BAB V
KESIMPULAN DAN SARAN

A. Kesimpulan

Hasil penelitian penapisan bakteri penghasil superoksida dismutase (SOD) dari air hutan mangrove Maron Edupark Semarang dapat disimpulakan bahwa :

Pertama, air hutan mangrove Maron Edupark Semarang terdapat isolat bakteri yang menghasilkan enzim superoksida dismutase (SOD).

Kedua, aktivitas enzim superoksida dismutase (SOD) tertinggi yaitu pada isolat bakteri AHM4 dengan persentase SOD sebesar 80,39%.

Ketiga, hasil identifikasi molekuler pada isolat bakteri AHM4 menunjukkan bahwa nama isolat bakteri adalah *Bacillus cereus* dengan homologi 99%.

B. Saran

Bakteri *Bacillus cereus* merupakan bakteri patogen yang berbahaya apabila terdapat dalam tubuh sehingga perlu dilakukan isolasi gen SOD yang kemudian dapat dilakukan kloning agar tidak berbahaya dalam tubuh dan dapat dimanfaatkan dan dilakukan pengujian keamanan pemakaian hasil kloning tersebut pada manusia.

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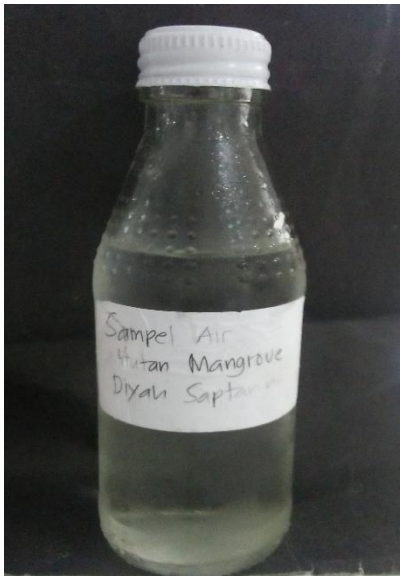
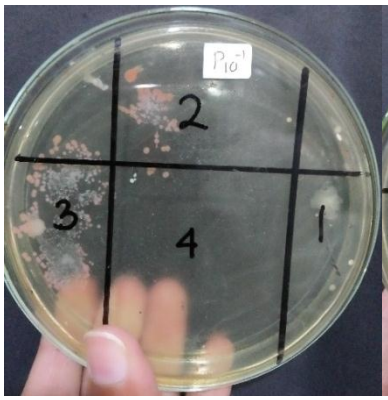
LAMPIRAN

