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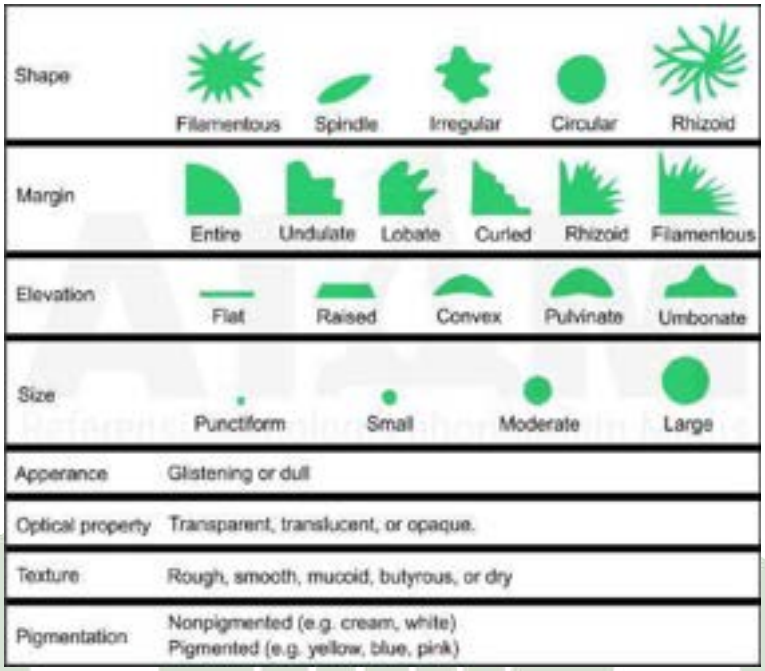
LAMPIRAN-LAMPIRAN

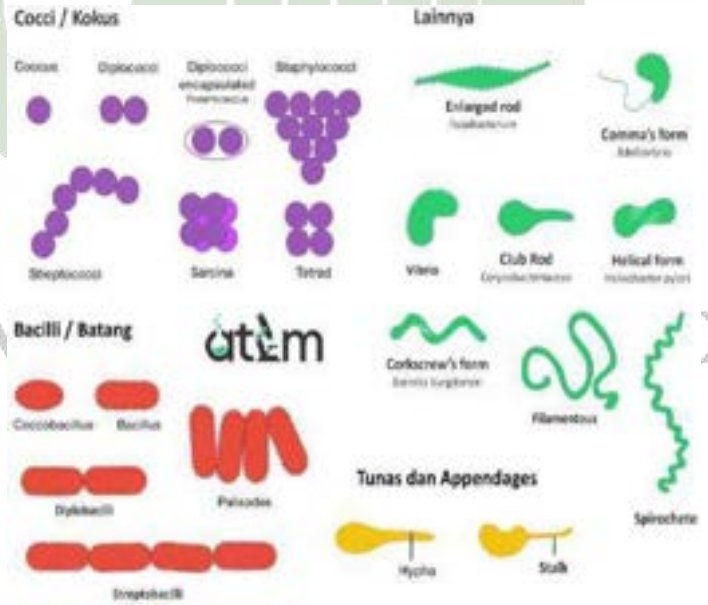
LAMPIRAN-LAMPIRAN

Lampiran 1. Karakteristik Morfologi Bakteri (Kurniawan, 2019)

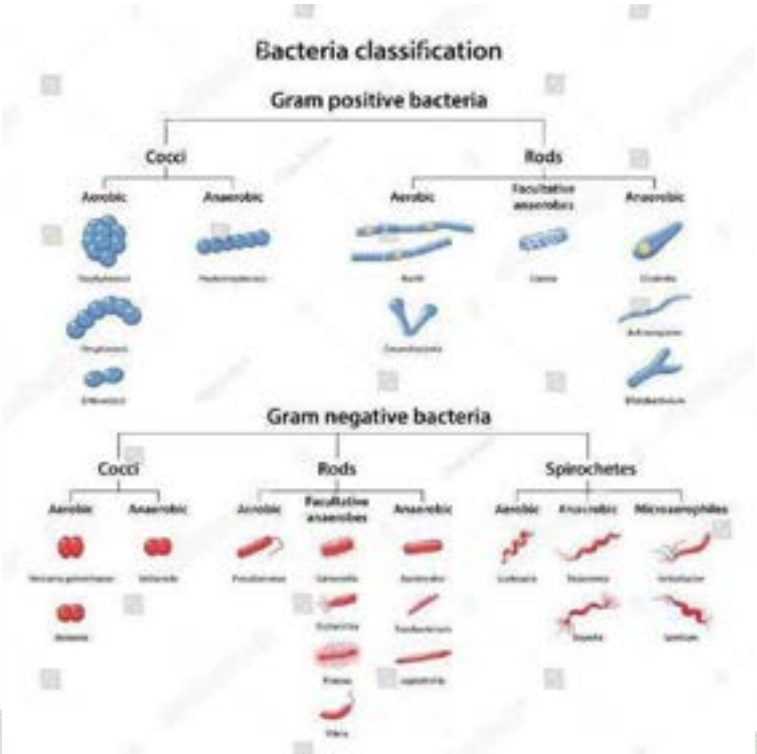
1. Karakteristik Morfologi Koloni Bakteri



2. Morfologi Sel Bakteri



3. Pewarnaan Gram



Lampiran 2. Penghitungan Konsentrasi Larutan (PPM)

- **Rumus :**

  1 PPM = 1 mg/kg atau 1 PPM = 1 mL/L

  PPM = Massa Zat Terlarut (mg) ÷ Volume larutan (L)

- **Penghitungan *Rhodamine B* 50 ppm**

  PPM = Massa Zat Terlarut (mg) ÷ Volume larutan (L)

  50 PPM= X mg ÷ 1 L

  X mg = 50 PPM × 1 L

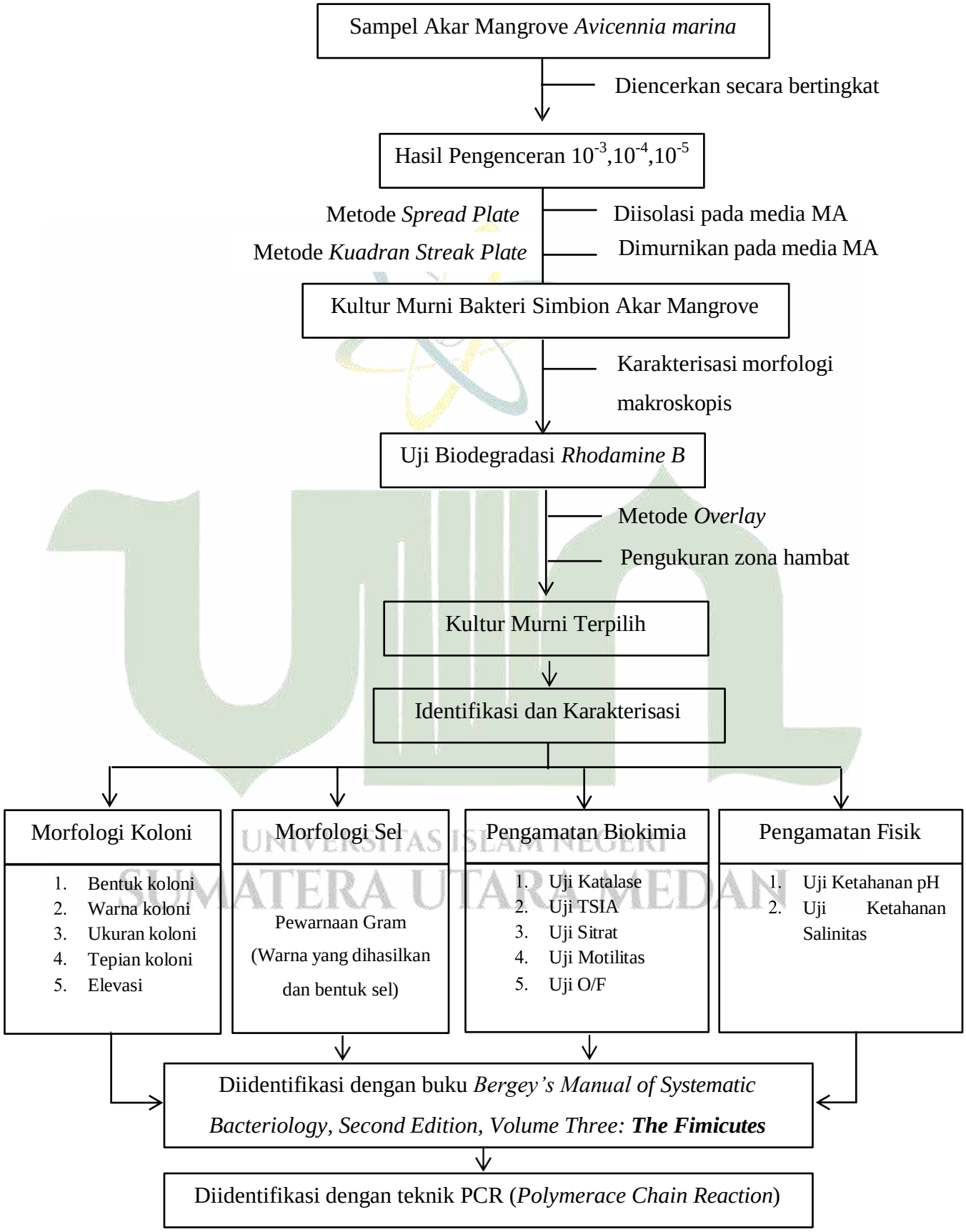
  X = 50 mg

  Sehingga, *Rhodamine B* yang digunakan sebanyak **50 mg** dan dilarutkan ke dalam aquades sebanyak **1 L** atau **5 mg** *Rhodamine B* dilarutkan ke dalam aquades sebanyak **100 mL** dengan penghitungan;

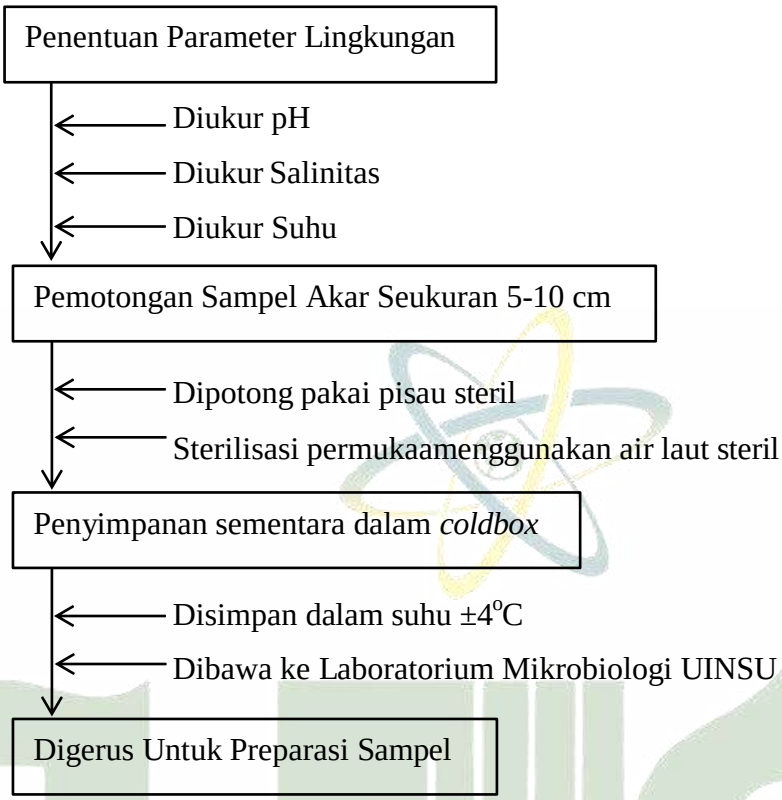
  50 mg/L → 50 mg/1000mL

  → 5 mg/100mL

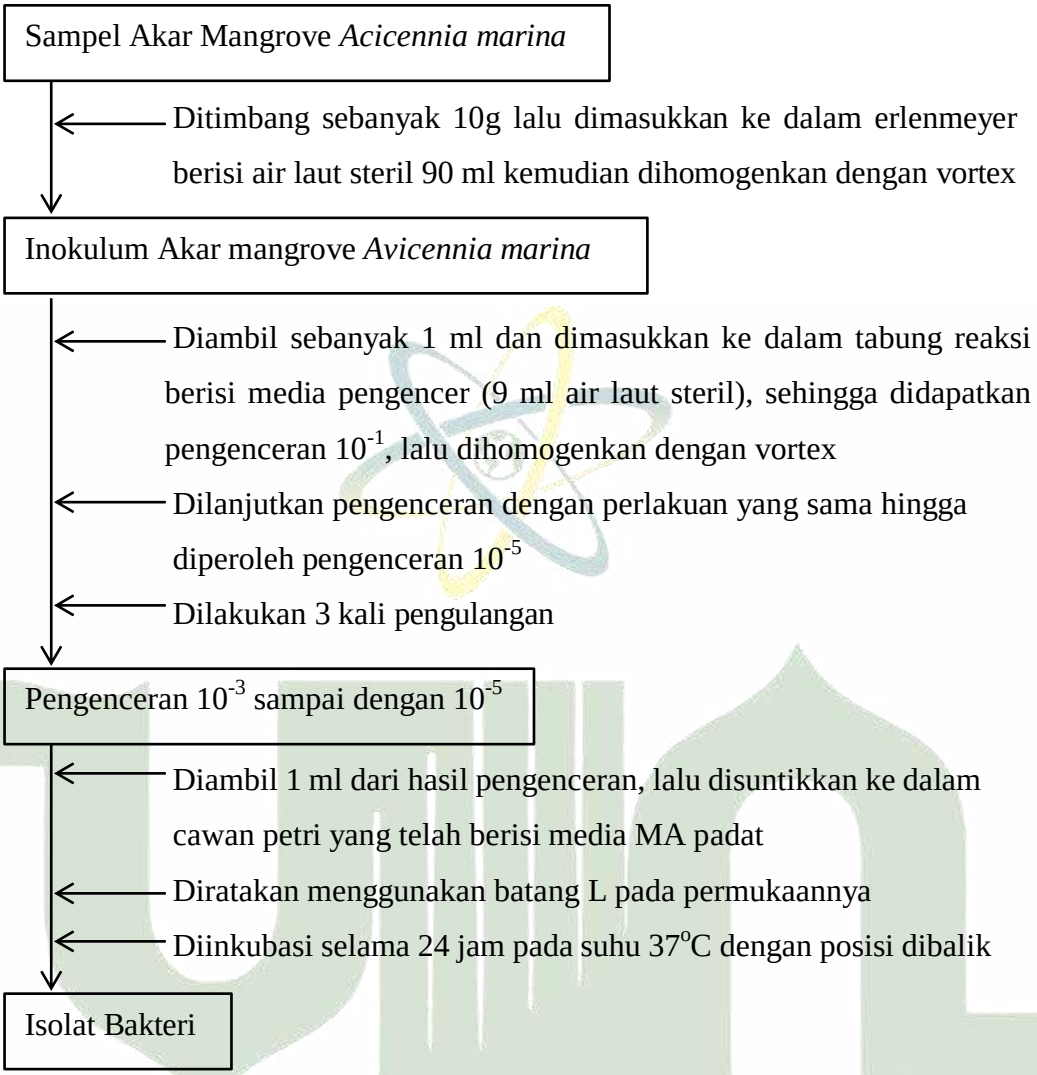
Lampiran 3. Skema Kerja



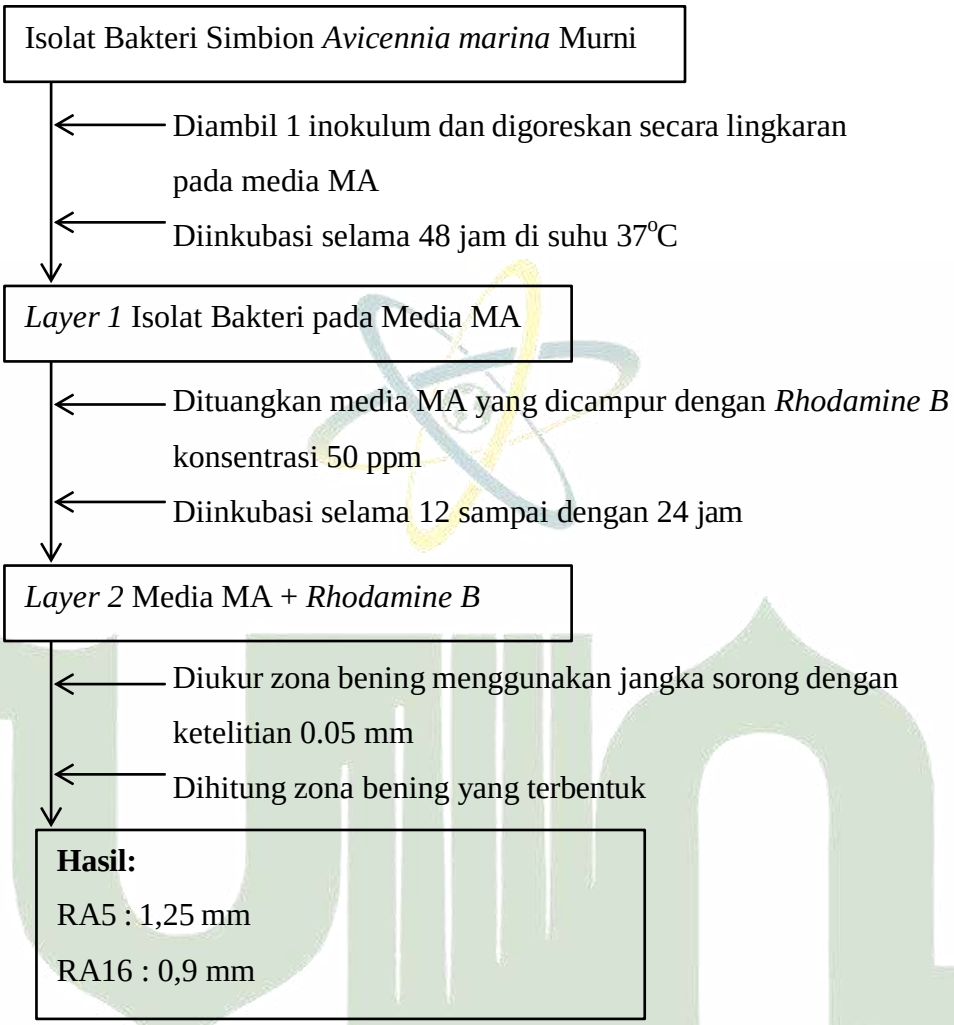
Lampiran 4. Skema Pengambilan Sampel Akar Mangrove *Avicennia marina*



