAAGCACTCCGCCTGGGGAGT
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GGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTC
TTGACATCCTCTGCCAATCCTAGAGATAGGATGTTCCCTTCGGGGACAGA
GTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTT
AAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTTAGTTG
GGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGCGGGGATGA
CGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATG
GATGGAACAAAGGGCAGCGAAGCCGCGAGGTCAAGCAAATCCCATAAAA
CCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAA
TCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCT
TGTACACACCGCCCGTCACACCACGAGAGTTGGTAACACCCGAAGTCGGT
GAGGTAACCTTTTGGAGCCAGCCGCCGAAGGTGGGACCAATGATTGGGGT
GAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACCTCCT
TTCTG

4. The 16S rDNA nucleotide sequence of MS3-4

TCAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCGGG
AAGCAAGCGGAAGCCTTCGGGTGGATGCTTGTGGAACGAGCGGCGGACG
GGTGAGTAACACGTGGGCAACCTGCCTGTAAGATGGGGATAACTCCGGG
AAACCGGGGCTAATACCGAATGAAGCGCGTCATCGCATGATGACGTGATG
AAAGGCGGCTTTTAGCTGTCACTTACAGATGGGCCCGCGGCGCATTAGTT
AGTTGGTGGGGTAAGAGCCTACCAAGGCGACGATGCGTAGCCGACCTGA
GAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGG
GAGGCAGCAGTAGGGAATCATCCGCAATGGACGAAAGTCTGACGGTGCA
ACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTCAGG
GAAGAACAAGTACCGTTTGAATAAGGCGGTACCGTGACGGTACCTGACCA
GAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGG
CAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCTCGCAGGCGGTCTTTT
AAGTCTGATGTGAAATCTCGCGGCTTAACCGCGAATGGTCATTGGAAACT
GGAGGACTTGAGTACAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTG
AAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGG
TCTGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGTTGAGTGCTAGGTGTTAGGGGGT
TTCCGCCCCTTAGTGCTGCAGTTAACGCATTAAGCACTCCGCCTGGGGAG
TACGGCCGCAAGGCTGAAACTCAAAAGAATTGACGGGGGCCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGT
CTTGACATCCTCTGCCAGCGCTGGAGACAGCGTGTTCCCTTCGGGGACAG
AGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGG
TTAAGTCCCGCAACGAGCGCAACCCTTAATCTTAGTTGCCAGCATTGAGTT
GGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATG
ACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAAT
GGATGGAACAGAGGGCAGCGAAGCCGCAAGGTGTAGCAAATCCCATAAA
ACCATTCTCAGTTCGGATNGCAGGCTGCAACTCGCCTGCATGAAGCCGGA
ATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCACGAGAGTTGGCAACACCCGAAGTCGG
TGAGGTAACCTTTTGGAGCCAGCCGCCGAAGGTGGGGCCAATGATTGGGG
TGAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCG