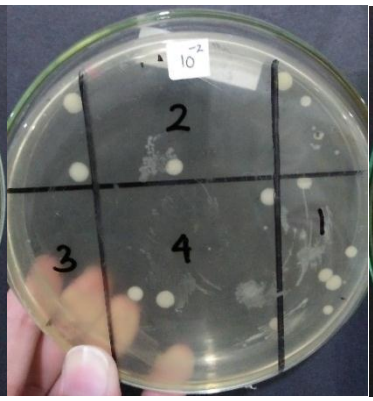
Lampiran 1. Sampel Air Hutan Mangrove



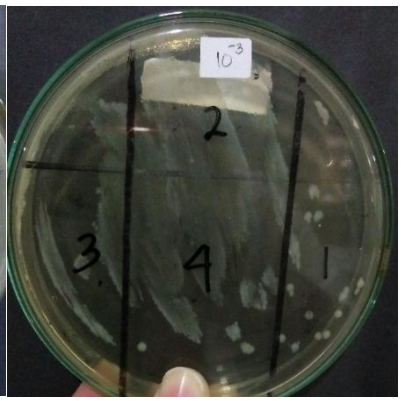
Lampiran 2. Hasil Isolasi Bakteri



Pengenceran 10^{-1}

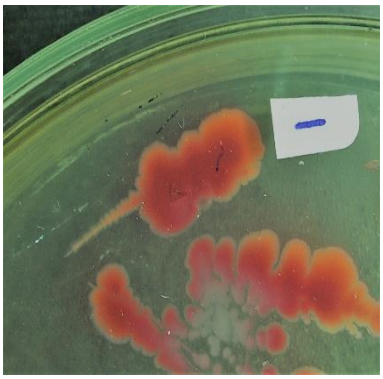
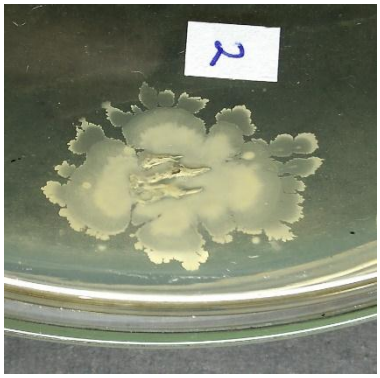
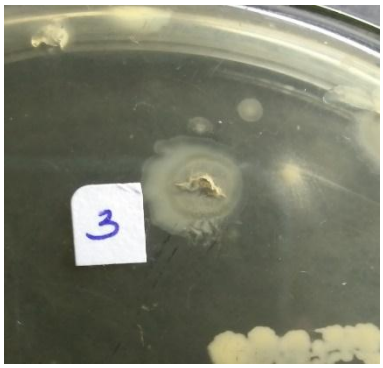
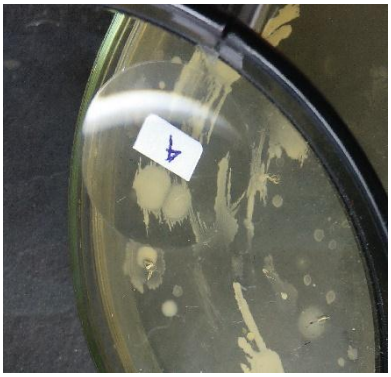


Pengenceran 10^{-2}

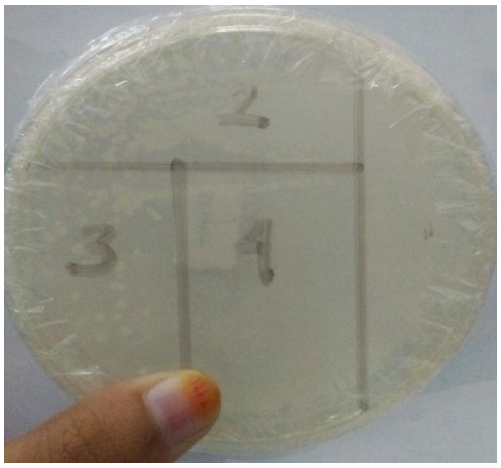


Pengenceran 10^{-3}

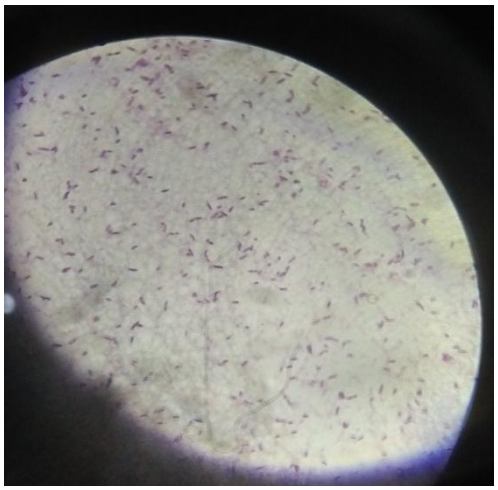