Lampiran 5. Skema Isolasi Bakteri Simbion Akar Mangrove *Avicennia marina*



Lampiran 6. Skema Pengamatan Biodegradasi *Rhodamine B* oleh Isolat Bakteri Simbion Akar *Mangrove marina*



Lampiran 7. Skema Pengamatan Morfologi, Biokimia, dan Ketahanan Fisik Isolat Bakteri Simbion Akar Mangrove *Avicennia marina*

1. Pemeriksaan Bentuk Koloni dan Pewarnaan Gram

Isolat Bakteri Simbion *Avicennia marina*
Menghasilkan Zona Bening

↓

← Diamati bentuk, tepian, elevasi dan warna koloni

Pewarnaan Gram :

← Dibilas gelas objek dengan alkohol 70%

← Diambil 1 ose biakan bakteri dan disuspensikan dengan aquades steril diatas gelas objek

← Dikeringkan dengan cara difiksasi

← Ditetesi kristal violet, didiamkan selama 1 menit lalu dibilas aquades

← Ditetesi lugol, didiamkan selama 1 menit lalu dibilas aquades

← Ditetesi alkohol 96% selama 5 detik

← Ditetesi safranin, didiamkan 1 menit lalu dibilas aquades

← Dikeringkan dengan cara difiksasi

← Diteteskan minyak imersi lalu diamati dengan perbesaran 100x pada mikroskop

Hasil :

RA5 : Basil, Gram Positif

RA16 : Coccus Gram Positif

2. Uji Katalase

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose

← Dioleskan pada permukaan gelas objek

Diberikan 2-3 tetes reagen H_2O_2 3% di atas isolat

← Diamati ada tidaknya gelembung yang terbentuk

Hasil :
RA5 : Katalase Positif
RA16 : Katalase Negatif

3. Uji TSIA

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose lurus

← Diinokulasikan ke dalam media TSIA dengan cara ditusukkan secara tegak lurus hingga pada bagian *butt*

← Diinkubasi selama 48 jam pada suhu 37^{o}C

Hasil :
RA5 : TSIA Positif
RA16 : TSIA Negatif

4. Uji Sitrat

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose lurus

← Diinokulasi ke dalam media SCA dengan cara ditusukkan secara tegak lurus

← Diinkubasi selama 24 jam pada suhu 37^{o}C

Hasil :
RA5 : Sitrat Negatif
RA16 : Sitrat Negatif

5. Uji Motilitas

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose lurus
← Diinokulasi ke dalam media SIM dengan cara ditusukkan secara tegak lurus
← Diinkubasi selama 24 jam pada suhu 37^{o}C

Hasil :
RA5 : Motilitas Positif
RA16 : Motilitas Negatif

6. Uji Oksidatif Fermentatif

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose lurus
← Diinokulasi ke dalam media OF dengan cara ditusukkan secara tegak lurus
← Salah satu ditutup parafin dan satunya tidak parafin
Diinkubasi selama 24 jam pada suhu 37^{o}C

Hasil :
RA5 : Fermentatif
RA16 : Fermentatif

7. Uji Ketahanan Salinitas

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose
← Diencerkan dengan NaCl kadar berbeda, 0%, 10%, 20%, dan 30%
← Ditanam pada media NB
← Diinkubasi selama 24 jam pada suhu 37^{o}C

Hasil :
RA5 : Halofilik
RA16 : Halofilik

8. Uji Ketahanan pH

Isolat Bakteri Simbion *Avicennia marina*

← Diambil sebanyak 1 ose

← Diencerkan dengan aquades yang telah diatur pH dengan kadar berbeda, 5, 6, 7, dan 8

← Ditanam pada media NB

← Diinkubasi selama 24 jam pada suhu 37^{o}C

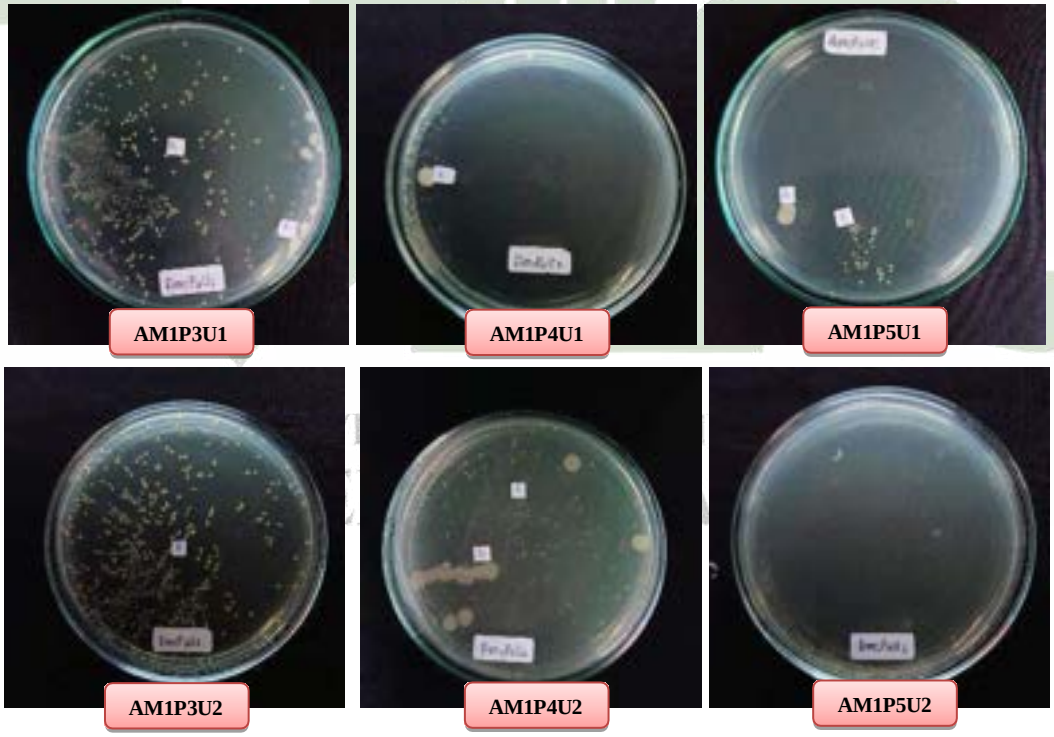
Hasil :
RA5 : Neutrofilik
RA16 : Neutrofilik

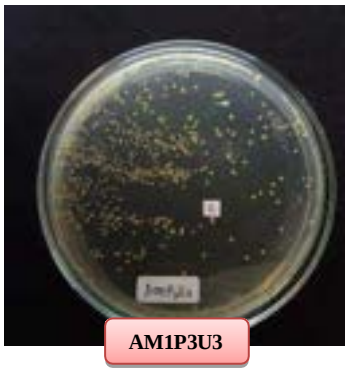
Lampiran 8. Pengenceran Sampel Akar *Avicennia marina* dengan Teknik Pengulangan Triplo

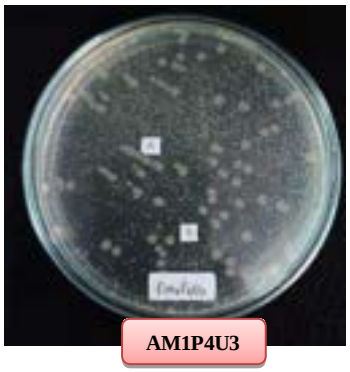
Titik 1	Titik 2
	

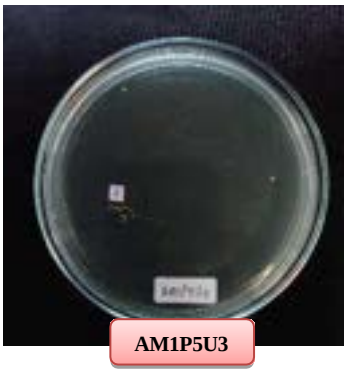
Lampiran 9. Isolasi Bakteri Simbion Akar Mangrove *Avicennia marina*