5. The 16S rDNA nucleotide sequence of TP2-8

GTTTGATCCTGGCTCAGGACGAACGCTGGCGGCGTGCCTAATACATCCAA
GTCGAGCGCGGGAAGCTTGTCTGATCCCTTCGGGGTGACGCGAGTGGAAC
GAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGG
GATAACTCCGGGAAACCGGGGCTAATACCGGATAATACATTGCTTCGCAT
GAAGCAATGTTGAAAGATGGCTTTGGCTATCACTTACAGATGGGCCCGCG
GCGCATTAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCAACGATGCGTA
GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAG
ACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCT
GACGGAGCAACGCCGCGTGAACGAAGAAGGTTTTCGGATCGTAAAGTTCT
GTTGTTAGGGAAGAACAAGTACCGTTCAAATAGGGCGGTACCTTGACGGT
ACCTATCGAGGAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGGGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGTAGGC
GGTTTCTTAAGTCTGATGTGAAATCTTGCGGCTCAACCGCAAGCGGCCATT
GGAAACTGAGGAACTTGAGTGCAGAAGAGGAGAGCGGAATTCCACGTGT
AGCGGTGAAATGCGTAGATATGTGGAGGAACACCAGTGGCGAAGGCGGC
TCTCTGGTCTGTAACTGACGCTGAGGTGCGAAAGCGTGGGGAGCGAACAG
GATTAGATACCCTGGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTT
AGGGGGTTTCCGCCCCTTAGTGCTGCAGTTAACGCATTAAGCACTCCGCCT
GGGGAGTACGGCCGCAAGGCTGAAACTCAAAAGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTA
CCAGGTCTTGACATCTTCGGATGTCCCTAGAGATAGGGAGTTCCCTTCGGG
GACCGAATGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATT
TAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGG
GGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCT
ACAATGGATGGAACAAAGGGCAGCGAAGCCGCGAGGTGTAGCAAATCCC
ATAAATCCATTCTCAGTTCGGATTGCAGGCTGCAACTCGCCTGTATGAAGC
CGGAATCGCTAGTAATCGTGGATCAGCATGCCACGGTGAATACGTTCCCG
GGCCTTGTACACACCGCCCGTCACACCACGAGAGTTGGCAACACCCGAAG
TCGGTGGGGTAACCTTTGGAGCCAGCCGCCGAAGGTGGGGCCAATGATTG
GGGTGAAGTCGTAACAAGGTAGCCGTATCGGAAGGTGCGGCTGGATCACC
TCC

6. The 16S rDNA nucleotide sequence of TPA3-2

GACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGCGGGAAGC
GAGCTGATCCCTTCGGGGTGACGCTCGTGGAACGAGCGGCGGACGGGTGA
GTAACACGTGGGCAACCTGCCTGTAAGATCGGGATAACTCCGGGAAACCG
GGGCTAATACCGGGTAATACTTTCTTTCGCATGAAGGAAAGTTGAAAGAT
GGCTTCTCGCTATCACTTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGT
GAGGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAG
CAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC
GTGAACGATGAAGGTCTTCGGATCGTAAAGTTCTGTTGTTAGGGAAGAAC
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CTGACGCTGAGGAGCGAAAGCGTGGGTAGCAAACAGGATTAGATACCCT
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GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACA
TCCTTGGACCACCCTAGAGATAGGGTCTTCCCTTCGGGGACCAAGTGACA
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CGCAACGAGCGCAACCCCTAATCTTAGTTGCCAGCATTCAGTTGGGCACT
CTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGCGGGGATGACGTCAA
ATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGGT
ACAAAGGGCAGCGAAGCCGCGAGGTGTAGCAAATCCCATAAAACCATTC
TCAGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATCGCTA
GTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACA
CACCGCCCGTCACACCACGAGAGTTGGCAACACCCGAAGTCGGTGAGGTA
ACCTTTTTGGAGCCAGCCGCCGAAGGTGGGGCCAATGATTGGGGTGAAGT
CGTAACAAGGTAGCCGTATCGGAAGGTGC

7. The 16S rDNA nucleotide sequence of N20-1

CAGGACGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGGATTGA
TGGGAGCTTGCTCCCTGATATCAGCGGCGGACGGGTGAGTAACACGTGGG
TAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCG
GATAACTCATTTCCTCGCATGAGGAAATGTTGAAAGGTGGCTTTTAGCTAC
CACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCT
CCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACT
GGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATC
TTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCCTTGAGTGATGAA
GGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCCCGTTC
GAATAGGGCGGCCACCTTGACGGTACCCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAAT
TATTGGGCGTAAAGCGCGCGCAGGTGGTTCCTTAAGTCTGATGTGAAAGC
CCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAG
AAGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAATGCGTAGATATTTGG
AGGAACACCAGTGGCGAAGGAGACTTTCTGGTCTGTAACTGACACTGAGG
CGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGC
CGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGC
AGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAA
CTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAA
TTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACC
CTAGAGATAGGGCTTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATG
GTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGC
GCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGATGACT
GCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCC
CCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGGGCAG
CGAGACCGCGAGGTTTAGCCAATCCCATAAAACCGTTCTCAGTTCGGATT
GTAGGCTGCAACTCGCCTACATGAAGCTGGAATCGCTAGTAATCGCGGAT
CAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCA
CACCACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCTTTTGGAGC
CAGCCGCCTAAGGTGGGACAGATGATTGGGGTG