Lampiran 3. Foto Hasil Identifikasi Bakteri secara Makroskopis

Koloni 1 	Koloni 2 
Koloni 3 	Koloni 4 
Koloni 5 	

Lampiran 4. Isolat AHM4 yang Dikirim ke Macrogen



Lampiran 5. Foto Identifikasi dengan Pewarnaan Gram



Lampiran 6. Hasil Identifikasi Molekuler dengan PCR 16S rDNA

Standard ID

16S rRNA Full Sequencing

macrogen

16S rRNA service report

Order Number : 190527FN-021
Sample name : 4D_contig_1

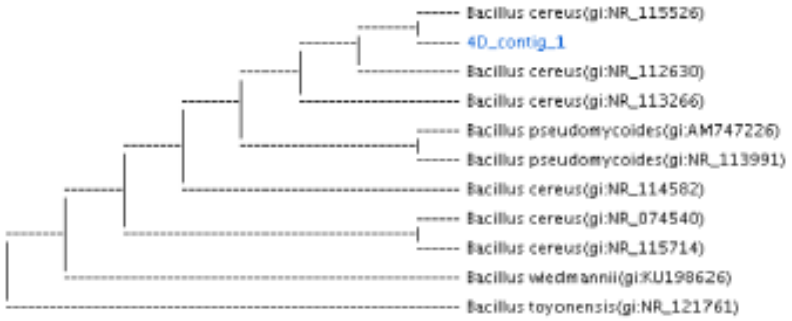
Information

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'