Titik 1. Muara Sungai

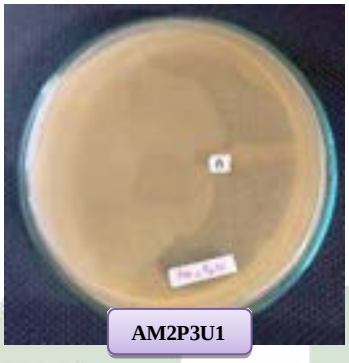



AM1P3U3


AM1P4U3

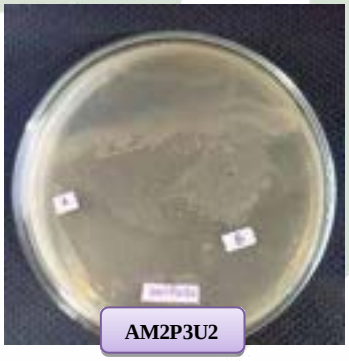

AM1P5U3

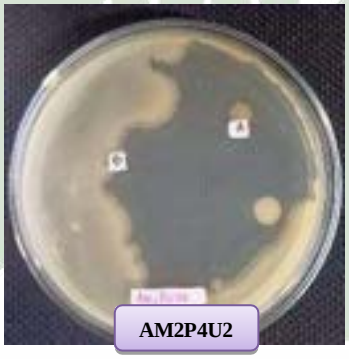
Titik 2. Pinggiran Pantai


AM2P3U1

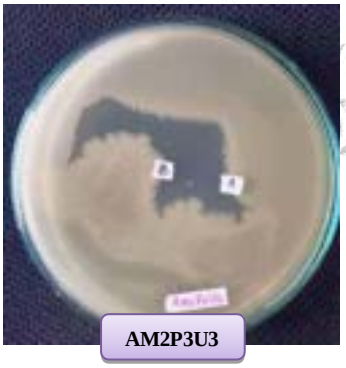

AM2P4U1


AM2P5U1

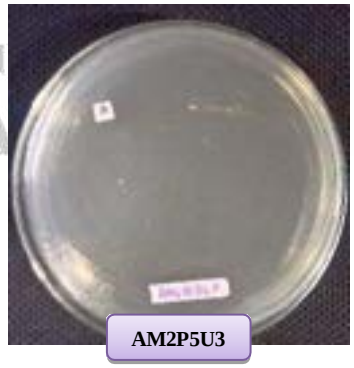

AM2P3U2


AM2P4U2

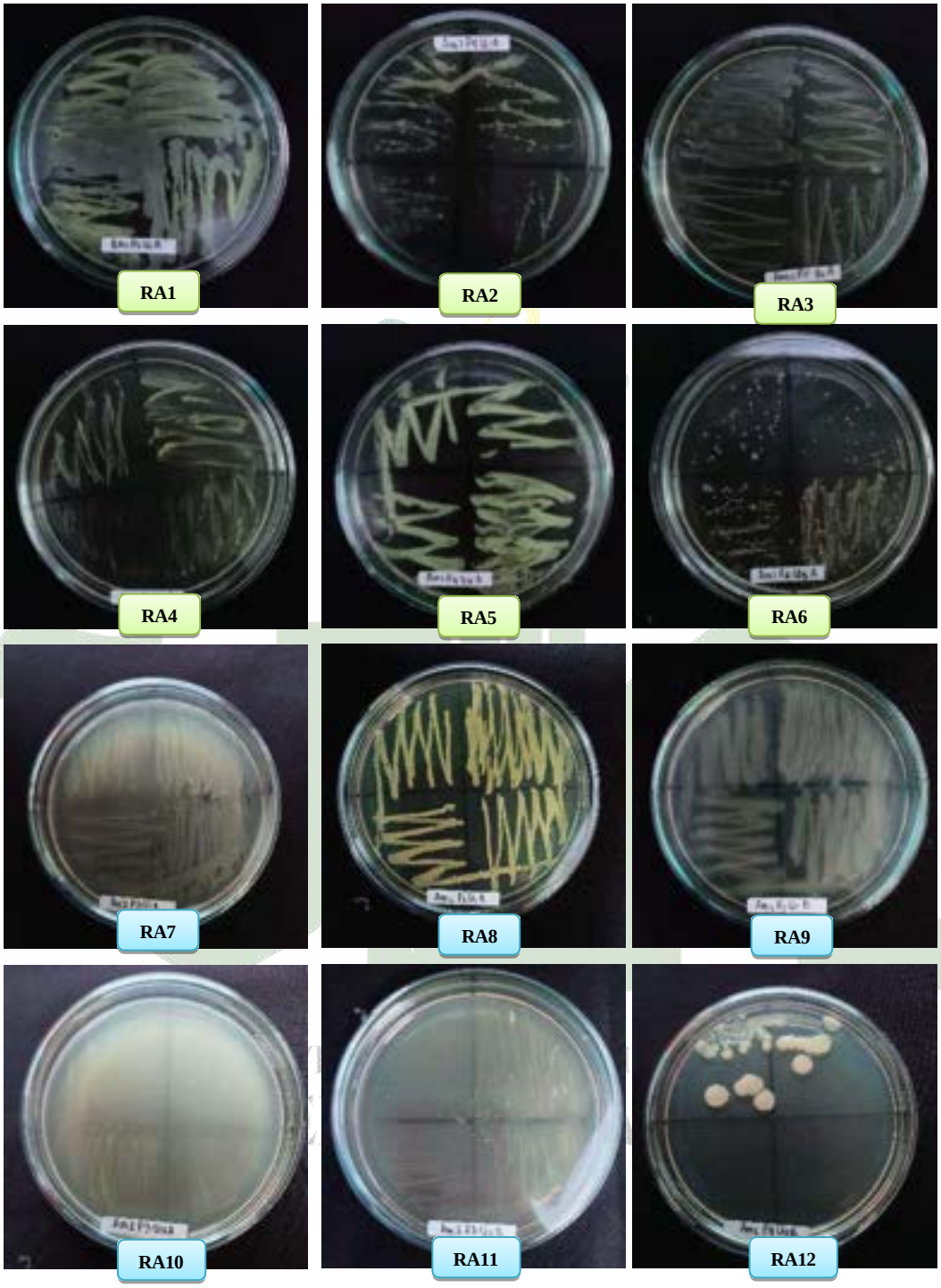

AM2P5U2

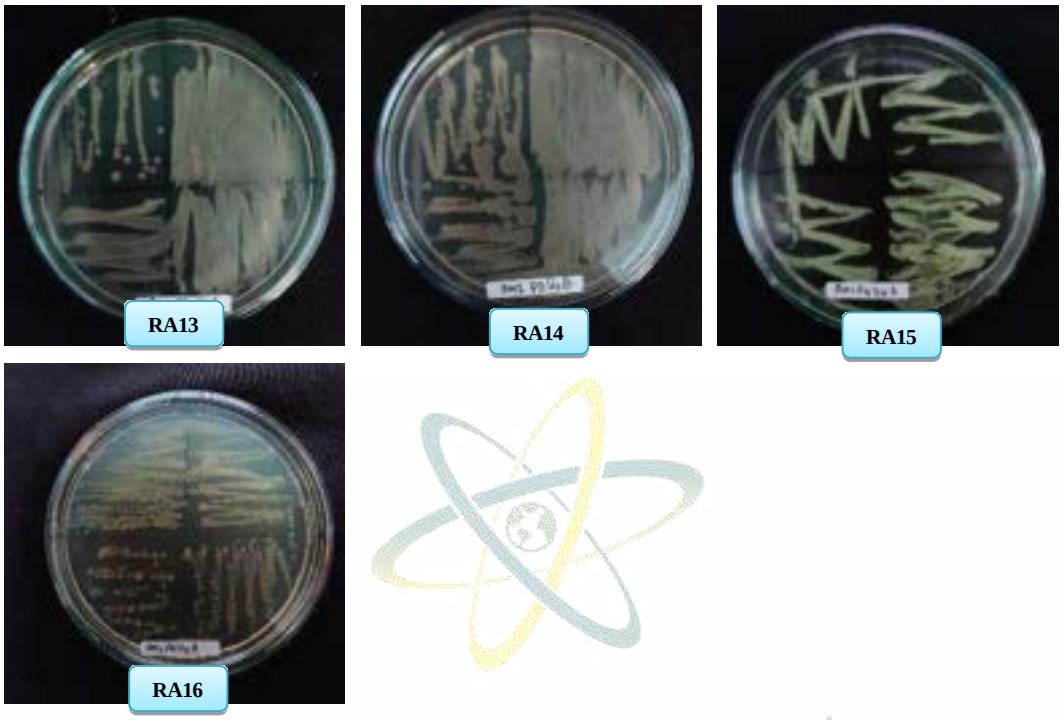

AM2P3U3


AM2P4U3

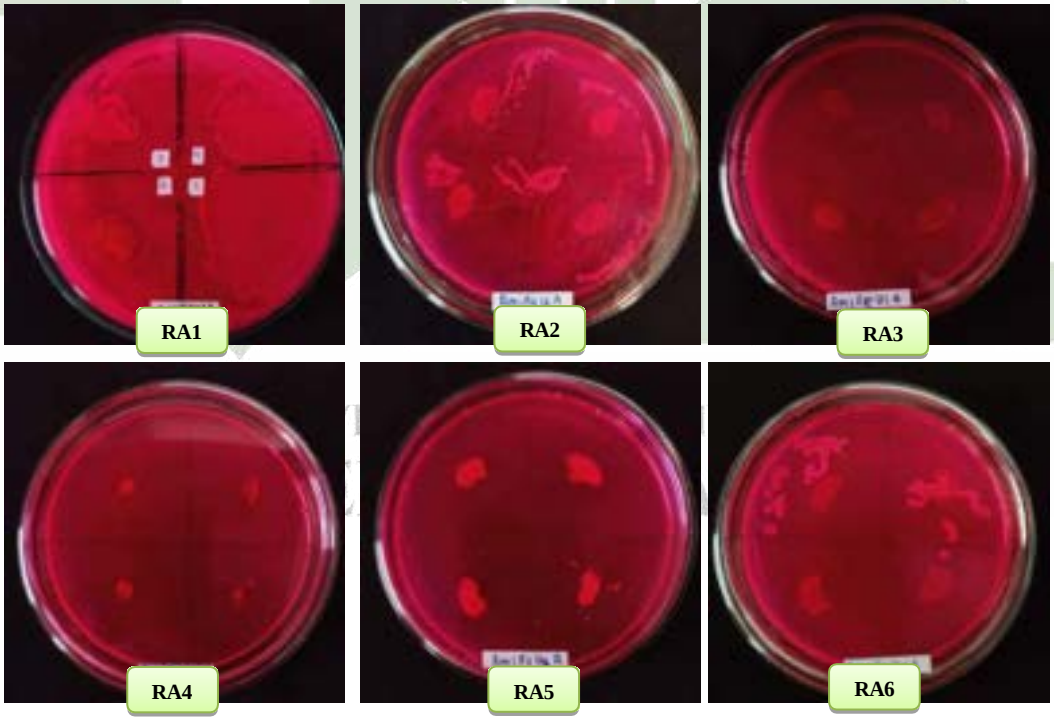

AM2P5U3

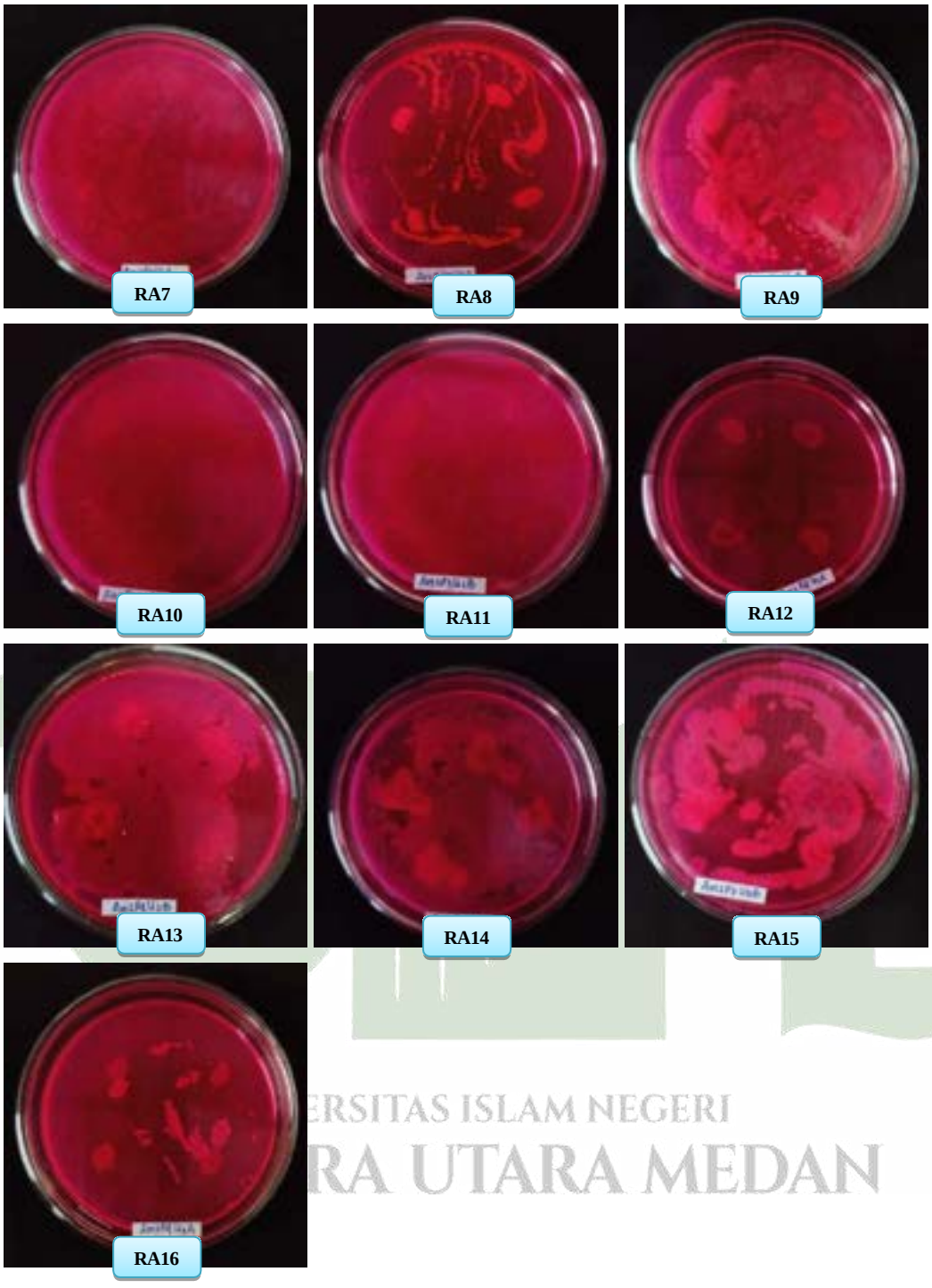
Lampiran 10. Kultur Murni Bakteri Simbion Akar Mangrove *Avicennia marina*





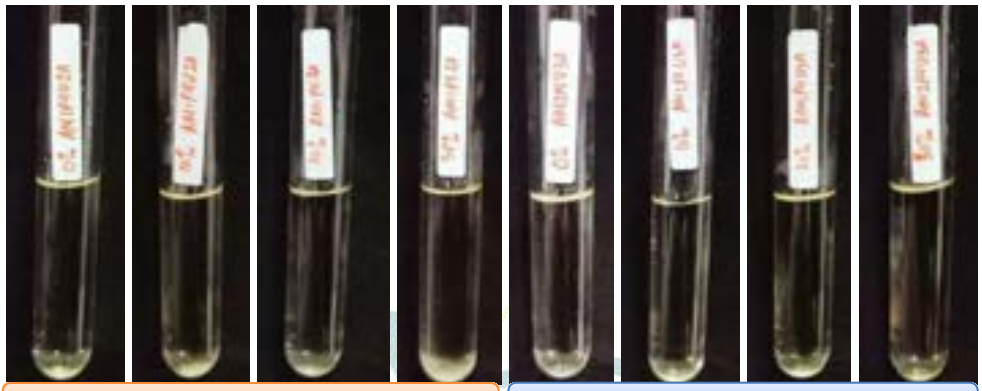
Lampiran 11. Hasil Uji Biodegradasi *Rhodamine B*



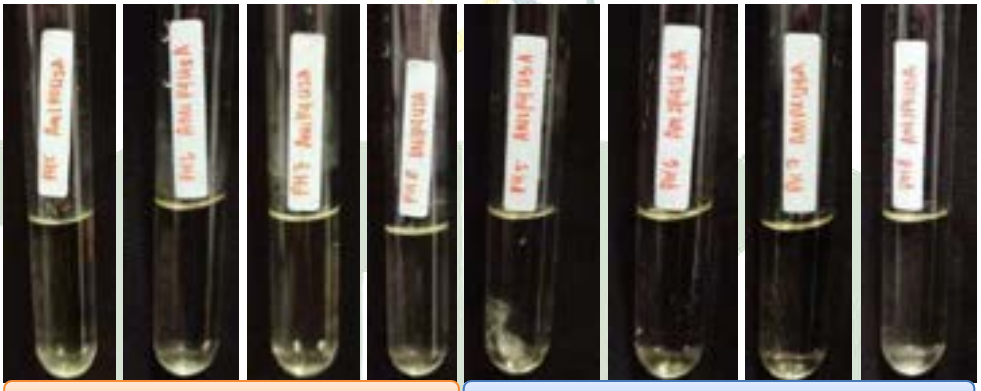


Lampiran 12. Uji Ketahanan Fisik

1. Ketahanan Salinitas



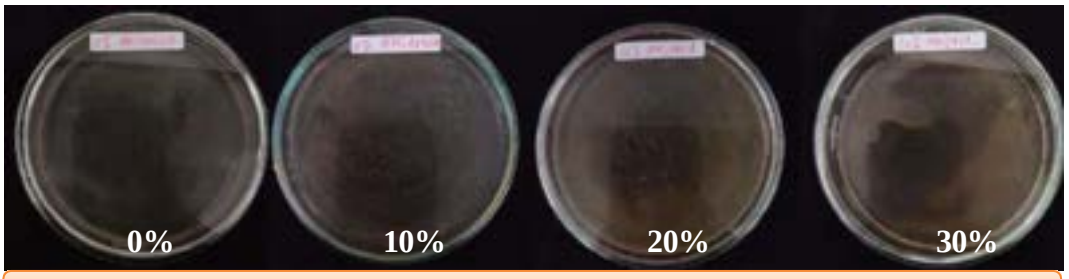
2. Ketahanan pH



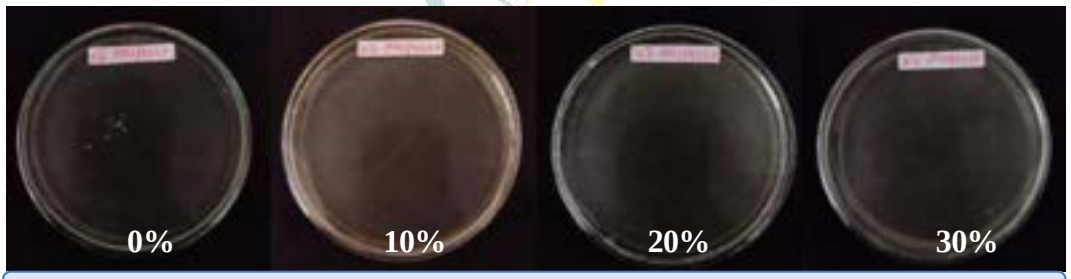
3. Pengukuran pH pada Media NB sebagai media Tanam Uji Ketahanan pH

4. Hasil Tanam Ulang Isolat Bakteri dari Perlakuan Fisik

Perlakuan Salinitas

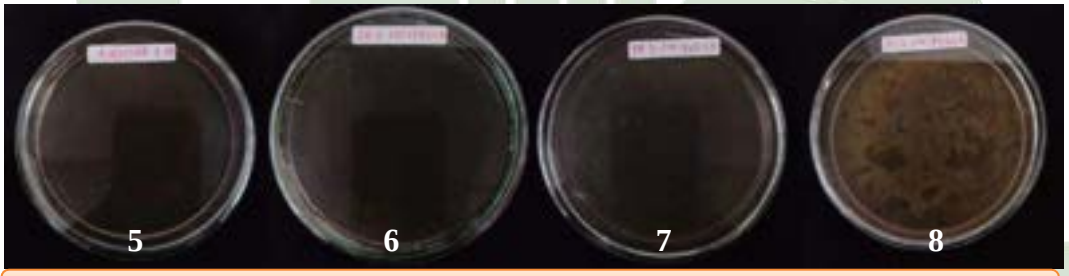


RA5

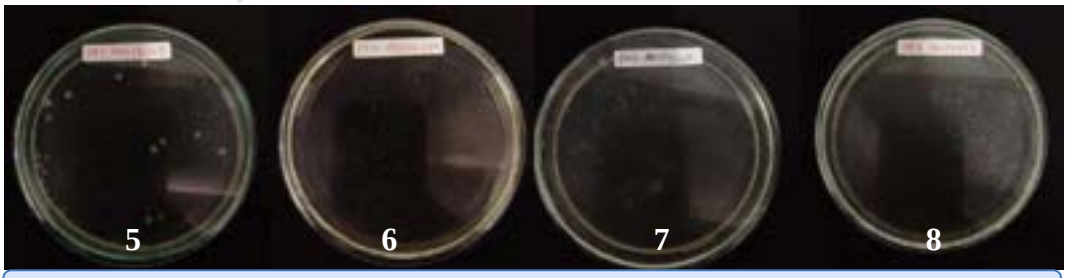


RA16

Perlakuan pH



RA5

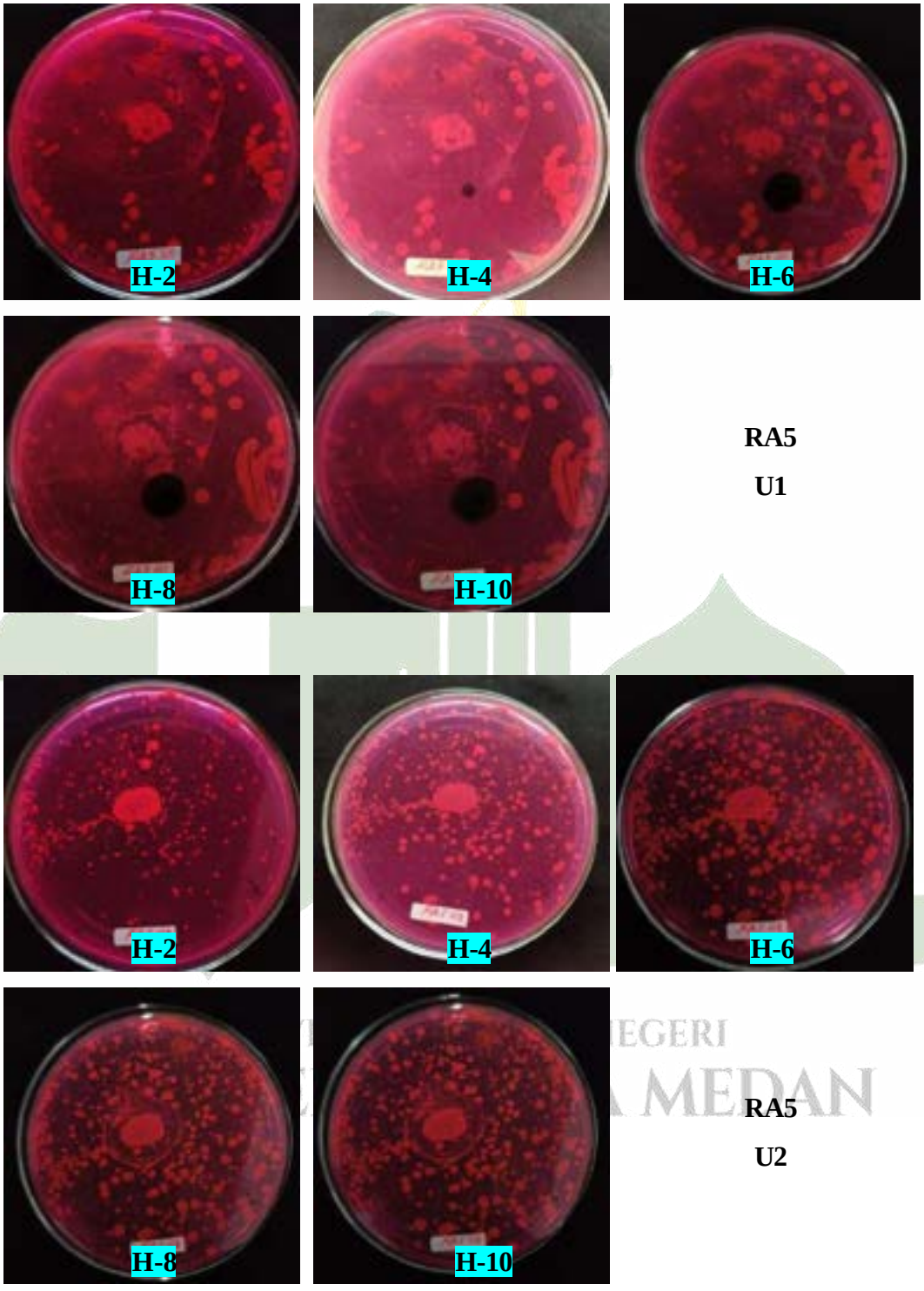


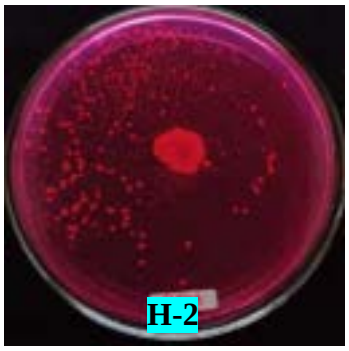
RA16

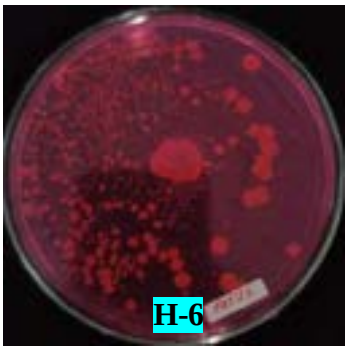
Lampiran 13. Konversi Kode Isolat

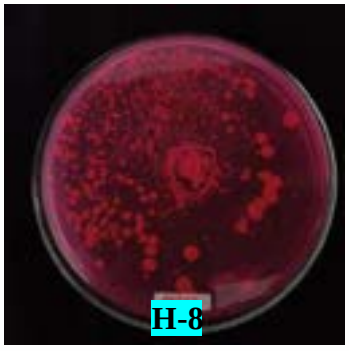
Kode Isolat Lama	Kode Isolat Baru
AM1P3U1A	RA1
AM1P4U1A	RA2
AM1P5U1A	RA3
AM1P3U2A	RA4
AM1P4U2A	RA5
AM1P4U3A	RA6
AM2P3U1A	RA7
AM2P5U1A	RA8
AM2P5U1B	RA9
AM2P3U2A	RA10
AM2P3U2B	RA11
AM2P4U2A	RA12
AM2P4U2B	RA13
AM2P3U3A	RA14
AM2P3U3B	RA15
AM2P4U3A	RA16