8. The 16S rDNA nucleotide sequence of ND1-1

TGAGTTTGATCCTGGCTCAGATTGAACGCCGGCGGCAGGCCTAACACATG
CAAGTCGAGCGGAAACGGCAGTATTGAAGCTTCGGTGGATTTACTGGACG
TCGAGCGGCGGACGGGTGAGTAACGGCTGGGAACCTGCCCTGACGAGGG
GGATAACCGTTGGAAACGACGGCTAATACCGCATAATGTCCTACGGACCA
AAGGTGGCCTCTACATGTAAGCTATCGCGTTGGGATGGGCCCAGTTAGGA
TTAGCTAGTTGGTAAGGTAATGGCTTACCAAGGCGACGATCCTTAGCTGG
TTTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTCCACAATGGGGGAGACCCTGATGC
AGCCATGCGGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAANCACTTTCAG
CAGTGAGGAAGGTGGTGTACTTAATACNTGCATGGCTTGACGTTAGCTGC
AGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGG
GTNCGAGCGTTAATCGGAATTACTGGGCGTAAAGCGCATGCAGGCGGTTT
GTTAAGTCAGATGTGAAAGCCCGGGGCTCAACCTCGGAACCGCATTTGAA
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TTGACATCCAGAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAGCTCT
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AAGTCCCGCAACGAGCGCAACCCTTATCCTTGTTTGCCAGCACGTCATGGT
GGGAACTCCAGGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGACG
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GGCAGATACAGAGGGCAGCGAAGCTGCGAAGTGGAGCGAATCCCTTAAA
GTTTGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGA
ATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCC
TTGTACACACCGCCCGTCACACCATGGGAGTGGGCTGCACCAGAAGTAGA
TAGCTTAACCTTCGGGAGGGCGTTTAGCCAACGGTGTGGTTC