Subject						Score		Identities	
Accession	Description	Length	Start	End	Coverage	Bit	E-Value	Match/Total	Pct.(%)
NR_074540.1	Bacillus cereus	1512	28	1503	97	2675	0.0	1467/1476	99

Kingdom	Family	Genus	Species
Bacteria	Bacillaceae	Bacillus	Bacillus cereus



Characterization

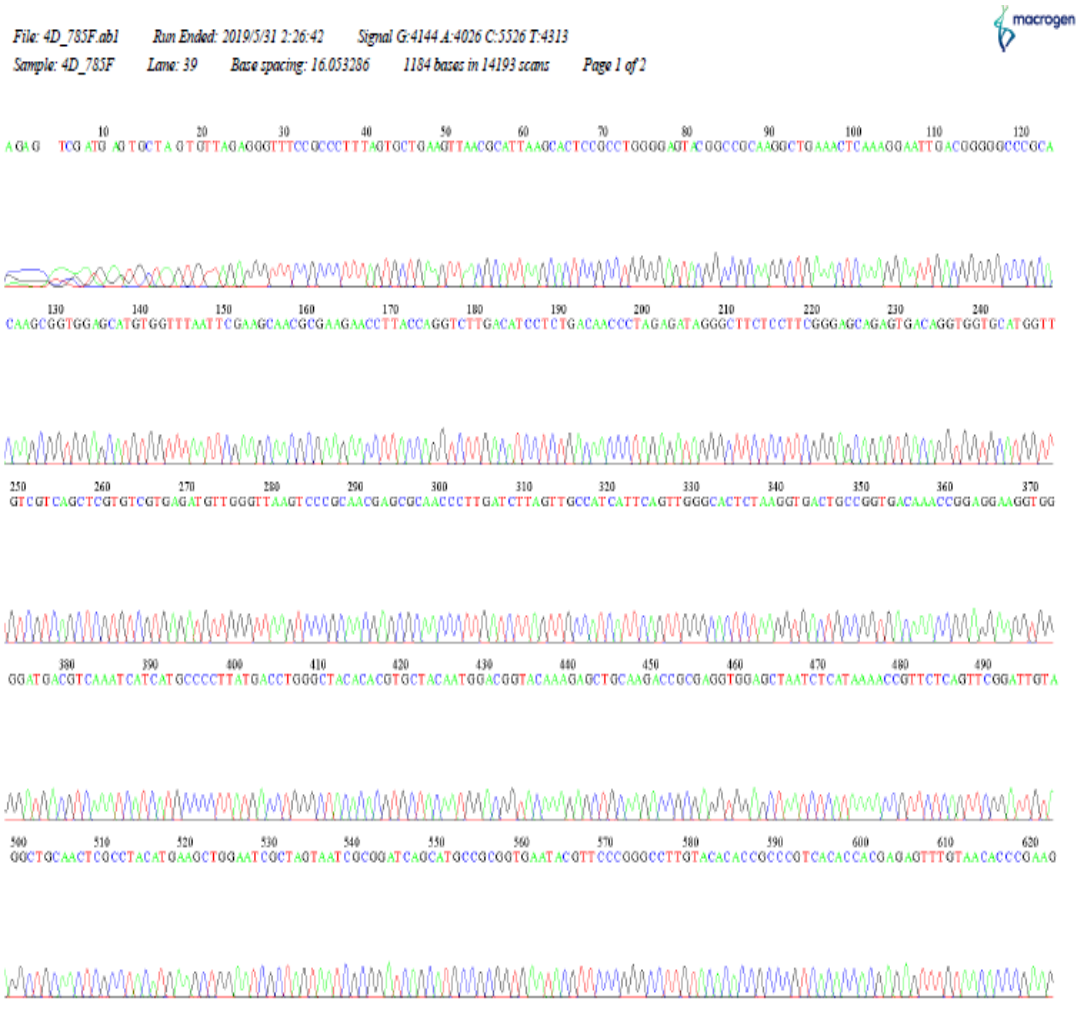
Bacilli cause an array of infections from ear infections to meningitis, and urinary tract infections to septicemia. Mostly they occur as secondary infections in immunodeficient hosts or otherwise compromised hosts. They may exacerbate previous infection by producing tissue-damaging toxins or metabolites that interfere with treatment.