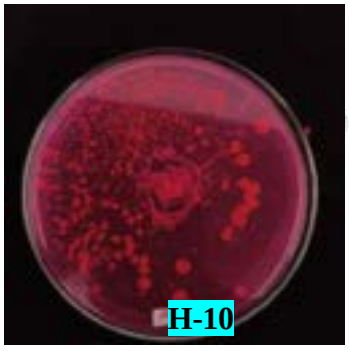
Lampiran 14. Uji Biodegradasi RhB oleh Bakteri Simbion Akar *A. marina*









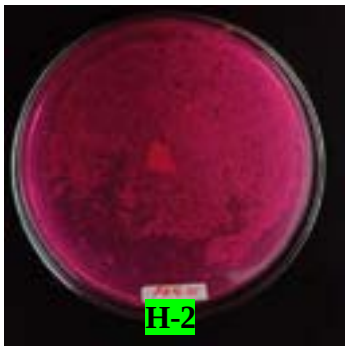


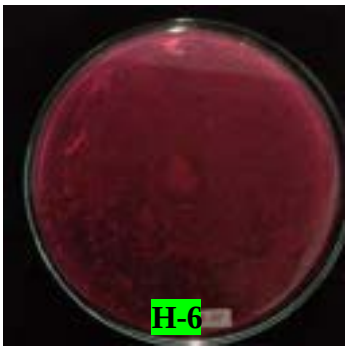
RA5

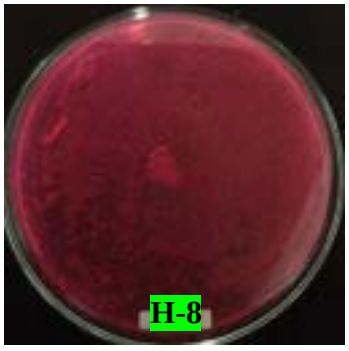
U3

RA5

U4

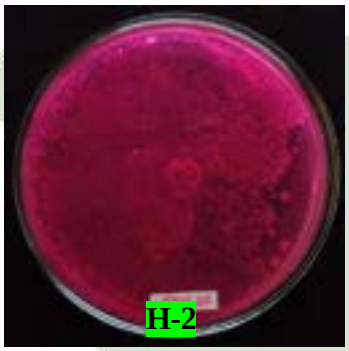


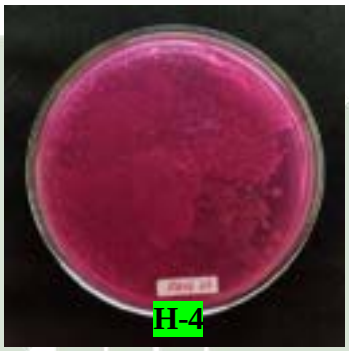


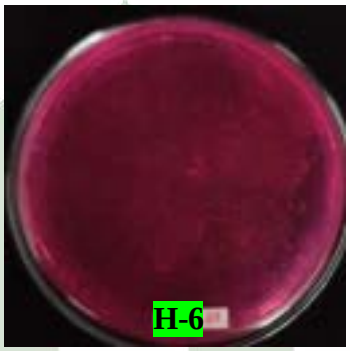


RA16

U1

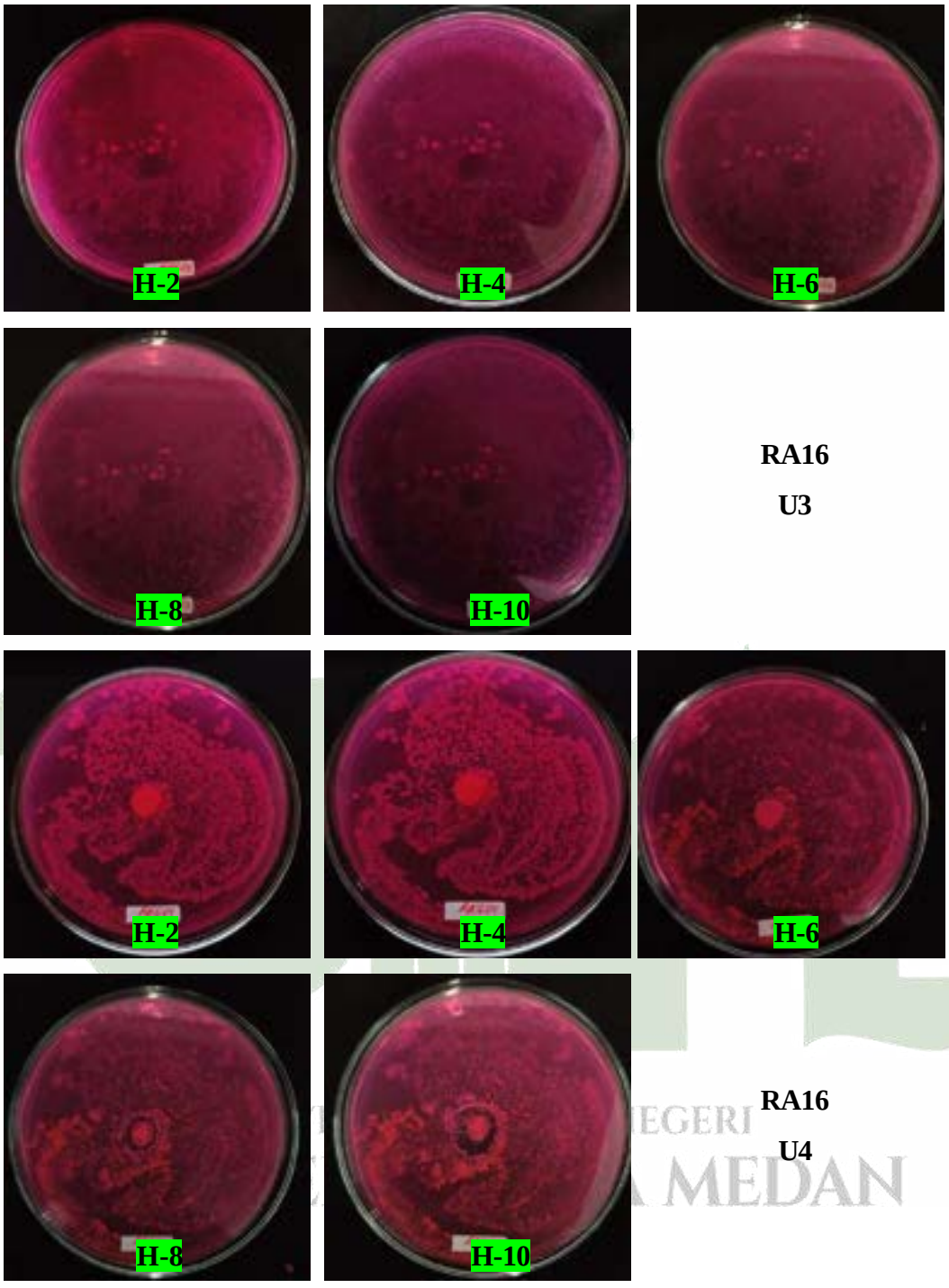






RA16

U2


H-2
H-4
H-6
H-8
H-10
RA16
U3
H-2
H-4
H-6
H-8
H-10
RA16
U4

Lampiran 15. Hasil Uji Biodegradasi *Rhodamine B*

Titik *Sampling*	Kode Isolat	Rata-rata Diameter Zona Hambat (mm)				
		Hari ke-2	Hari ke-4	Hari ke-6	Hari ke-8	Hari ke-10
1	RA1	-	-	-	-	-
	RA2	-	-	-	-	-
	RA3	-	-	-	-	-
	RA4	-	-	-	-	-
	RA5	-	0,075	0,3	1	1,25
	RA6	-	-	-	-	-
2	RA7	-	-	-	-	-
	RA8	-	-	-	-	-
	RA9	-	-	-	-	-
	RA10	-	-	-	-	-
	RA11	-	-	-	-	-
	RA12	-	-	-	-	-
	RA13	-	-	-	-	-
	RA14	-	-	-	-	-
	RA15	-	-	-	-	-
	RA16	-	-	0,4	0,7	0,9

Lampiran 16. Dokumentasi Kegiatan Selama Penelitian

1. Sterilisasi Air Laut, Cawan Petri, dan Tabung Reaksi


2. Pengambilan Sampel Akar *Avicennia marina*

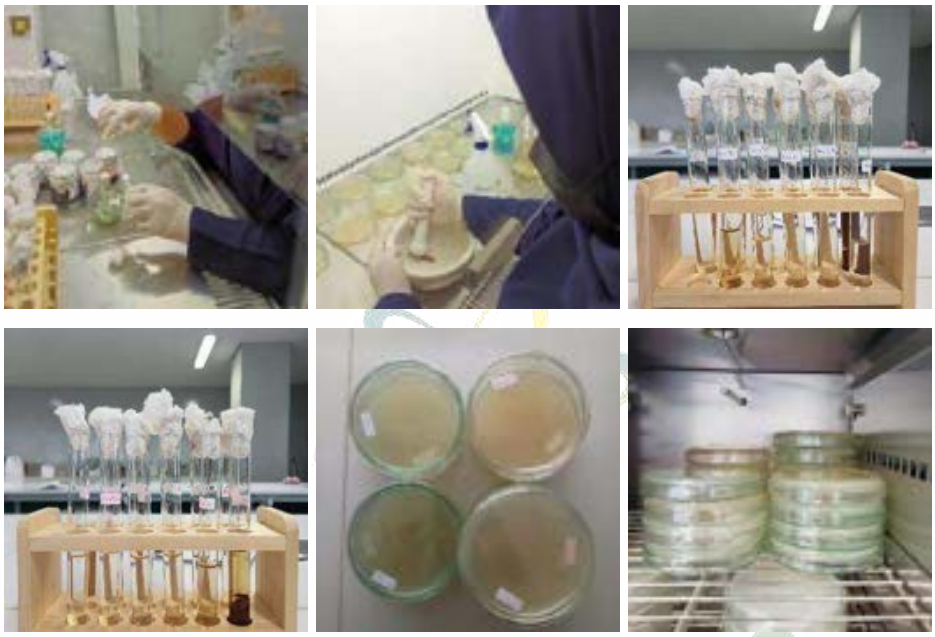





3. Penyaringan Air Laut dan Pembuatan Media



4. Pengenceran dan Isolasi Sampel Bakteri Simbion



5. Pemurnian Bakteri Simbion

6. Pembuatan Media MA + *Rhodamine B* 5ppm

7. Uji Biodegradasi *Rhodamine B* 5ppm

8. Uji Pewarnaan Gram dan Uji Biokimia

Standard ID

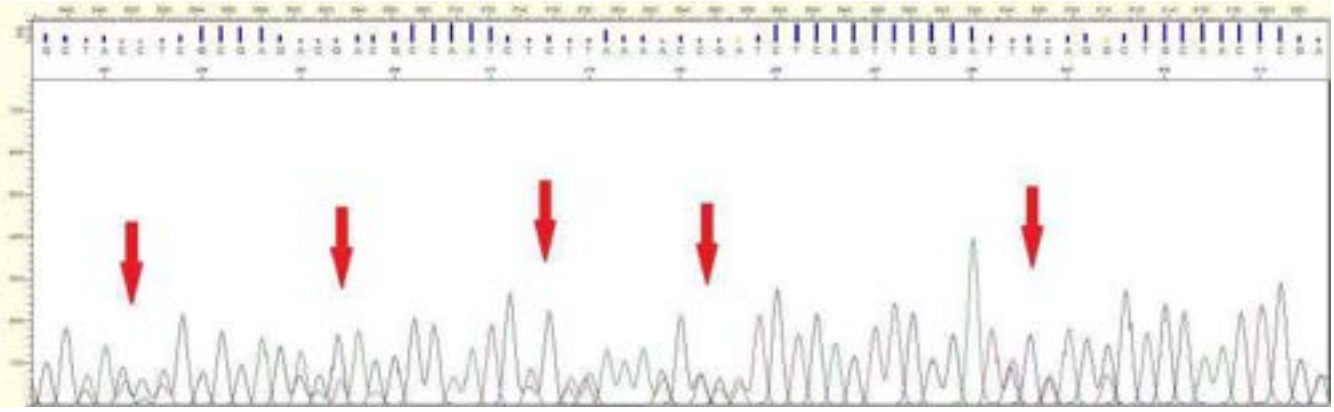
16S rRNA service report

Order Number : HC00614272
Sample name : AM2P4U3A

Information

Primer Information

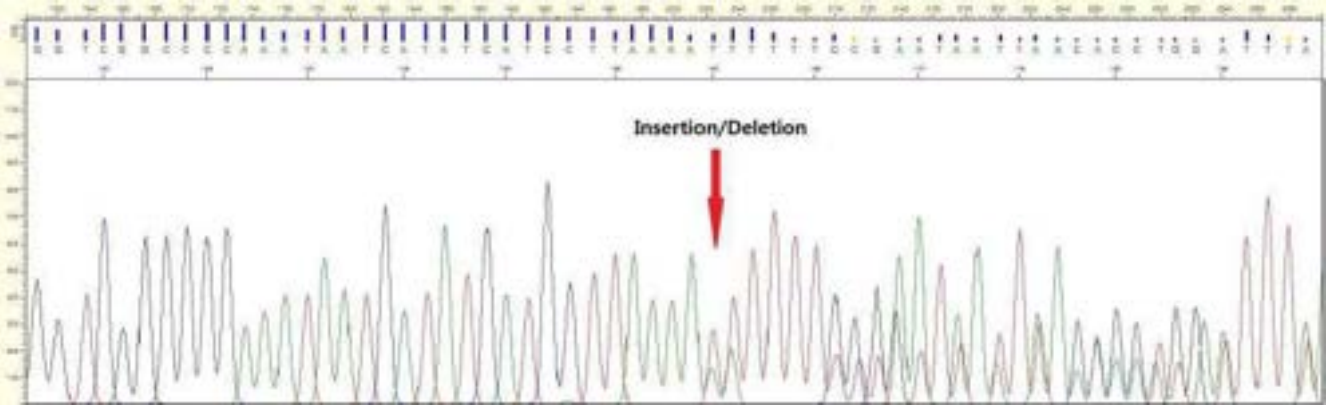
Sequencing Primer Name Primer Sequences	PCR Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'	27F 5' (AGA GTT TGA TCM TGG CTC AG) 3
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'	1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'



Mixed Template

Multiple sequencing peaks are observed at the same location when two or more strains were present in the template DNA.

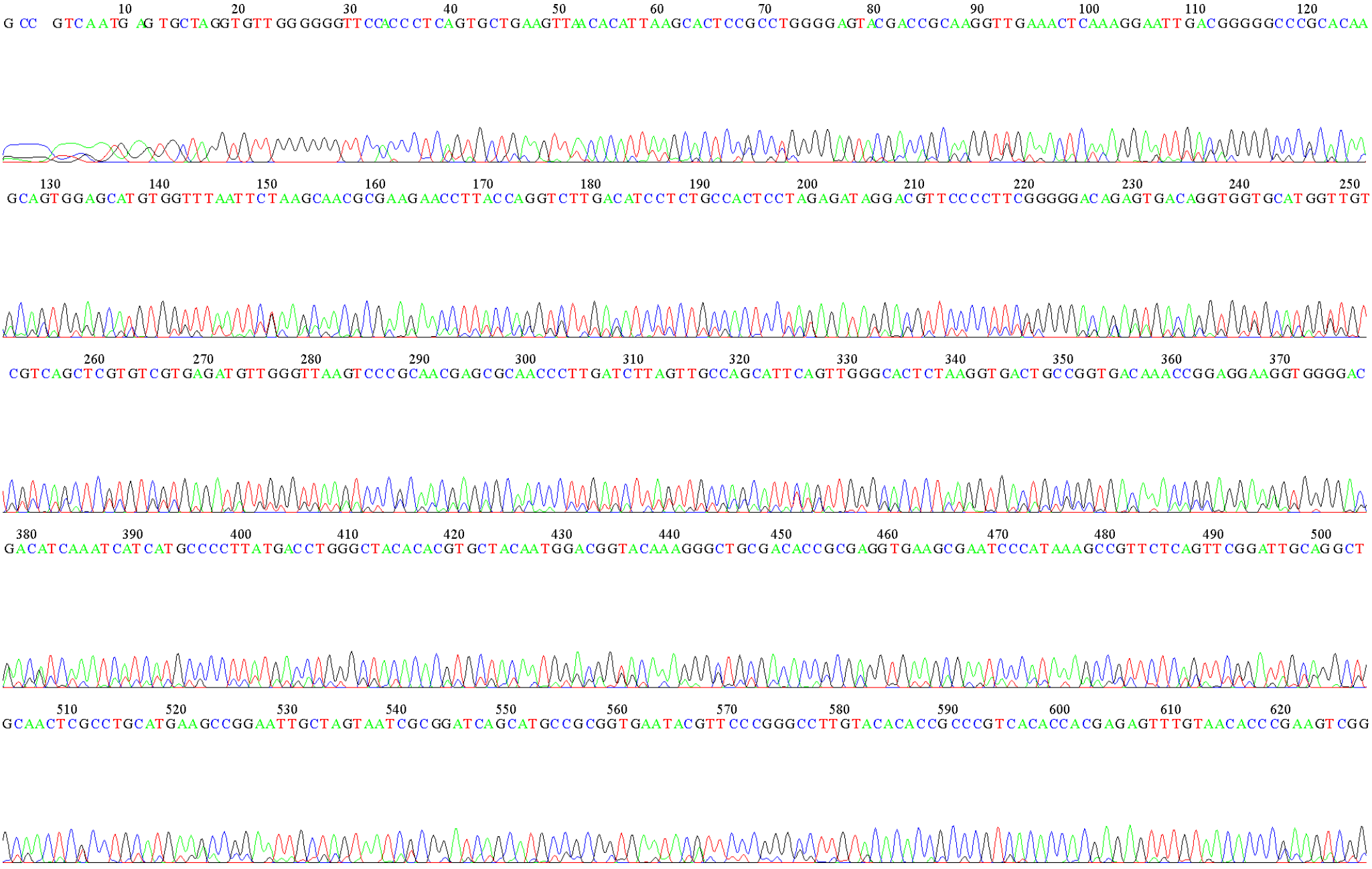
Troubleshooting: Prepare the template DNA from pure culture so that only one DNA template is present.