9. The 16S rDNA nucleotide sequence of R5-7

GCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTA
ACAGGTCCAGCTTGCTGGACGCTGACGAGCGGCGGACGGGTGAGTAATGC
ATAGGAATCTACCCAGTCGTGGGGGATAACCTGGGGAAACCCAGGCTAAT
ACCGCATACGTCCTACGGGAGAAAGCGGGGGCTCTTCGGACCTCGCGCGA
TTGGATGAGCCTATGTCGGATTAGCTGGTTGGTGGGGTAACGGCTCACCA
AGGCGACGATCCGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT
GAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTGGGGAATATTGGAC
AATGGGCGAAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGCCTTTC
GGGTTGTAAAGCACTTTCAGTGGGAAAGAAGGCTTGTCGGCCAATACCCG
GCAAGAGCGACATCACCCACAGAAGAAGCACCGGCTAACTCCGTGCCAG
CAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCGCGTAGGCGGCTTGTCACGCCGGGTGTGAAAGCCCCGGGCTCA
ACCTGGGAACGGCATCCGGAACGGGCAGGCTAGAGTGCAGGAGAGGAAG
GTAGAATTCCCGGTGTAGCGGTGAAATGCGTAGAGATCGGGAGGAATACC
AGTGGCGAAGGCGGCCTTCTGGACTGAAACTGACCGCTGAGGTGCGAAA
GGCGTGGGTAGCAAACAGGATTAGATACCCTGGTAATCCACGCCGTAAAC
GATGTCGACTAGCCGTTGGTCCCTTGAGGACTTAGTGGCGCAGTTAACGC
GATAAGTCGACCGCCTGGGGAGTACGGCCCCAAGGTTAAAACTCAAATGA
ATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCA
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CTCCTGTTGAGAAATGTTGGGTTAAGTCCCGTAACGAGCGCAACCTTTGTC
CCTATTTGCCAGCGATTCGGTCGGGAACTCTAGGGAGACTCCGGGTGACA
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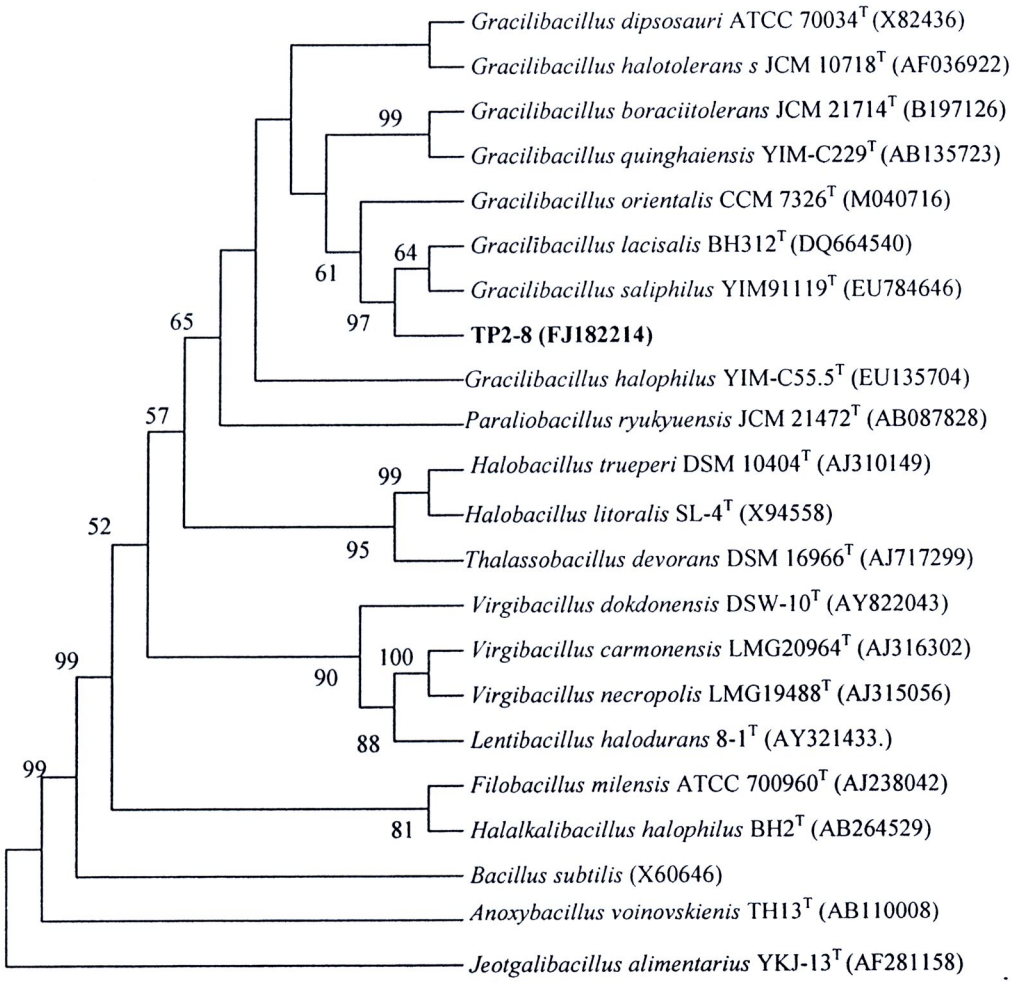
GGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAG
TGGACTGCACCAGAAGTGGTTAGCCTAACTTCGGAGGGCGATCACNACGG
TGT