Bacillus cereus causes two types of food poisoning in humans including diarrhoeal syndrome and emetic syndrome. Food poisoning results from its production of enterotoxins in the gastrointestinal tract. The dosage of ingested B. cereus spores leading to diarrhoeal syndrome is 1057107 g 1 of ingested food, and 1057108 g 1 of ingested food for emetic syndrome. Enterotoxins associated with diarrhoeal syndrome are unresistant to the acidic conditions of the stomach.

Lampiran 7. Hasil Identifikasi Bakteri Penghasil SOD dengan Uji Sekuensing 16S rDNA

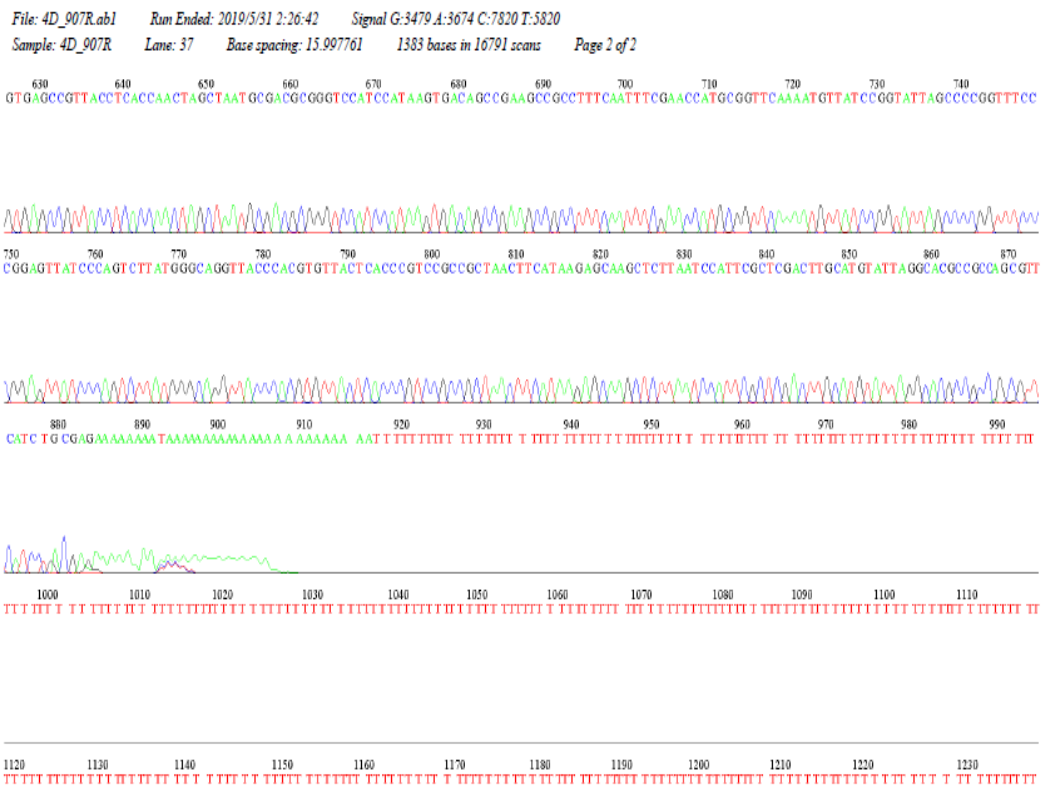
Name	Length	Start	End	Description	Lin	AC	Length	Start	End	Bit	Raw	E-valu	Match	Total	Pct(%)	
4D_contig_1	1501	25	1499	Bacillus cereus strain ATCC 145	htt	NR_07454	1512	28	1503	2675	1448	00.00	1467	1476	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus cereus strain CCM 2010	htt	NR_11571	1535	21	1496	2675	1448	00.00	1467	1476	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus cereus ATCC 14579, con	htt	AE016877.	5411809	9215	10690	2675	1448	00.00	1467	1476	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus cereus strain IAM 1260	htt	NR_11552	1486	1	1476	2675	1448	00.00	1467	1476	99	Plus/Plus
4D_contig_1	1501	26	1499	Bacillus cereus strain NBRC 153	htt	NR_11263	1476	1	1475	2673	1447	00.00	1466	1475	99	Plus/Plus
4D_contig_1	1501	25	1497	Bacillus cereus strain JCM 2152	htt	NR_11326	1474	1	1474	2671	1446	00.00	1465	1474	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus wiedmannii strain FSL \	htt	KU198626.	1540	21	1496	2669	1445	00.00	1466	1476	99	Plus/Plus
4D_contig_1	1501	25	1495	Bacillus cereus strain ATCC 145	htt	NR_11458	1482	11	1482	2667	1444	00.00	1463	1472	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus thuringiensis strain AT	htt	CP021061.	5427594	361609	363084	2658	1439	00.00	1464	1476	99	Plus/Plus
4D_contig_1	1501	25	1499	Bacillus toyonensis strain BCT-7	htt	NR_12176	1544	18	1493	2652	1436	00.00	1463	1476	99	Plus/Plus