Insertion/Deletion

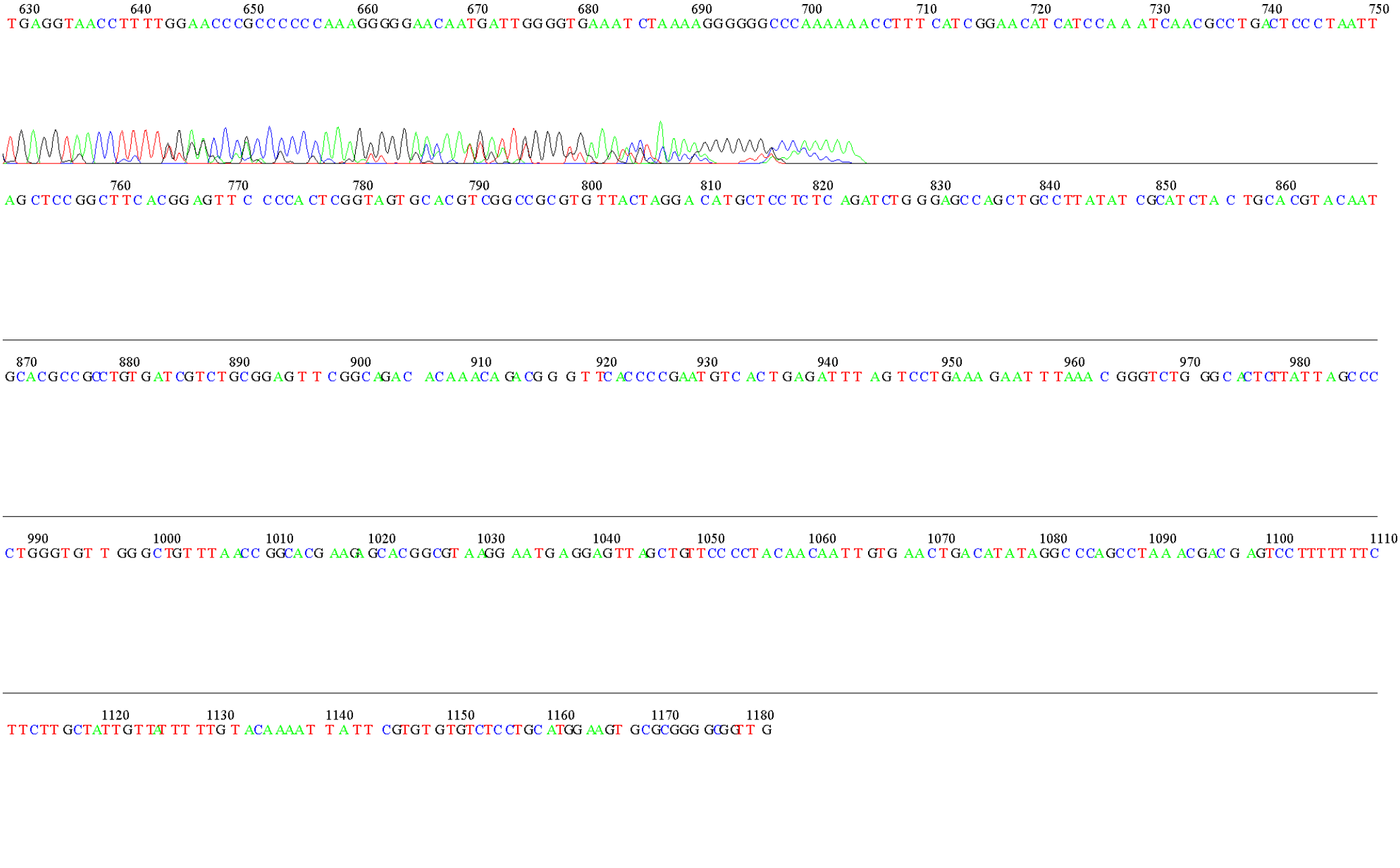
Multiple sequencing peaks are observed after certain position, when insertion/deletion is occurred.

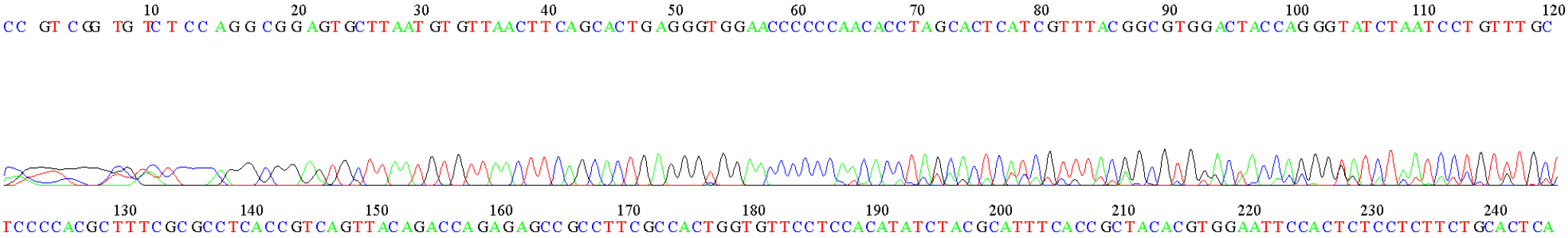
Troubleshooting: Cloning is recommended before sequencing.


10 20 30 40 50 60 70 80 90 100 110 120
G CC GTCAATG AG TGCTAGGTGTT GGGGGGTTCCACCCTCAGTGCTGAAGTTAACACATTAAGCACTCCGCCTGGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA
130 140 150 160 170 180 190 200 210 220 230 240 250
GCAGTGGAGCATGTGGTTTAATTCTAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGCCACTCCTAGAGATAGGACGTTCCCCTTCGGGGGACAGAGTGACAGGTGGTGCATGGTTGT
260 270 280 290 300 310 320 330 340 350 360 370
CGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCACTCTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGAC
380 390 400 410 420 430 440 450 460 470 480 490 500
GACATCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGACGGTACAAAGGGCTGCGACACCGCGAGGTGAAGCGAATCCCATAAAGCCGTTCTCAGTTCGGATTGCAGGCT
510 520 530 540 550 560 570 580 590 600 610 620
GCAACTCGCCTGCATGAAGCCGGAATTGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGTCGG

File: AM2P4U3A_785F.ab1 *Run Ended: 2022/12/22 5:46:46* *Signal G:1529 A:2000 C:3050 T:1957*

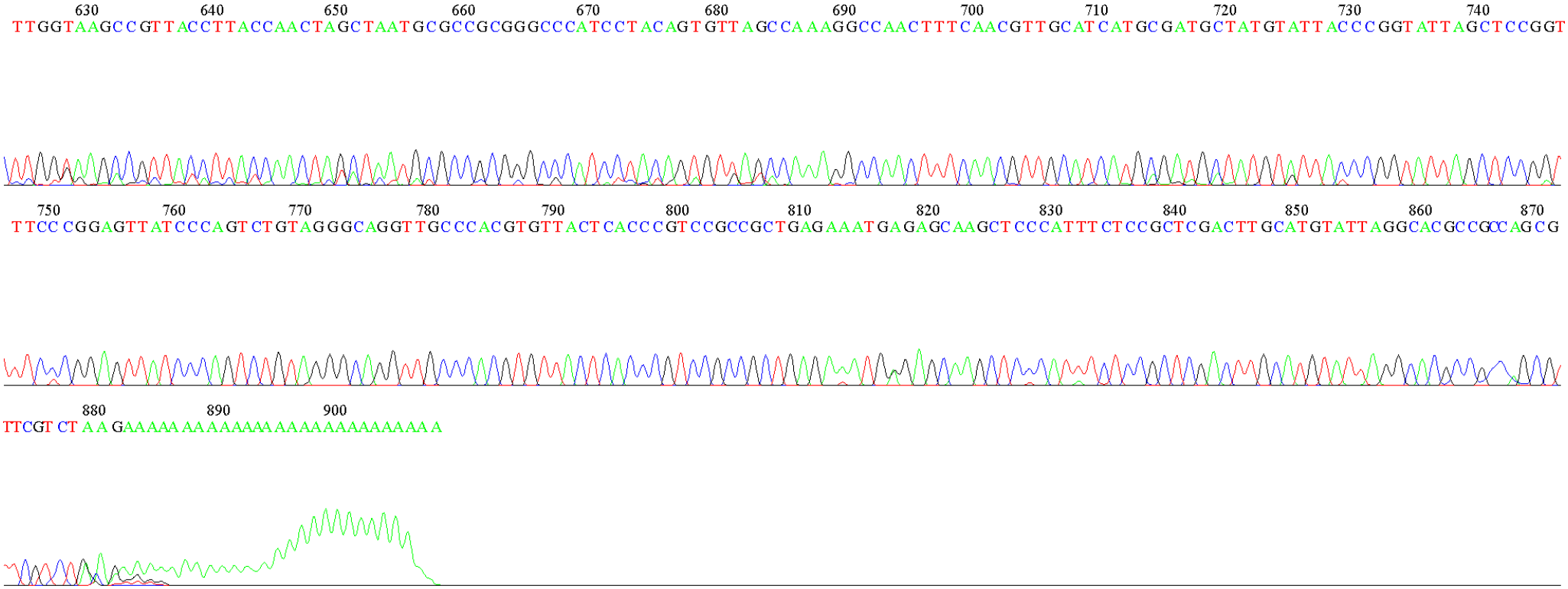
Sample: AM2P4U3A_785F *Lane: 7* *Base spacing: 16.6341* *1181 bases in 14335 scans* *Page 2 of 2*




10 20 30 40 50 60 70 80 90 100 110 120
CC GT C GG TG TC T CC AG G CG G AGT GCTT AAT GT GT T AACTT CA GCACT G AGGGT GG AACCCCCC AACACCT A GCACT CAT C GT T T AC GGC GT GG ACT AC CAG GG T AT CT AAT C CT GT T T GC
130 140 150 160 170 180 190 200 210 220 230 240
TCCCCACGCTTTCGC GCCTCACC GTCA GTT AC AGACCA GA GA GCC GCCTTC GCCACT GGT GTTCCTCCACAT AT CT AC GCATTT CACC GCT ACAC GT GGAATTCCACTCTCCTCTTCT GCACT CA
250 260 270 280 290 300 310 320 330 340 350 360 370
AGTCCCCCAGTTTCCAAT GGCCCTCCACGGTT GAGCC GT GGGCTTT CACATCACACTTAAGAGACC GCCT GC GC GC GCTTTAC GCCCAATAATTCC GGACAACGCTC GCCACCTACGTATTACC GC G
380 390 400 410 420 430 440 450 460 470 480 490
GCTGCTGGCACGT AGTTAGCC GT GGCTTTCT GGTTAGGTACC GTCAAGGTACC GCCCTATTC GAACGGT ACTT GTT CTTCCCTAACAACAGAGCTTTACGACCC GAAGGCCTTC GTCACT CACGC GG
500 510 520 530 540 550 560 570 580 590 600 610 620
C GTT GCT CC GT CAGACTTTC GT CCATT GC GGAAGATT CCCTACT GCT GCCTCCCGT AGGAGT CT GGGCC GT GT CT CAGT CCCAGT GT GGCC GATCACCCTCTCAGGTC GGCT AC GCAT C GT GGCC

File: AM2P4U3A_907R.ab1 *Run Ended: 2022/12/22 5:46:46* *Signal G:1747 A:2163 C:4863 T:3132*

Sample: AM2P4U3A_907R *Lane: 5* *Base spacing: 16.78715* *909 bases in 14039 scans* *Page 2 of 2*



BERGEY'S MANUAL OF

Systematic Bacteriology

Second Edition

Volume Three

The *Firmicutes*

Paul De Vos, George M. Garrity, Dorothy Jones, Noel R. Krieg, Wolfgang Ludwig, Fred A. Rainey, Karl-Heinz Schleifer and William B. Whitman

EDITORS, VOLUME THREE

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MANAGING EDITOR

Springer

Genus I. **Bacillus** Cohn 1872, 174[AL]

NIALL A. LOGAN AND PAUL DE VOS

Ba.cil′lus. N.L. masc. n. *Bacillus* a rodlet.

Cells rod-shaped, straight or slightly curved, occurring singly and in pairs, some in chains, and occasionally as long filaments. Endospores are formed, no more than one to a cell; these spores are very resistant to many adverse conditions. Gram-positive, or Gram-positive only in early stages of growth, or Gram-negative. A *meso*-DAP direct murein cross-linkage type is commonest, but L-Lys-D-Glu, Orn-D-Glu and L-Orn-D-Asp have occasionally been reported. Motile by means of peritrichous or degenerately peritrichous flagella, or nonmotile. Aerobes or facultative anaerobes, but a few species are described as strictly anaerobic. The terminal electron acceptor is oxygen, replaceable by alternatives in some species. Most species will grow on routine media such as nutrient agar and blood agar. Colony morphology and size very variable between and within species. A wide diversity of physiological abilities is exhibited, ranging from psychrophilic to thermophilic, and acidophilic to alkaliphilic; some strains are salt tolerant and some are halophilic. Catalase is produced by most species. Oxidase-positive or -negative. Chemo-organotrophic; two species are facultative chemolithotrophs: prototrophs to auxotrophs requiring several growth factors. Mostly isolated from soil, or from environments that may have been contaminated directly or indirectly by soil, but also found in water, food and clinical specimens. The resistance of the spores to heat, radiation, disinfectants, and desiccation results in species being troublesome contaminants in operating rooms, on surgical dressings, in pharmaceutical products and in foods. Most species have little or no pathogenic potential and are rarely associated with disease in humans or other animals; an exception is *Bacillus anthracis*, the agent of anthrax; several other species may cause food poisoning and opportunistic infections, and strains of *Bacillus thuringiensis* are pathogenic to invertebrates.

DNA G + C content (mol%): 32–66 (**T_m**).

Type species: **Bacillus subtilis** Cohn 1872, 174[AL].

Further descriptive information

Phylogeny. A phylogenetic tree, based on 16S rDNA sequences, is shown in Figure 8. The tree includes 142 named Bacillus species as listed in this chapter (but excludes *Bacillus laevolacticus* and *Bacillus tequilensis). Bacillus tusciae* and *Bacillus schlegelii* lie at the edge of the tree, and their respective closest neighbors, on the basis of 16S rDNA gene sequence comparisons, are an unknown *Alicyclobacillus* species and *Aneurinibacillus*.

It is well known that 16S rDNA sequences do not always allow species to be discriminated, and that DNA–DNA hybridizations may be needed for this. However, sequences of other genes (the so-called core genes) may be more appropriate for discriminating these relatively recent branchings of the evolutionary tree that correspond to bacterial species. The ad hoc committee for the re-evaluation of the species definition in bacteriology (Stackebrandt et al., 2002) advised that genetic differences of the so-called core genes should be explored in order to come to a finer "bacterial species concept" in the future. The groupings (phylogenetic trees) that are obtained from comparisons either of sequences of individual core genes, or of concatenated gene sequences of several core genes, need to be validated against the phylogenetic species concept (Wayne et al., 1987). Recent data (Wang et al., 2007a) clearly show that in the *Bacillus subtilis* group, within which species delineation is very difficult, core genes such as *gyrB* allow differentiation on a genetic basis. A debate began recently concerning the impact of these new findings of genome analysis on bacterial taxonomy (Buckley and Roberts, 2007). Analysis of whole-genome sequences showed that about 80% of an individual genome may be shared by all pathogenic isolates of *Streptococcus agalactiae* (Tettelin et al., 2005), indicating that in closely related strains belonging to the same species, at least, a vast amount of the genetic information is shared. The interested reader is referred to the literature (e.g., Kunin et al., 2005, 2007; Dagan and Martin, 2006).