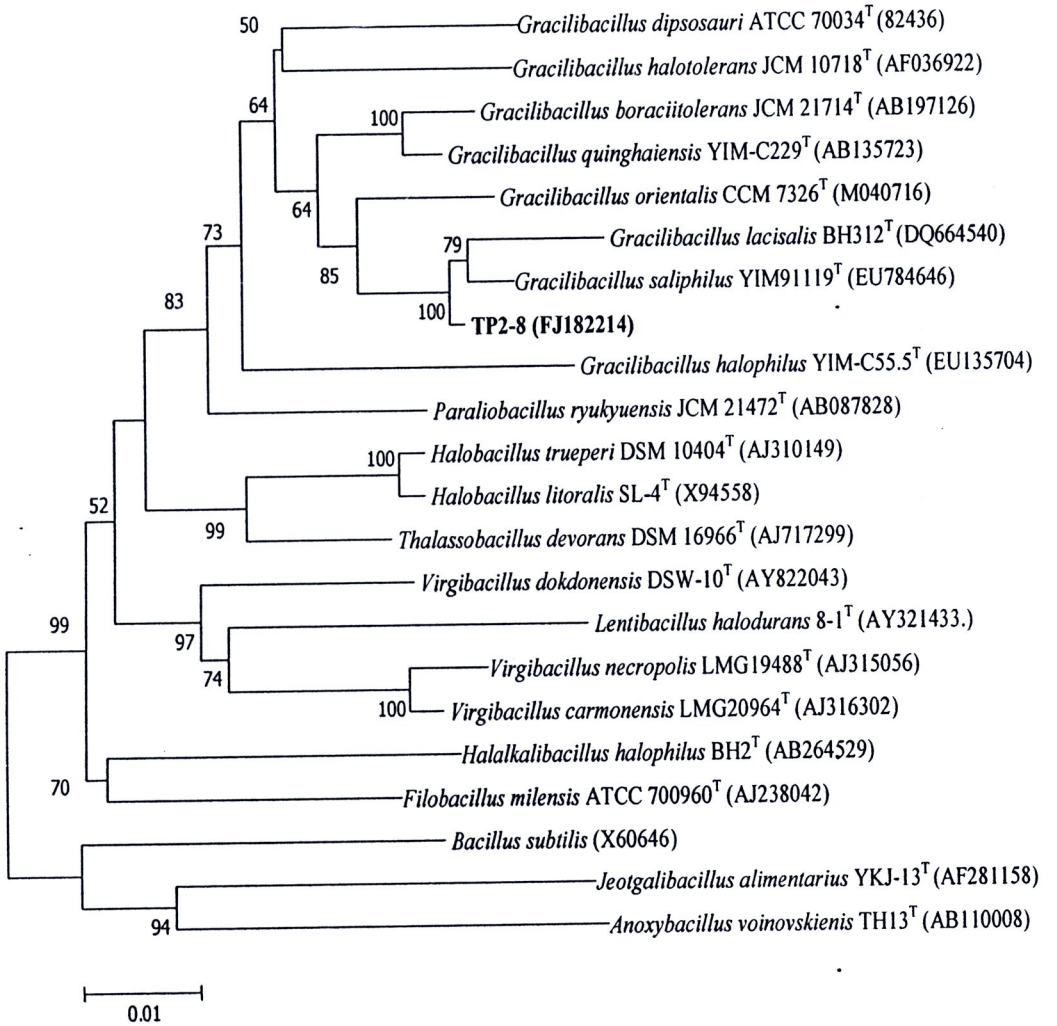
APPENDIX F

PHYLOGENETIC TREE OF TP2-8 AND RELATED TAXA

1. Phylogenetic tree showing the relationships between strain TP2-8, *Gracilibacillus* species and related taxa based on 16S rRNA gene sequences. The branching pattern was generated by the maximum-parsimony method. Bootstrap percentages ≥ 52%, based on 1000 replications are shown at the nodes.