Lampiran 8. Sampel AHM4 dengan Forward

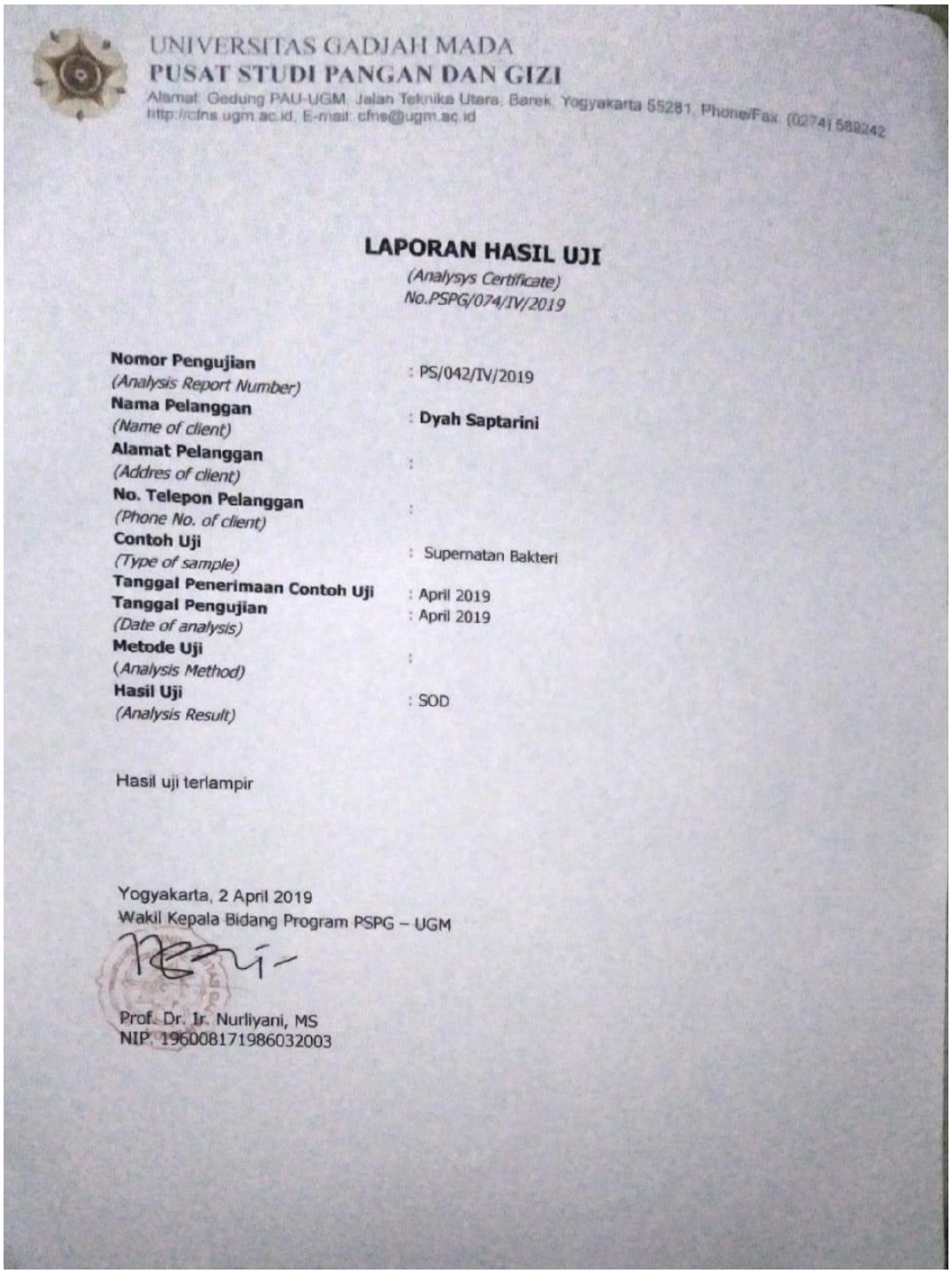



File: 4D_785F.ab1 Run Ended: 2019/5/31 2:26:42 Signal G:4144 A:4026 C:5526 T:4313
Sample: 4D_785F Lane: 39 Base spacing: 16.053286 1184 bases in 14193 scans Page 2 of 2

Lampiran 9. Sampel AHM4 dengan Reverse


File: 4D_907R.ab1 Run Ended: 2019/5/31 2:26:42 Signal G:3479 A:3674 C:7820 T:5820
Sample: 4D_907R Lane: 37 Base spacing: 15.997761 1383 bases in 16791 scans Page 2 of 2

Lampiran 10. Tanda Bukti Uji Aktivitas Superoksida Dismutase



UNIVERSITAS GADJAH MADA
PUSAT STUDI PANGAN DAN GIZI
Alamat: Gedung PAU-UGM, Jalan Teknika Utara, Barek, Yogyakarta 55281, Phone/Fax. (0274) 589242
http://cfns.ugm.ac.id, E-mail: cfns@ugm.ac.id