Cell morphology. *Bacillus* cells may occur singly and in pairs, in chains (which may be of great length), and as filaments. Trichome-forming "*Arthromitus*" strains from sow bug or wood louse (*Porcellio scaber*) guts, with endospore-forming filaments over 100 µm long and up to 180 cells per filament in animals cultivated in darkness, have been identified as *Bacillus cereus* (Jorgensen et al., 1997) and similar filamentous organisms have been isolated from moths, roaches and termites (Margulis et al., 1998; see *Habitats*, below). The rod-shaped cells of *Bacillus* species are usually round-ended, but the cells of members of the *Bacillus cereus* group have often been described as squared. Cell diameters range from 0.4 to 1.8 µm and lengths from 0.9 to 10.0 µm, but the cells of a particular strain are usually quite regular in size, and individual species normally have dimensions within fairly narrow limits. For example, cells of *Bacillus pumilus* are typically 0.6–0.7 by 2.0–3.0 µm, while those of *Bacillus megaterium* are usually 1.2–1.5 by 2.0–5.0 µm. Pleomorphism, showing as cells and filaments with swollen regions, and entirely swollen cells, may be observed in cultures grown in suboptimal conditions; this is seen, for example, in cultures of *Bacillus fumarioli* grown on relatively rich media (Logan et al., 2000), and such stressed cultures sporulate poorly. *Bacillus* cytoplasm may stain uniformly or be vacuolate; vacuolation (the presence of inclusions is visible by phase-contrast microscopy as areas less refractive than spores, and in Gram-stained preparations by unstained globules) is enhanced in some species (*Bacillus cereus* and *Bacillus megaterium*, for example) by cultivation on an agar medium containing a fermentable carbohydrate such as glucose, so that copious storage material is produced.

Sporangial morphologies are characteristic of species, and so often valuable in identification (see *Life cycle*, below), but an individual strain may show some variation and produce, for example, both oval and spherical spores. The commonest spore shape is ellipsoidal or oval, but shapes range from frankly cylindrical through ellipsoidal to spherical, and irregular forms such as kidney- or banana-shaped spores may be seen in some species. The position of the spore is also characteristic; the most

pseudalcaliphilus, *Bacillus pseudofirmus*, and *Bacillus vedderi*. The two closely related species *Bacillus cohnii* (alkaliphilic) and *Bacillus halmapalus* (alkalitolerant) do not belong in this phylogenetic group, and lie nearer to *Bacillus cereus:* the cross-linkage of *Bacillus cohnii* is L-Orn-D-Asp (Spanka and Fritze, 1993), while *Bacillus halmapalus* has been shown to lack DAP (Nielsen et al., 1994).

Other cell-wall polymers have attracted less attention than murein, and the small amounts of reported data for a few strains do not allow the taxonomic values, if any, of these components to be recognized; the subject has been reviewed by Naumova and Shashkov (1997). Teichoic acids have been found in *Bacillus coagulans*, *Bacillus licheniformis* and *Bacillus subtilis*, and teichuronic acids have been found in *Bacillus licheniformis*, *Bacillus megaterium* and *Bacillus subtilis*. Aono and Ohtani (1990) and Aono et al. (1993) found the acidic polymers teichuronic acid and teichuronopeptide in the cell walls of alkaliphilic *Bacillus* strains and suggested that these components might be important in alkalophily as mutants deficient in them grew poorly at high pH. Fox et al. (1998) described the use of gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry in the investigation of teichoic acids and teichuronic acids in *Bacillus* species.

Naumova and Shashkov (1997) also reviewed studies on sugar-phosphate polymers (found in *Bacillus pumilus* and *Bacillus subtilis*) and anionic polysaccharides (found in *Bacillus cereus* and *Bacillus megaterium*), but again the information is too sparse to reveal any taxonomic implications.

Capsules. Gram-positive bacteria may produce two kinds of capsule, composed of polyglutamic acid or polysaccharide, but their production by *Bacillus* species has not appeared to be of much taxonomic value. Although most *Bacillus subtilis* strains do not produce significant capsular material in the laboratory, the genome sequence of strain 168 indicates that this organism possesses the genes encoding both types of capsule (Foster and Popham, 2002). The production of poly-γ-glutamic acid by "*Bacillus subtilis* var. *natto*" during the stationary phase of growth is economically important in the manufacture of the fermented soybean product natto (Ueda, 1989).

The poly-γ-D-glutamic acid capsule of *Bacillus anthracis* is encoded by the three plasmid pXO2 genes *capA*, *capB*, and *capC*, and it is an important virulence factor for this organism as non-capsulate strains are avirulent (see *Pathogenicity*, below). The sequences of the enzymes encoded by the three genes suggest that they are membrane-associated (Mock and Fouet, 2001). The capsule is produced *in vivo* and when grown in appropriate conditions in the laboratory (see *Procedures for testing special characters*, below). *Bacillus anthracis* is a member of the *Bacillus cereus* group of closely related species, but none of the species besides *Bacillus anthracis* appears to produce this capsule. Although homologs of *Bacillus anthracis* virulence plasmid pXO1 genes were found in half of a set of 19 other members of the *Bacillus cereus* group in hybridization experiments, few pXO2 genes were found that hybridized with genomic DNA from the 19 *Bacillus cereus* group strains (Read et al., 2003). The capsule of *Bacillus anthracis* was reviewed by Mock and Fouet (2001). Other *Bacillus* species, outside the *Bacillus cereus* group, are known to produce poly-γ-glutamic acid. Synthesis by *Bacillus licheniformis* is carried out by a membrane-associated complex that catalyzes glutamic acid racemization, polymerization, and membrane translocation (Gardner and Troy, 1979); as with "*Bacillus subtilis* var. *natto*", production of the capsular material is induced during the stationary phase (Foster and Popham, 2002). While D-glutamic acid is the predominant stereoisomer incorporated into the polymer, the ratio of D- and L-glutamic acids may vary according to the rate at which D-glutamic acid is being formed in the *Bacillus subtilis* cell (Aschiuchi et al., 1999), but in *Bacillus licheniformis* two glutamyl polypeptides are formed, one of each isomer, and the ratio is influenced by the concentrations of certain metal ions in the growth medium (Thorne, 1993). *Bacillus megaterium* is also known to produce poly-γ-glutamic acid, and can form a capsule comprising both polysaccharide and polypeptide, with the former at the cell poles and equators and the latter located laterally (Guex-Holzer and Tomcsik, 1956). Applications of bacterial poly-γ-glutamic acid are reviewed by Shih and Van (2001).

Carbohydrate polymers are formed by several *Bacillus* species, dextrans and levans being produced extracellularly by *Bacillus licheniformis* and *Bacillus subtilis* from sucrose (Claus and Berkeley, 1986), but true polysaccharide capsules have not been reported for *Bacillus subtilis*. The *Bacillus subtilis* genome contains two operons and some additional genes that show great similarity to capsule synthesis loci in *Staphylococcus aureus* and *Streptococcus pneumoniae*, but it is not known if they are truly genes for capsule synthesis (Foster and Popham, 2002). The extracellular polysaccharides of *Bacillus licheniformis* and *Bacillus subtilis* are of economic importance in the spoilage of bread and alcoholic beverages by "ropiness." Analysis of the polysaccharide of a *Bacillus licheniformis* from ropy cider found that it wasaheteropolymercontainingover80%mannose(Larpinetal., 2002). Aubert (1951) assumed that a heteropolysaccharide of D-glucose, D-galactose and D-ribose extractable from *Bacillus megaterium* KM with hot water was probably capsular material, but Cassity and Kolodziej (1984) concluded that a heteropolysaccharide of D-glucose, D-xylose, D-galactose, and L-arabinose produced by another strain of this species was intracellular and that it was used as a source of carbon and energy during sporulation. Several polysaccharides from *Bacillus* strains have been found to cross-react with antisera to capsules from other genera: *Bacillus mycoides* with *Streptococcus pneumoniae* type III, and *Bacillus pumilus* with *Haemophilus influenzae* type b and with *Neisseria meningitidis* group A (Myerowitz et al., 1973).

Flagella. Many species of *Bacillus* are motile by means of peritrichous flagella, which are not usually numerous and may be very few in number. Flagellation has not been considered a particularly useful taxonomic character for the genus, but the presence or absence of motility continues to be indicated in most species descriptions, and it is of some value in identification. For example, *Bacillus anthracis* and *Bacillus mycoides* are nonmotile, while most *Bacillus cereus* strains are motile. The flagella of *Bacillus thuringiensis* may bind to insect cells and be important in virulence (Zhang et al., 1995). The value of H-antigens in the typing of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus sphaericus*, and other aspects of *Bacillus* flagellar antigens, are discussed in Antigens and vaccines, below. The flagella of *Bacillus subtilis* are well characterized, and reviews may be found in Sonenshein et al. (1993) and in Aizawa et al. (2002).

S-layers. Surface or S-layers are two-dimensional arrays composed of protein or glycoprotein molecules. The S-layer proteins assemble themselves into very stable structures which have oblique, square or hexagonal lattice symmetries, are 5–25 nm thick, and contain pores of 2–8 nm in diameter (Sleytr et al., 2001). The phylogenetic origins of the S-layers of some *Bacillus cereus* group strains was investigated by Mignot et al. (2001), and the possession of an S-layer was found to be largely restricted to a genetically clustered subgroup of clinical and insect isolates, suggesting a role in pathogenicity and the influence of ecological pressures to maintain the layer. It has been shown that the S-layer of *Bacillus cereus* is involved in the adhesion of the organism to host cell molecules, and polymorphonuclear leukocytes, as well as enhancing the organism's radiation resistance (Kotiranta et al., 2000). However, S-layers are apparently of no value as taxonomic markers, as in some species, including *Bacillus cereus*, their presence is strain-dependent (Kotiranta et al., 1998; Sleytr et al., 2001). The S-layer of *Bacillus anthracis* is reviewed by Mock and Fouet (2001).

Colony characteristics. *Bacillus* species show a very wide range of colonial morphologies, both within and between species, and of course medium composition and other incubation conditions have a strong influence. Despite this diversity, however, *Bacillus* colonies on routine media are not generally difficult to recognize. Some species have characteristic yet seemingly infinitely variable colonial morphologies: colonies of *Bacillus cereus* and relatives are very variable, but readily recognized (Figure 9a, b, h): they are characteristically large (2–7 mm in diameter) and vary in shape from circular to irregular, with entire to undulate, crenate or fimbriate edges; they have matt or granular textures, but smooth and moist colonies are not uncommon. Although colonies of *Bacillus anthracis* and *Bacillus cereus* can be similar in appearance, those of the former are generally smaller, non-hemolytic, may show more spiking or tailing along the lines of inoculation streaks, and are very tenacious as compared with the usually more butyrous consistency of *Bacillus cereus* and *Bacillus thuringiensis* colonies, so that they may be pulled into standing peaks with a loop. The colonies of *Bacillus mycoides* differ from those of other members of the *Bacillus cereus* group; they are characteristically rhizoid or hairy-looking and adherent, and they readily cover the whole agar surface (Figure 9d).

The colonies of other species vary from moist and glossy (Figure 9c, e, f) through granular to wrinkled (Figure 9h); shapes vary from round to irregular, sometimes spreading, with entire through undulate or crenate to fimbriate edges. After 24–48 h incubation, colonial sizes of mesophilic strains typically range from 1 to 5 mm; color commonly ranges from buff or creamy-gray to off-white, but occasional strains may produce black, brown, orange, pink or yellow pigments; such pigmentation tends to be characteristic of species or subspecies. Elevations range from effuse through raised to convex. Consistency is usually butyrous, but mucoid and dry, adherent colonies are not uncommon. Hemolysis may be absent, slight or marked, partial or complete. *Bacillus subtilis* (Figure 9g) and *Bacillus licheniformis* produce similar colonies which are exceptionally variable in appearance and often appear to be mixed cultures – the colonies are irregular in shape and of moderate (2–4 mm) diameter, and range in consistency from moist and butyrous or mucoid (with margins varying from undulate to fimbriate), through membranous with an underlying mucoid matrix (with or without mucoid beading at the surface), to a rough and crusty appearance as they dry. The "licheniform" colonies of *Bacillus licheniformis* tend to be quite adherent. Rotating and migrating microcolonies (Figure 9i), which may show spreading growth (the V morphotype, see below), were observed macroscopically in about 13% of strains received as *Bacillus circulans* (Logan et al., 1985) but this very heterogeneous species has undergone radical taxonomic revision, and organisms producing motile microcolonies are now allocated to *Paenibacillus cookii*, *Paenibacillus glucanolyticus*, *Paenibacillus lautus*, and some unidentified *Paenibacillus* species (Alexander and Priest, 1989; Logan et al., 2004a). Most of the colonial morphologies illustrated here are shown in color by Logan and Turnbull (2003).

Matsushita et al. (1998, 1999) have constructed a mathematical model to explain some of the morphological variation

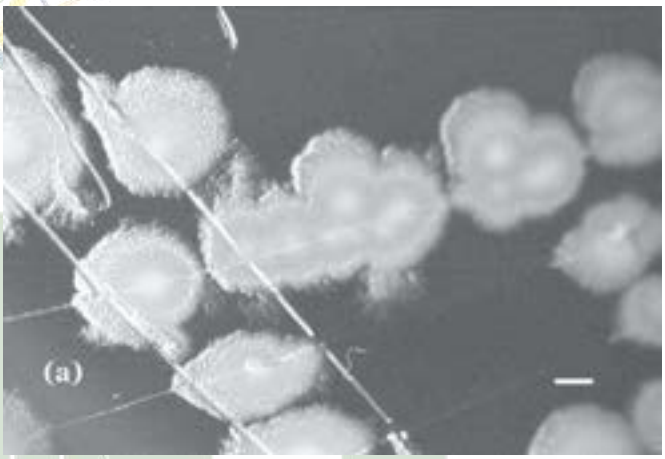

FIGURE 9. Colonies of endospore-forming bacteria on blood agar [parts (a)–(c), (e)–(f), (h)] and nutrient agar [parts (d), (g), (i)] after 24–36 h at 37 °C. These figures illustrate some of the diversity of colonial appearance within the genus, but the appearances shown should not be regarded as necessarily typical of the species illustrated. Bars for parts (a)–(f) and (h)–(i) = 2 mm; bar for (g) = 4 mm. (a) *Bacillus anthracis*: circular to irregular colonies with entire to undulate, crenate and fimbriate edges, and granular surface textures; (b) *Bacillus cereus*: irregular, with undulate, crenate and fimbriate edges, and matt or granular textures; (c) *Bacillus megaterium*: glossy, round to irregular colonies with entire to undulate margins; (d) *Bacillus mycoides*: rhizoid or hairy-looking, adherent colonies which may readily cover the whole agar surface; (e) *Bacillus pumilus*: wrinkled, irregular colonies with undulate margins; (f) *Bacillus sphaericus*: smooth, glossy, round to irregular colonies with entire to undulate margins; (g) *Bacillus subtilis*: irregular colonies that may give the appearance of a mixed culture. They range in consistency from moist through butyrous or mucoid to membranous, with an underlying mucoid matrix (with or without mucoid beading at the surface), and become rough and crusty in appearance as they dry. Margins vary from undulate to fimbriate; (h) *Bacillus thuringiensis*: circular to irregular colonies with entire or undulate edges, and matt to granular surface textures; (i) Motile, spreading microcolonies sometimes seen in strains that were previously assigned to *Bacillus circulans*, but which are now usually allocated to *Paenibacillus* species (see text). Photographs prepared by N. A. Logan.

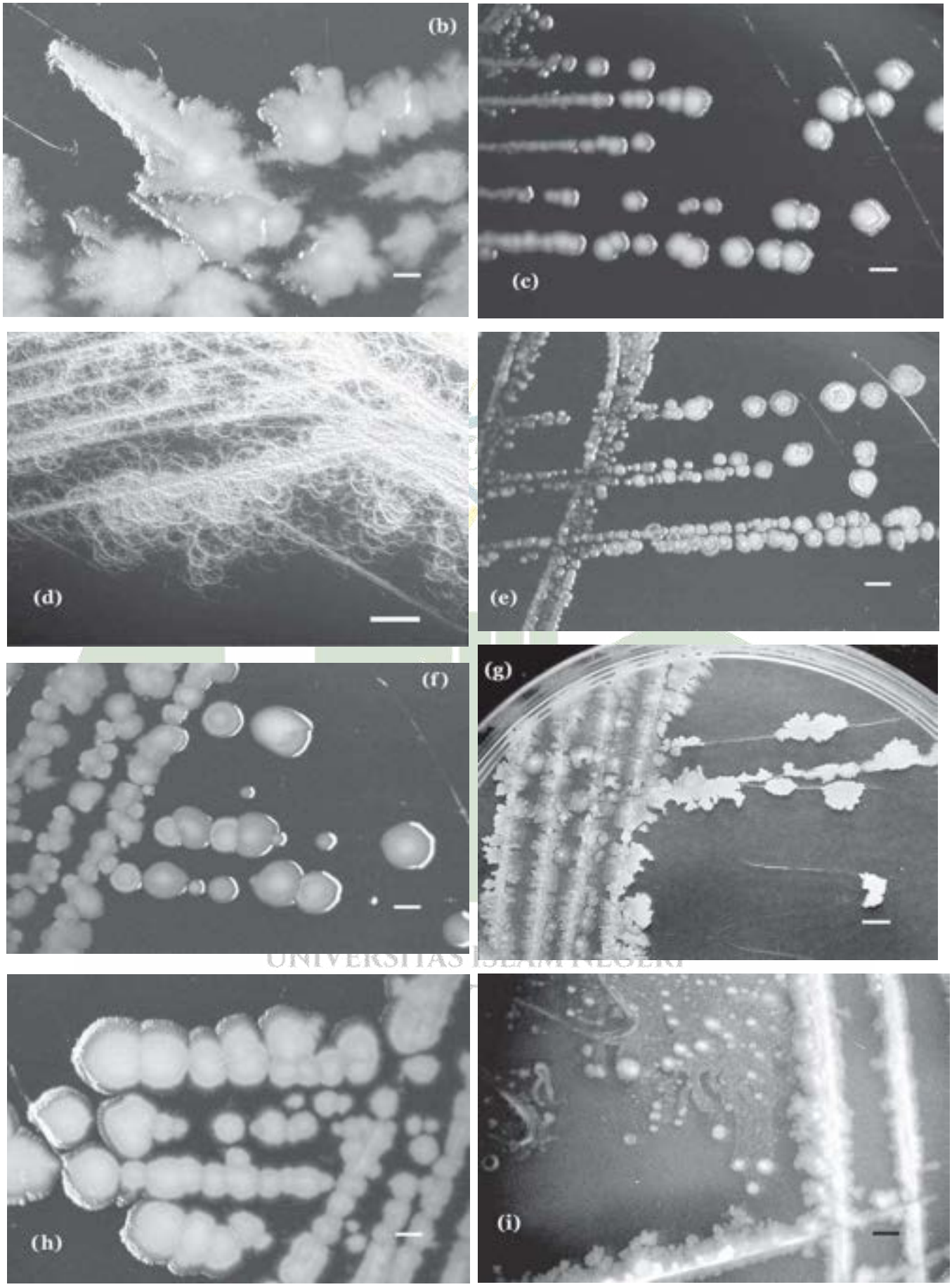

FIGURE 9. (continued)

with a strain of *Klebsiella terrigena* was found to increase nitrogen fixation by the latter, probably owing to the protection of nitrogenase by the phenolic compounds it excreted (Zlotnikov et al., 2001); a *Bacillus cereus* strain was found to stimulate nodulation in legumes, so enhancing nitrogen fixation by bradyrhizobia (Vessey and Buss, 2002).