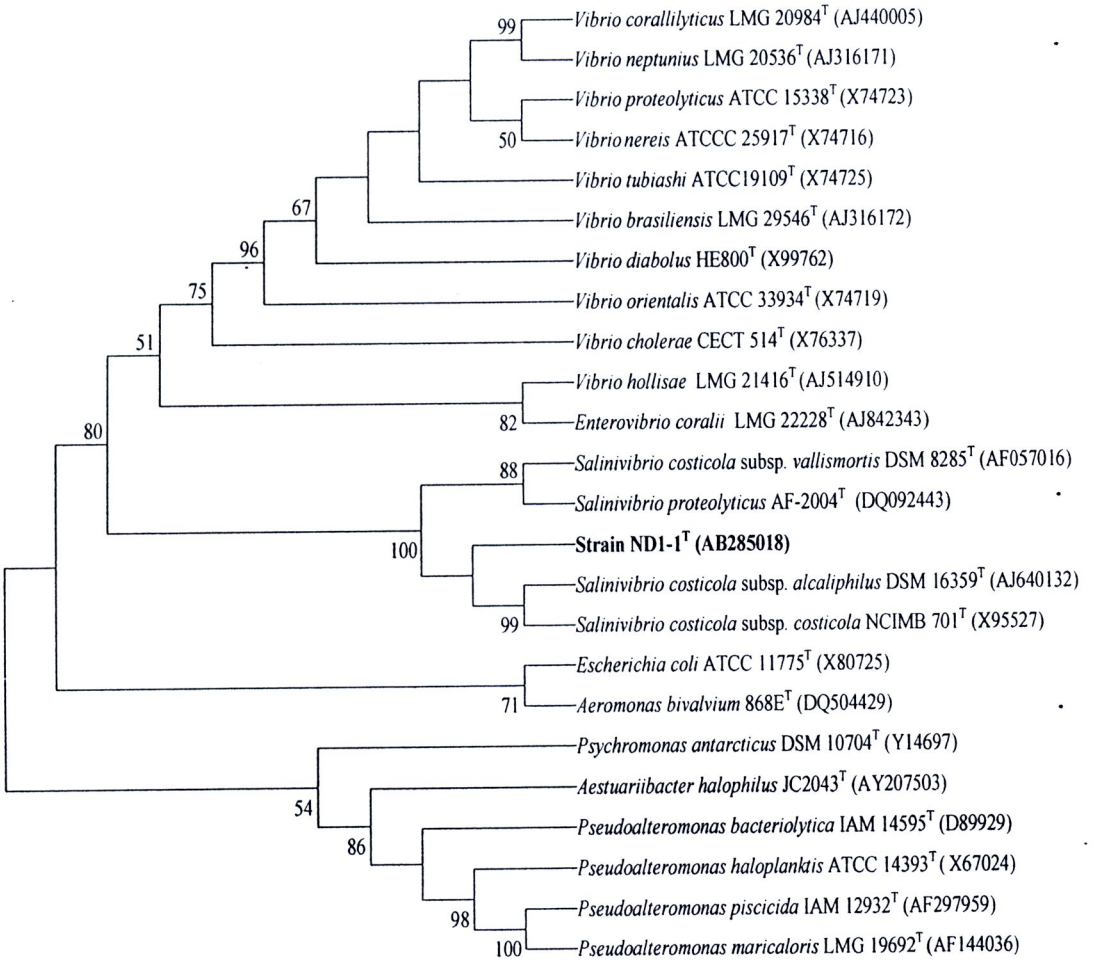
2. Phylogenetic tree showing the relationships between strain TP2-8^T, *Gracilibacillus* species and related taxa based on 16S rRNA gene sequences. The branching pattern was generated by the maximum-likelihood method. Bootstrap percentages ≥ 50%, based on 1000 replications are shown at the nodes. Bar, 0.01 substitutions per nucleotide position.