LAPORAN HASIL UJI
(Analysys Certificate)
No.PSPG/074/IV/2019

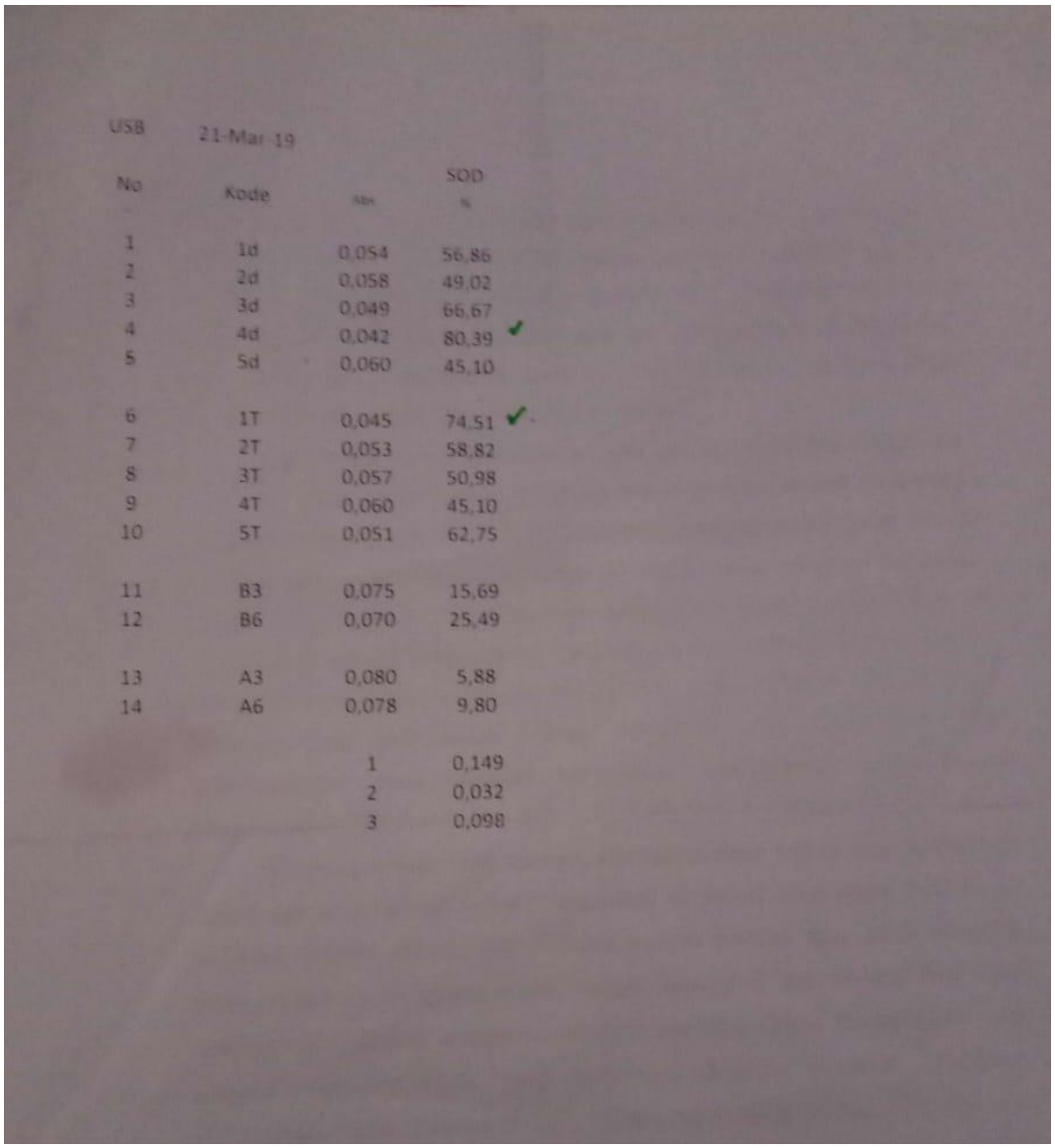
Nomor Pengujian *(Analysis Report Number)*	: PS/042/IV/2019
Nama Pelanggan *(Name of client)*	: **Dyah Saptarini**
Alamat Pelanggan *(Addres of client)*	:
No. Telepon Pelanggan *(Phone No. of client)*	:
Contoh Uji *(Type of sample)*	: Supernatan Bakteri
Tanggal Penerimaan Contoh Uji	: April 2019
Tanggal Pengujian *(Date of analysis)*	: April 2019
Metode Uji *(Analysis Method)*	:
Hasil Uji *(Analysis Result)*	: SOD

Hasil uji terlampir

Yogyakarta, 2 April 2019
Wakil Kepala Bidang Program PSPG – UGM

Prof. Dr. Ir. Nurliyani, MS
NIP. 196008171986032003

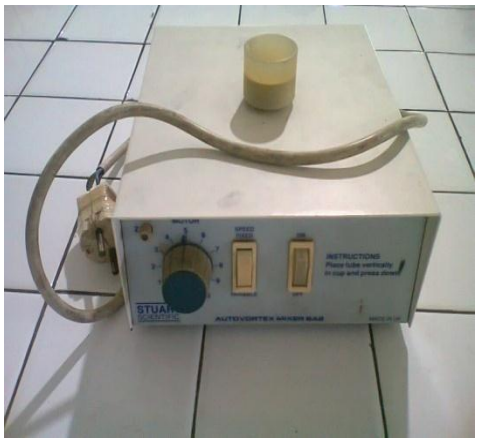
Lampiran 11. Hasil Uji Aktivitas Superoksida Dismutase



USB 21-Mar-19

No	Kode	Abs	SOD %
1	1d	0,054	56,86
2	2d	0,058	49,02
3	3d	0,049	66,67
4	4d	0,042	80,39 ✔
5	5d	0,060	45,10
6	1T	0,045	74,51 ✔
7	2T	0,053	58,82
8	3T	0,057	50,98
9	4T	0,060	45,10
10	5T	0,051	62,75
11	B3	0,075	15,69
12	B6	0,070	25,49
13	A3	0,080	5,88
14	A6	0,078	9,80
		1	0,149
		2	0,032
		3	0,098