Little comprehensive information is available on the vitamin requirements of individual *Bacillus* species. Many do not require such growth factors, but yeast extract will often stimulate better growth. Adams and Stokes (1968) studied the requirements of the psychrophiles *Bacillus insolitus* and *Bacillus psychrosaccharolyticus:* the former required biotin and thiamine, while the latter needed niacin and thiamine, and biotin was essential or stimulator, depending upon the strain. Among spherical-spored species, *Bacillus neidei* and *Bacillus sphaericus* require both biotin and thiamin for growth, but *Bacillus pycnus* does not. In the presence of molybdate, *Bacillus niacini* can use nicotinate (niacin) as sole source of carbon, nitrogen and energy. *Bacillus sporothermodurans* and *Bacillus subterraneus* require biotin and thiamin for growth, but neither require cystine. For some species, such as *Bacillus thermoamylovorans*, vitamins and nucleic acid derivatives will stimulate growth, but are not essential.

Growth temperature ranges vary appreciably between the strains of species, and maxima and minima may be extended beyond the usual limits of a species for strains found in unusually hot or cold environments. Isolates of *Bacillus licheniformis* and *Bacillus megaterium* from an Antarctic geothermal lake, for example, were found to have maxima of 68 °C and 63 °C, 13 °C and 18 °C, respectively, higher than the previously published limits for these species (Llarch et al., 1997). The vast majority of established species are mesophiles, with optima between 25 °C and 40 °C and typically around 30 °C, minima in the range 5–20 °C, and maxima of 35–55 °C. Several species, *Bacillus coagulans, Bacillus fumarioli, Bacillus infernus, Bacillus methanolicus, Bacillus okuhidensis, Bacillus smithii, Bacillus thermoamylovorans* and *Bacillus tusciae*, have higher growth temperature optima, ranging from 40 °C to 55 °C and above, with minima in the range 25–40 °C and maxima of 55–65 °C, and may be regarded as only moderately thermophilic. With minimum temperatures for growth of 37 °C and above, optima in the range 55–70 °C and maxima of 65–75 °C, *Bacillus schlegelii* and *Bacillus thermocloacae* may be regarded as true thermophiles. *Bacillus psychrodurans, Bacillus psychrosaccharolyticus,* and *Bacillus psychrotolerans* grow and sporulate around 0 °C and have maximum growth temperatures between 30 °C and 35 °C, while *Bacillus insolitus*, with a maximum growth temperature of 25 °C, an optimum of 20 °C and a minimum below 0 °C, is a true psychophile. Growth temperature ranges and optima are given for most species in the *List of species of the genus*, below, and the differential characters of species with optimum temperatures of 50 °C and above are shown in Table 8.

Although aerobic growth has long been a defining character of members of the genus, some 20 species are facultatively anaerobic, and the definition was undermined by the discoveries of *Bacillus infernus* and *Bacillus arseniciselenatis*, which are strictly anaerobic (Boone et al., 1995; Switzer Blum et al., 1998). Nitrate respiration is a common property in the genus. Although *Bacillus subtilis* has long been regarded as a strict aerobe, which will like many *Bacillus* species, however, grow anaerobically using nitrate or nitrite as an electron acceptor, it has recently been shown to grow by fermentation in the absence of electron acceptors (Clements et al., 2002; Nakano and Zuber, 2002) (see *Metabolism* and *metabolic pathways*, below).

Survival. Spores are readily formed by strains of many species, but it is a mistake to assume that a primary culture or subculture in or on a routine growth medium will automatically yield spores if stored on the bench or in the incubator. *Bacillus* strains will not sporulate under all cultural conditions, and if conditions are not suitable for sporulation the culture may die (see *Life cycle*, above). Most strains will sporulate if grown for a few days on a routine, solid growth medium supplemented with 5 mg/l manganese sulfate; failure to sporulate on such a medium may be addressed by cultivating on a nutritionally weaker, manganese-supplemented, medium. Repeated subculture of a strain sometimes leads to the production of fewer spores or the complete loss of ability to sporulate; some strains, however, appear able to survive for long periods in refrigerated cultures, even though they have not sporulated.

It is best to grow the organism on nutrient agar containing manganese for a few days, and refrigerate when microscopy shows that most cells have sporulated. For most species sporulated cultures, sealed after incubation, can survive in a refrigerator for many years.

Metabolism and metabolic pathways. The majority of information on the metabolism and biochemistry of *Bacillus* species relates to *Bacillus subtilis* alone or to comparisons of this with other species, and further valuable information has been forthcoming from studies aimed at the optimization of various industrial processes employing several other aerobic endospore-forming species.

It is now established that *Bacillus subtilis*, which was long regarded as a strict aerobe, is capable of growing anaerobically, not only with nitrate as electron acceptor but also by fermentation in the absence of electron acceptors. This species and its close relatives apparently cannot use other electron acceptors such as dimethyl sulfoxide, fumarate and trimethylamine N-oxide, and have been considered to lie in an intermediate position between the true facultative anaerobes now allocated to *Paenibacillus* and the aerobes of the *Bacillus sphaericus* group (Priest, 1993), which are strictly oxidative. *Bacillus cereus, Bacillus licheniformis* and *Bacillus thuringiensis* can ferment carbohydrates in the absence of exogenous electron acceptors, and many *Bacillus* species can use nitrate as an electron acceptor in the absence of oxygen, but several species such as *Bacillus megaterium* and *Bacillus pumilus* are unable to do this. *Bacillus subtilis* uses pyruvate dehydrogenase for conversion of pyruvate to acetyl-coenzyme A in anaerobic as well as in aerobic conditions and fermentation is stimulated by pyruvate. The fermentation is of the mixed acid-butanediol type, and products include acetate, acetoin, 2,3 butanediol, ethanol and lactate (Nakano et al., 1997); *Bacillus licheniformis* also carries out a mixed acid fermentation (Shariati et al., 1995). During nitrate respiration, *Bacillus subtilis* reduces nitrate to nitrite and ammonium, and, unlike the denitrifier *Bacillus licheniformis*, it does not produce the gaseous products NO, N_2O and N_2 (Nakano and Zuber, 2002). A homolog of the *Bacillus subtilis* gene encoding membrane-bound respiratory nitrate reductase is found in *Bacillus anthracis* (Nakano and Zuber, 2002). *Bacillus licheniformis* shows poor anaerobic growth on fumarate, but it can

TABLE 3. (continued)

Characteristic	19. *B. circulans*	20. *B. clarkii*[1]	21. *B. clausii*	22. *B. coagulans*	23. *B. cohnii*[1]	24. *B. decolorationis*	25. *B. deramificans*	26. *B. endophyticus*	27. *B. farraginis*	28. *B. fastidiosus*	29. *B. firmus*	30. *B. flexus*	31. *B. fordii*	32. *B. fortis*	33. *B. fumarioli*[1]	34. *B. funiculus*	35. *B. fusiformis*[3]	36. *B. galactosidilyticus*	37. *B. gelatini*
Pigmented colonies:																			
Yellow-pink-red	–	+[l]	–	–	–	–	–	+[h]	–	d[h]	+[h]	–	–	–	–	–	–	–	–
Dark brown/black	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Motility	–			+	+	+	+	–	+	+	+		+	+	+	+	+	+	+
Cell diameter >1.0 μm	–	–	–	–	–	–	d	v	–	+	–	–	–	–	–	+	–	–	–
Spore formation:	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ellipsoidal		l	l	l	l	l	l		l	l	l	l	l	l	l	l		l	l
Cylindrical	–	–	–	–	–	–	–	–		–	d	–			+	–	–	–	–
Spherical	–	–	–	v	–	v	+	–		–	–	–			–	–	+	–	–
Borne terminally	v	–	–	v	+	–	–	v		v	–	–			–	–	d	–	–
Swell sporangia	–	d	v	+	+	+	+	–	v	–	v	–	v	+	–	–	+	+	–
Parasporal crystals	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Catalase	–			+	+	+		+	+	+	+		+	+	+	+		+	+
Aerobic growth	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic growth	–			+		–	+	–	–	–	+	–	–	–	–	–	–	+	–
Voges-Proskauer	–	ng		d	ng	–	–/w	–	–	ng	–	–	–	–	+	+	–	–	–
Acid from:																			
L-Arabinose	–	ng		d	ng	–	–	+	–	ng	–	–	–	–	–k		–	d/w	–
D-Glucose	–	ng		+	ng	w	w	+	–	ng	+	+	–	–	+[k]	+	–	+	+
Glycogen	–	ng		–	ng	–	–	–	–	ng	d		–	–	–k		–	–	–
D-Mannitol	–	ng		–	ng	d/w	–	+	–	ng	+	+	–	–	+[k]		–	d/w	+
D-Mannose	–	ng		+	ng	w	d/w	+	–	ng	–	–	–	–	+[k]		–	d/w	+
Methyl β-xyloside	–	ng		–	ng	–	–		–	ng	–		–	–	–k		–	–	–
Salicin	–	ng		d	ng	w	w		–	ng	–	d	–	–	–k		–	d/w	–
Starch	–	ng		+	ng	–	d/w		–	ng	+		–	–	–k		–	d/w	–
D-Xylose	–	ng		d	ng	–	d/w	d	–	ng	d	–	–	–	–k		–	d/w	+
Hydrolysis of:																			
Casein	w	+	+	–	d	+	–	–	–	–	w	+	–	–	–	–	+	w	+
Gelatin	d	+	+	d	+	+	–	–	–	–	+	+	–	–	+	–	+	–	+
Starch	w	–	+	+	+			–	–	–	+	+	–	–		+	–		
Utilization of:																			
Citrate	–			–		–	–	+	–	–	–	+	–	–	–	–	+	–	–
Propionate								–	–	–	–		d/w	–					
Egg yolk reaction	–			–				–		–	–	–					–		
Nitrate reduction	d	+	+	–	+	+	d	–	–	–	+	–	–	–	–	+	–	+	–
Growth at pH:																			
5	d	–	–	+	–	–	–		–	–	–	+	–	–	+	+	–	–	+
6	–	–	–	+	–	d	+		+	–	+	+	+	+	+	+	+	+	+
7			l	l		l	l	l	l	d	l	l	l	l		l	l	l	l
8	–		+	+		–	+		+	+	+	+	+	+	–	+	+	+	+
9	–	+		+	+	–	+		+	+	+	+	+	–	–	+	+	+	+
10	d	+	–	+	+	–	d				+				–	–	–	+	d
Growth in NaCl:																			
2%			+	+	+	+		+	+	+	+	+	+	+			+		+
5%		+	+	–	+	+		+	+	+	+	+	+	+			+		+
7%		+	+	–		+		+	+	–	+	+	+	+			+		+
10%		+	d	–	–	+		+		–		+							+
NaCl required for growth		+												–	–	–	–	–	–
Growth at:																			
5 °C		–	–		–	+		–		–		–			–	–	–		
10 °C		–	–		+	+		+		+		–			–	+			
20 °C		+	+		+	+		+	w	+	+	+	w	w	–	+	+		
30 °C	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
40 °C	–	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+
50 °C	–	–	+	d	–	–	+	–		–	d	–			+	–	–	–	+
55 °C	d	–	–	d	–	–	d	–		–	–	–			+	–	–	–	+
65 °C	–	–	–	–	–	–	–	–		–	–	–			–	–	–	–	–
Growth with lysozyme present								+								+			
Respiratory growth with As(V)																			
Respiratory growth with Se(IV) or Se(VI)																			
Autotrophic with $H_2 + CO_2$ or CO		–	–	–	–			–		–		–			–	–	–		
Degradation of tyrosine										–		–							
Deamination of phenylalanine		l															d		
Allantoin or urate required	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–

(continued)

TABLE 3. (continued)

Characteristics	38. *B. gibsonii*	39. *B. halmapalus*	40. *B. halodurans*[a]	41. *B. halophilus*	42. *B. horikoshii*	43. *B. hwajinpoensis*[d]	44. *B. hwajinpoensis*	45. *B. indicus*	46. *B. infernus*[g]	47. *B. insolitus*[g]	48. *B. jeotgali*	49. *B. krulwichiae*[d]	50. *B. lentus*	51. *B. licheniformis*[k]	52. *B. lautiferensis*	53. *B. macyae*	54. *B. marisflavi*	55. *B. megaterium*	56. *B. methanolicus*[l]
Pigmented colonies:																			
Yellow-pink-red	+[h]	−	−	−	−	−	+[c]	+[c]	−	−	+[h]	−	−	−	−	−	+[h]	−[h]	−
Dark brown/black	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−[h]	−
Motility				+		+	−	−	−	+	+	+	+	+	+	+	+	+	−
Cell diameter >1.0 µm	−	−	−	−	−	−	+	+	−	v	v	−	−	−	−	−	−	+	d
Spore formation:	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Ellipsoidal	−	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+
Cylindrical	−	−	−	−	−	−	−			+	−	−	−	v	−		−	−	−
Spherical										+								v	
Borne terminally	−	−	−	−	−	−	v			+		−	−	−	v		−	−	−
Swell sporangia	−	−	+	−	v	+	+	+		−	+	−	v	−	v	−	+	−	+
Parasporal crystals	−	−	−	−	−	−	−	−		−	−	−	−	−	−	−	−	−	−
Catalase				+		+	+	+	−	+	+	+	+	+	w	+	+	+	+
Acrobic growth	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+
Anaerobic growth				−		−	−		+	−	+	+	−	+	+	+	−	−	−
Voges-Proskauer				−			−	−		−			−	+	+			−m	
Acid from:																			
L-Arabinose							−		−	−	−		+/w	+	−		−	+	
D-Glucose				+		+	+		+	−	+	+	+/w	+	+		+	+	+
Glycogen											+		−	+	−		−	+	−
D-Mannitol				−			+			−	−	+	+/w	+	−		+	+	+
D-Mannose				+			+		−		−	−	+/w	+	−		+	−	
Methyl β-xyloside											−		+/w	−	−		−	−	
Salicin				+							−		+/w	+	+		+	+	−
Starch											+		+/w	+	w		−	+	−
D-Xylose				+		+	−		−	−	−	+	+/w	+	−		+	+	
Hydrolysis of:	−	+	+	−	+	+	+		−	ng	−	d	−	+	v		+	+	−
Casein			+									d							
Gelatin	−	+	+	−	+	+	+	+	−	−	+	+	+	+			−	+	d
Starch																			
Utilization of:				−				−	−	−			d	+	−	−		+	
Citrate				−					−				−	+					
Propionate				−									−	−	−			−	
Egg yolk reaction	d	−	d	−	−	+	+	−	+	−	−	+	+	+	−	+		d	−
Nitrate reduction																			
Growth at pH:	−	−	−	−	−	−	+		−		−	−	d		−		+		
5	−	−	−	+	−	−	+	+	−		+	−	+	+	+		+	+	−
6	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
7	−	+	+	+	+	+			+		+	+	+		−		−		
8	−	−	+	−	−	+			−			+	+		−		+		
9	−	−	+	−	−	+			−			+	+		−		+		
10	−	−	+	−	−	+			−			+	d		−				
Growth in NaCl:																			
2%	−		+	−	+	d	+	+	+	d	+	+	+	+		+	+	+	d
5%	−	−	+	+	+	+	+	−	+	−	+	+	+	+		−	+	+	−
7%	−	−	+	+	+	+	+		+	−	+	+		+		−	+	+	
10%	d	−	+	+	−	+	+		+	−	+	+				−	+	−	
NaCl required for growth	−	−	−	+	−	d	+		−	−	+	−	−	−	−		−	−	−
Growth at:																			
5 °C	−	−	−	−	−	−			−	+	−			−	−		−	d	−
10 °C	−	+	−	−	+	−	+		−	+	+	−	+	−	−		+	d	−
20 °C	−	+	+	+	+	+	+	+	−	+	+		+	+	+		+	+	−
30 °C	−	+	+	+	+	+	+	+	−	−	+		+	+	+	+	+	+	−
40 °C	−	+	+	+	+	+	+	−	−	−	+	+	−	+	+		+	d	+
50 °C	−	−	+	+	−	−	−		+	−	−	−		+	−		−	d	+
55 °C	−	−	+	−	−	−	−		+	−	−	−		d	−		−	d	+
65 °C	−	−		−	−	−	−		−	−	−	−		−	−		−	−	−
Growth with lysozyme present										−				d				−	
Respiratory growth with As(V)																+			
Respiratory growth with Se(IV) or Se(VI)																−			
Autotrophic with $H_2 + CO_2$ or CO	−	−	−	−	−	−			−	−	−			−	−			−	−
Degradation of tyrosine				−						−	−			−			−	d	
Deamination of phenylalanine	−	−	−	−	−	−				d				−				+	
Allantoin or urate required	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−

(continued)

tive organisms may multiply readily in a variety of foods and may cause diarrheal and emetic food poisoning syndromes. Growth in milk may result in "bitty cream defect". Occasionally causes opportunistic infections in man and other animals. Certain endospore-forming, trichome-forming bacteria that occur in the alimentary tracts of animals, some of which have been called "*Arthromitus*", have been identified as *Bacillus cereus*; see *Cell morphology* and *Habitats*, in *Further descriptive information*, above.