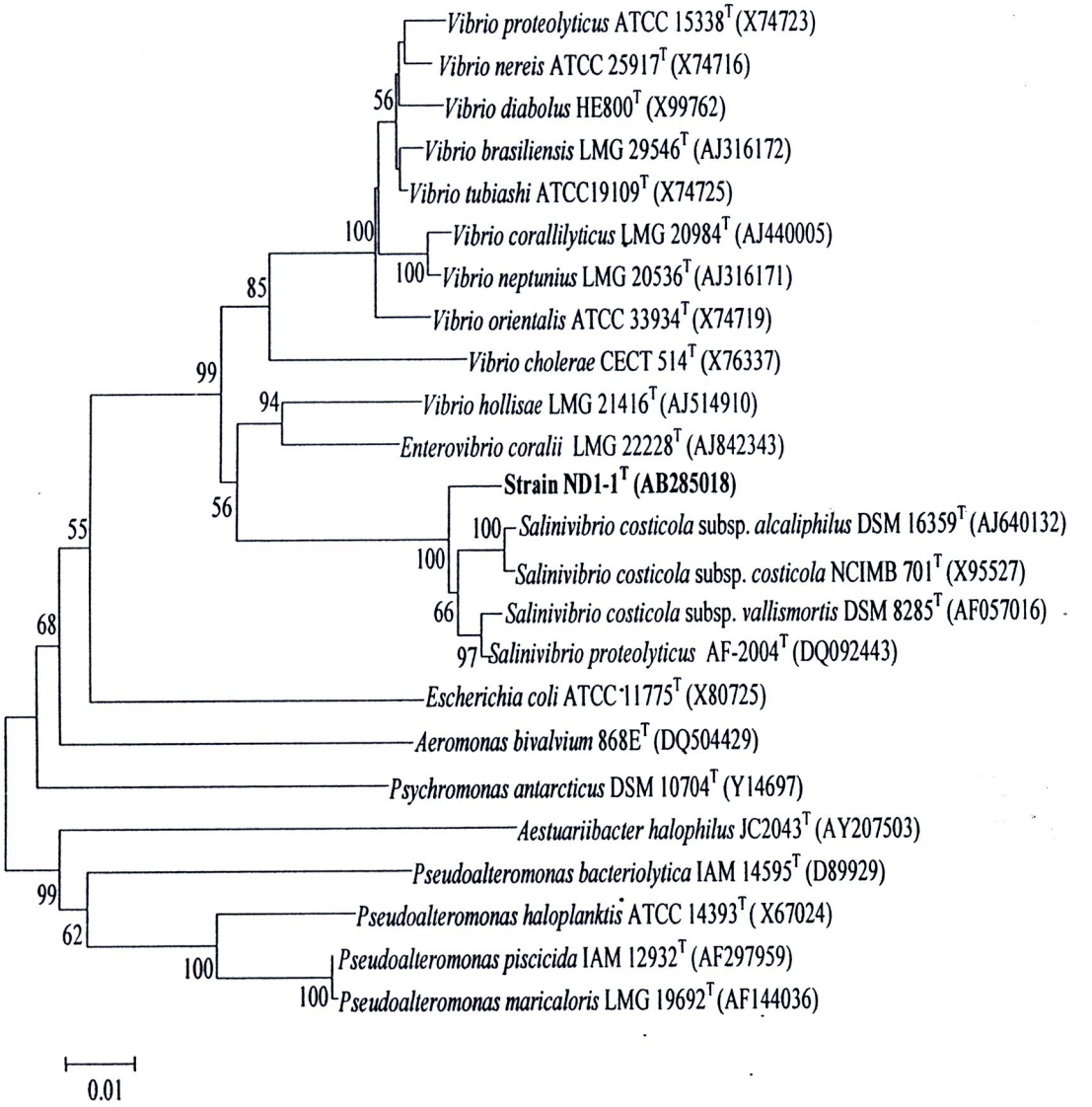
APPENDIX G

PHYLOGENETIC TREE OF ND1-1 AND RELATED TAXA

1. Phylogenetic tree showing the relationships between strain ND1-1, *Salinivibrio* species and related taxa based on 16S rRNA gene sequences. The branching pattern was generated by the maximum-parsimony method. Bootstrap percentages ≥ 50%, based on 1000 replications are shown at the nodes.



2. Phylogenetic tree showing the relationships between strain ND1-1, *Salinivibrio* species and related taxa based on 16S rRNA gene sequences. The branching pattern was generated by the maximum-likelihood method. Bootstrap percentages ≥ 50%, based on 1000 replications are shown at the nodes. Bar, 0.01 substitutions per nucleotide position.



BIOGRAPHY

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Education Attainment

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1999-2002	Bachelor of science (Biotechnology)	Mahasarakham University
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Publications:

Chamroensaksri, N., Akaracharanya, A., Visessanguan, W. and Tanasupawat, S. 2008. Characterization of halophilic bacterium NB2-1 from pla-ra and its protease production. J. Food Biochem. 32(4): 536-555.

Chamroensaksri, N., Tanasupawat, S., Akaracharanya, A., Visessanguan, W., Kudo, T. and Itoh, T. 2008. *Salinivibrio siamensis* sp. nov., from fermented fish (pla-ra) in Thailand. Int. J. Syst. Evol. Microbiol. (Paper in press: IJSEM paper no.IJS/2008/001768).

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