DNA G + C content (mol%): 31.7–40.1 (T_m) for 11 strains, 34.7–38.0 (Bd), and 35.7 (T_m), 36.2 (Bd) for the type strain.

Type strain: ATCC 14579, DSM 31, JCM 2152, LMG 6923, NCIMB 9373, NRRL B-3711, IAM 12605.

EMBL/GenBank accession number (16S rRNA gene): D16266 (IAM 12605).

Additional remarks: Phenotypically similar to other members of the *Bacillus cereus* group: *Bacillus anthracis*, *Bacillus mycoides*, *Bacillus thuringiensis* and *Bacillus weihenstephanensis*. For distinguishing characters see the individual species descriptions and Table 7. Another member of the group, *Bacillus pseudomycoides*, is separated from *Bacillus cereus* only by DNA relatedness and some differences in fatty acid composition. Genetic evidence supports the recognition of members of the *Bacillus cereus* group as one species, given that differentiation often relies on the presence of virulence characters which are carried by extrachromosomal mobile genetic elements (Turnbull et al., 2002), but practical considerations argue against such a move.

19. **Bacillus circulans** Jordan 1890, 821[AL]

cir′cu.lans. L. part. adj. *circulans* circling.

For many years this species accommodated a wide variety of phenotypically unrelated strains. It was referred to by Gibson and Topping (1938) as a complex rather than a species, and later investigators agreed with this description. Strains were frequently allocated to this species on account of their distinctive motile microcolonies (Figure 9i); however, Jordan named his isolate for the circular motion that he saw in the interior of colonies observed under low magnification, rather than because of motile microcolonies. Jordan's original strain is considered lost, but Ford's isolate 26, that he believed to be of the same species as Jordan's strain, is available. Smith and Clark (1938) observed the rotary motion within the colonies of Ford's strain and noted also the production of motile microcolonies. Despite a few discrepancies between Jordan's and Ford's descriptions of their strains, Smith et al. (1952) considered that Ford's strain 26 could be accepted as authentic and this became the type strain. The production of motile microcolonies is more characteristic of strains now allocated to *Paenibacillus* (see *Further descriptive information*, *Colony characteristics*, above).

Further grounds for the allocation of later isolates to this species were the production of sporangia swollen by subterminal to terminal ellipsoidal spores, and their being very active in the production of acid from a very wide range of carbohydrates. DNA relatedness studies revealed at least 10 homology groups among strains labeled *Bacillus circulans*, and it became clear that the phenotypic and genotypic heterogeneity of the complex had resulted from the allocation of unrelated strains to the species (Nakamura and Swezey, 1983). This work led to the allocation of members of several of the homology groups to new or revived species which were subsequently assigned to *Paenibacillus: Paenibacillus amylolyticus*, *Paenibacillus lautus*, *Paenibacillus pabuli* and *Paenibacillus validus*. A further group of strains previously assigned to *Bacillus circulans* was proposed as the new species *Bacillus* (now *Paenibacillus) glucanolyticus* on the basis of a numerical taxonomic study (Alexander and Priest, 1989). However, many misnamed strains remain allocated to *Bacillus circulans* and await reallocation, and authentic strains of this species are in the minority in most collections.

The description which follows is based upon the type strain and several other strains which have been shown by amplified rDNA restriction analysis, polyacrylamide gel electrophoresis of whole-cell proteins, and various phenotypic characters (De Vos, Logan and colleagues, unpublished data) to be closely related to the type strain. Phylogenetic studies indicate that *Bacillus circulans*, *Bacillus firmus* and *Bacillus lentus* are related.

Facultatively anaerobic, motile, straight, round-ended, occasionally slightly tapered and curved rods 0.6–0.8 µm in diameter, appearing singly or in pairs and occasionally short chains. Endospores are ellipsoidal and lie terminally or subterminally in swollen sporangia (Figure 9c). Colonies grown for 2 d on TSA at 30 °C are 1–3 mm in diameter, opaque, cream-colored, slightly convex, with eggshell surface textures and irregular margins that may spike along the streak lines. Optimum temperature lies between 30 °C and 37 °C; maximum temperature for growth lies between 50 °C and 55 °C. The optimum pH for growth is 7.0. Minimum pH for growth lies between 4.0 and 5.0. The maximum pH lies between 9 and 10. Casein and starch are weakly hydrolyzed. In the API 20E strip, *o*-nitrophenyl-β-D-galactopyranoside hydrolysis is positive and urease production, hydrolysis of gelatin and nitrate reduction are occasionally positive. Arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase production, citrate utilization, hydrogen sulfide, tryptophan deaminase and indole production and Voges–Proskauer reaction are negative. In the API 50CH gallery using the CHB suspension medium, hydrolysis of esculin is positive, and acid without gas is produced from a very wide range of carbohydrates: Production of acid without gas is variable for: adonitol, D-arabitol, 2-keto- and 5-keto-D-gluconate, rhamnose and ribose; the type strain is positive for adonitol, rhamnose and ribose. Acid production is negative for the following substrates: D-arabinose, dulcitol, erythritol, D-fucose, L-fucose, L-sorbose, D-tagatose and L-xylose. In the variable results, the type strain scores positive for: adonitol, rhamnose and ribose. Occasional strains may produce acid without gas from D-lyxose.

Source: sewage, soil, food and infant bile.

DNA G + C content (mol%): 35.7 (T_m), 36.2 (Bd) for the type strain.

Type strain: ATCC 4513, DSM 11, JCM 2504, LMG 13261, IAM 12462.

EMBL/GenBank accession number (16S rRNA gene): D78312 (IAM 12462).

20. **Bacillus clarkii** Nielsen, Fritze and Priest 1995b, 879[VP] (Ef-

Type strain: Nielsen PN-121, ATCC 700161, DSM 8719, LMG 17946.

EMBL/GenBank accession number (16S rRNA gene): AB043865 (DSM 8719).

43. **Bacillus horti** Yumoto, Yamazaki, Sawabe, Nakano, Kawasaki, Ezura and Shinano 1998, 570[VP]

hor′ti. L. masc. n. *hortus* garden; L. gen. n. *horti* from the garden.

Alkaliphilic, strictly aerobic, Gram-negative, motile rods, 0.6–0.8 by 1.5–6.0 μm, forming ellipsoidal, subterminal spores in swollen sporangia. Description is based upon two isolates. Colonies on complex medium at pH 10 are white. Grow occurs at pH 7, with optimum growth at pH 8–10. Grows in presence of 3–11% NaCl but not at 12% NaCl. Growth occurs between 15 °C and 40 °C; no growth at 10 and 45 °C. Catalase- and oxidase-positive. Nitrate is reduced to nitrite, *o*-nitrophenyl-β-D-galactopyranoside is hydrolyzed and H_2S is produced at pH 7. Acid is produced without gas from glucose and a narrow range of other carbohydrates. Casein, gelatin, starch and DNA are hydrolyzed; Tween 20, 40, 60 and 80 and urea are not. See Table 6.

Source: garden soil in Japan.

DNA G + C content (mol%): 40.9% for the type strain and 40.2 for another strain (HPLC).

Type strain: K13, ATCC 700778, DSM 12751, JCM 9943, LMG 18497.

EMBL/GenBank accession number (16S rRNA gene): D87035 (K13).

44. **Bacillus hwajinpoensis** Yoon, Kim, Kang, Oh and Park 2004b, 807[VP]

hwa.jin.po.en′sis. N.L. adj. *hwajinpoensis* of Hwajinpo, a beach of the East Sea in Korea, where the type strain was isolated.

Aerobic, nonmotile rods, 1.0–1.3 μm in diameter and 2.5–4.0 μm long. Gram-positive, but Gram-variable in older cultures. Description is based on a single isolate. Ellipsoidal endospores are borne centrally or terminally in swollen sporangia. Colonies are smooth, circular to slightly irregular, slightly raised, light yellow in color and 2–4 mm in diameter after 3 d cultivation at 30 °C on marine agar. Optimum growth temperature is 30–35 °C. Growth occurs at 10 and 40 °C but not at 4 °C or above 41 °C. Optimum pH for growth is 6.0–7.0. Growth is observed at pH 5.0, but not at pH 4.5. NaCl is required for growth. Optimal growth occurs in the presence of 2–5% NaCl. Growth occurs in the presence of 19% NaCl but is inhibited by 20% NaCl. No anaerobic growth on marine agar. Esculin is hydrolyzed. Hypoxanthine, tyrosine, urea and xanthine are not hydrolyzed. Acid is produced from D-mannitol and stachyose. Cell-wall peptidoglycan contains *meso*-diaminopimelic acid. Predominant menaquinone is MK-7. Major fatty acid is $C_{15:0\ anteiso.}$

Source: sea water of the East Sea in Korea.

DNA G + C content (mol%): 40.9 (HPLC).

Type strain: SW-72, KCCM 41641, JCM 11807.

EMBL/GenBank accession number (16S rRNA gene): AF541966 (SW-72).

45. **Bacillus indicus** Suresh, Prabagaran, Sengupta and Shivaji 2004, 1374[VP]

in′di.cus. L. masc. adj. *indicus* pertaining to India, Indian.

Cells are aerobic, Gram-positive, nonmotile rods measuring approximately 0.9–1.2 μm wide and 3.3–5.3 μm long. Description is based upon a single isolate. Produces subterminal endospores in a slightly swollen sporangium. Colonies on nutrient agar are yellowish-orange pigmented, circular, raised, smooth, convex and 3.0–4.0 mm in diameter. The pigment in acetone exhibits three absorption maxima at 404, 428 and 451 nm, characteristic of carotenoids. Grows in the range of 15–37 °C (optimum 30 °C) but not at 40 °C. Grows between pH 6 and 7 and tolerates up to 2.0% (w/v) NaCl. Positive for catalase, gelatinase, amylase, arginine dihydrolase and esculin. Does not hydrolyze Tween 20 or urea. Does not reduce nitrate to nitrite and is negative for indole production, Voges–Proskauer test and citrate utilization. Utilizes D-cellobiose, *meso*-erythritol, inositol, lactose, D-melibiose, D-maltose, D-mannose, sucrose, L-rhamnose, D-ribose, raffinose, L-arginine, L-tryptophan and L-tyrosine as sole carbon sources. The major fatty acids are $C_{14:0\ iso}$ (10.9%), $C_{15:0\ iso}$ (33.5%), $C_{15:0\ anteiso}$ (19.3%), $C_{16:0\ iso}$ (11.0%), $C_{16:0}$ (5.9%) and $C_{17:0\ iso}$ (10.8%). The main proportion of the polar lipids consists of phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine. The major respiratory quinone is MK-7. The cell wall is an A4β-murein with ornithine as the diamino acid and aspartic acid as the interpeptide bridge.

Source: sand of an arsenic-contaminated aquifer in West Bengal, India.

DNA G + C content (mol%): 41.2 (T_m).

Type strain: Sd/3, MTCC 4374, DSM 15820.

GenBank/EMBL accession number (16S rRNA gene): AJ583158 (Sd/3).

46. **Bacillus infernus** Boone, Liu, Zhao, Balkwill, Drake, Stevens and Aldrich 1995, 447[VP]

in.fer′nus. N.L. adj. *infernus* that which comes from below (the ground).

Strictly anaerobic, thermophilic, nonmotile rods 0.7–0.8 by 4–8 μm. Cell-wall morphology is Gram-positive but the Gram reaction is ambiguous. Endospores not observed, but their presence has been inferred from the survival of heat-treated cultures. Growth occurs at 45–60 °C but not at 40 or 65 °C; optimum temperature for growth is about 61 °C. Optimum pH for growth is about 7.3; grows well at pH 8.1 but does not grow at pH 9.2. The type strain is halotolerant; other strains have not been tested for this property. Grows fermentatively with glucose as substrate, but not with a range of other carbohydrates, alcohols and organic acids. Respiratory growth uses formate or lactate as electron donors and MnO_2, Fe^{3+}, trimethylamine oxide, and nitrate as electron acceptors. Nitrate is reduced to nitrite but not to ammonia or dinitrogen. Sulfate and thiosulfate are not reduced. Casein, gelatin and starch are not hydrolyzed. See Table 8.

Source: a shale core taken from 2.7 km below the land surface in the Taylorsville Triassic Basin in Virginia, USA.

DNA G + C content (mol%): not reported.

Type strain: Boone TH-23, DSM 10277, SMCC/W 479.

EMBL/GenBank accession number (16S rRNA gene): U20385 (Boone TH-23).

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY

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FAMILY XIII. BACILLACEAE FISCHER, 1895.

(Jahrb. f. wiss. Bot., *27*, 1895, 139.)

Ba.cil.la'ce.ae. M.L. noun *Bacillus* type genus of the family; *-aceae* ending to denote a family; M.L. fem.pl.n. *Bacillaceae* the *Bacillus* family.

Rod-shaped cells capable of producing endospores which are cylindrical, ellipsoidal or spherical, and which are located in the center of the cell, subterminally or terminally. Sporangia do not differ from the vegetative cells except when bulged by spores larger than the cell diameter; such sporangia are spindle-shaped when spores are central and wedge- or drumstick-shaped when spores are terminal. Motile by means of peritrichous flagella or non-motile. Usually Gram-positive. Pigment formation is rare. Gelatin is frequently hydrolyzed. Sugars are generally fermented, sometimes with the production of visible gas. Aerobic, facultatively anaerobic; anaerobic; or anaerobic, aerotolerant. Some species are capable of growth at 55° C. Mostly saprophytes, commonly found in soil; a few are animal or insect parasites or pathogens.

Key to the genera of family **Bacillaceae.**

I. Aerobic or facultatively anaerobic; catalase-positive.
Genus I. *Bacillus*, p. 613.

II. Anaerobic or aerotolerant; catalase not known to be produced.
Genus II. *Clostridium*, p. 634.

Genus I. **Bacillus** *Cohn, 1872.**

(Beiträge z. Biol. d. Pflanzen, *1*, Heft 2, 1872, 146 and 175.)

Ba.cil'lus. L. dim.noun *bacillum* a small rod; M.L. noun *Bacillus* a rodlet.

Rod-shaped cells, sometimes in chains, capable of producing endospores. Sporangia do not differ from the vegetative cells except when bulged by spores larger than the cell diameter; such sporangia are spindle-shaped when the spores are central and wedge- or drumstick-shaped when the spores are terminal. Motile by means of peritrichous flagella or non-motile. Gram-positive, some species being Gram-variable or Gram-negative. Some species usually occur in the rough stage, forming a pellicle on broth, whereas other species are smooth and the rough stage is rarely seen. Usually proteins are decomposed with the production of ammonia. [redacted] Catalase-positive. Aerobic or facultatively anaerobic. Maximum temperatures for growth vary greatly, not only between species but also between strains of the same species. Variations in other characters frequently occur within a species. Mostly saprophytes, commonly found in soil; a few are animal, especially insect, parasites or pathogens.

The type species is *Bacillus subtilis* Cohn *emend.* Prazmowski.

Key to the species of genus **Bacillus.**

I. Sporangia not definitely swollen.† Spores ellipsoidal to cylindrical, central to terminal. Spore walls thin and not easily stained. Gram-positive.

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* Revised by Dr. Nathan R. Smith, St. Armands Key, Sarasota, Florida, and Dr. Ruth E. Gordon, N. J. Agricultural Experiment Station, New Brunswick, N. J., September, 1954. The arrangement and the descriptions of the species, unless otherwise noted, have been taken from the work of Smith, Gordon and Clark (Agricultural Monograph 16, U. S. Department of Agriculture, 1952).

† Nearly 50 per cent of the strains of *Bacillus coagulans* studied by Smith, Gordon and Clark (*op. cit.*, 1952) had definitely swollen sporangia; the species was placed in group I because of other characteristics.

RIWAYAT HIDUP

Rizka Annisa lahir di Kota Medan Sumatera Utara pada tanggal 06 April 2000 dari pasangan bapak **Mardin Nasution** dan ibu **Sopia Trisdalina Pulungan** sebagai anak ke-3 dari 3 bersaudara. Penulis menempuh pendidikan dari sekolah dasar di SDN 060899 Medan pada tahun 2007 dan lulus pada tahun 2012. Pada tahun 2012 melanjutkan pendidikan ke jenjang menengah pertama di SMPN 36 Medan dan lulus pada tahun 2015. Penulis melanjutkan pendidikannya di SMAN 13 Medan dan lulus pada tahun 2018. Pada tahun yang sama penulis melanjutkan pendidikannya di Program Studi Biologi Fakultas Sains dan Teknologi UINSU dan lulus pada tahun 2023.

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Akhir kata semoga skripsi tersebut dapat bermanfaat bagi seluruh pembaca terutama bermanfaat di bidang sains dan teknologi, serta penulis mengucapkan banyak-banyak terima kasih untuk semua orang yang telah membersamai sampai terselesaikannya tugas akhir skripsi ini.