Lampiran 12. Alat Praktikum



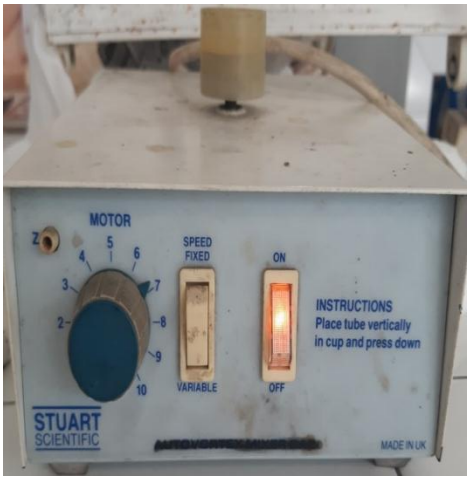
Vortex

Inkas

Inkubator

Oven

Autoclave



Vortex

Jarum Ose dan Jarum Ent

Lampu Spiritus

Micropipet

Gelas Ukur

Beker Gelas

Mikroskop Binokuler

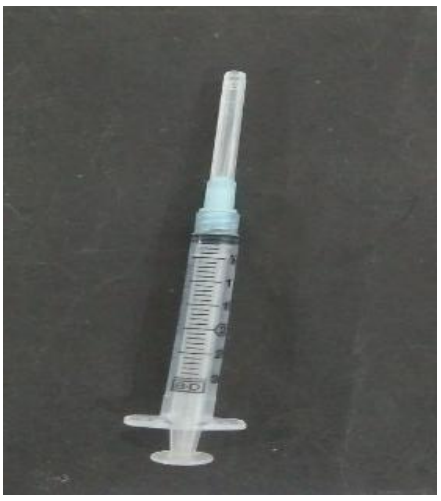
Alat sentrifugasi

Sonikator

Pipet volume

Kapas lidi

Batang pengaduk



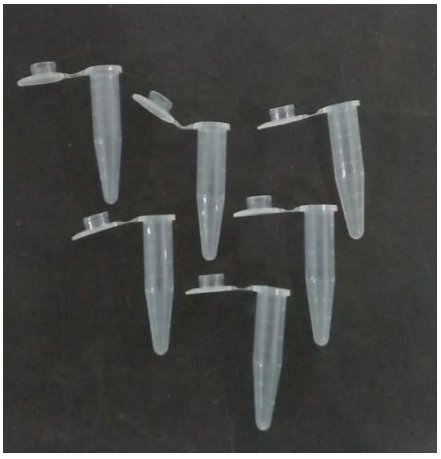
Spuit 1 ml

Objek glass

Mikrot tip

Timbangan analitik

Ice box



Gel Ice **Mikrotube**

Rak tabung reaksi **Cawan petri disposible**

Lampiran 13. Perhitungan Persentase SOD

Keterangan : Blank 1 = 0,149

Blank 2 = 0,032

Blank 3 = 0,098

Rumus : % SOD : $\frac{(\text{A blank 1}-\text{A blank 3})-(\text{A sampel}-\text{A blank 2})}{\text{A blank 1}-\text{A blank 3}}$ x 100%

1. Isolat AHM1 : $\frac{(0{,}149-0{,}098)-(0{,}054-0{,}032)}{0{,}149-0{,}098}$ x 100%

: 56,86 %

2. Isolat AHM2 : $\frac{(0{,}149-0{,}098)-(0{,}058-0{,}032)}{0{,}149-0{,}098}$ x 100%

: 49,02 %

3. Isolat AHM3 : $\frac{(0{,}149-0{,}098)-(0{,}049-0{,}032)}{0{,}149-0{,}098}$ x 100%

: 66,67 %

4. Isolat AHM4 : $\frac{(0{,}149-0{,}098)-(0{,}042-0{,}032)}{0{,}149-0{,}098}$ x 100%

: 80,39 %

5. Isolat AHM5 : $\frac{(0{,}149-0{,}098)-(0{,}060-0{,}032)}{0{,}149-0{,}098}$ x 100%

: 45,10 %

Lampiran 14. Komposisi dan Pembuatan Media

1. *Brain Heart Infusion* (BHI)

Komposisi :	Sari otak anak sapi	12	gram
	Sari jantung sapi	5	gram
	Proteose pepton	10	gram
	Bacto dextrose	2	gram
	NaCl	5	gram
	Dinatrium fosfor	2,5	gram
	Bacto agar	15	gram
	Aquadestilata	ad 1 L pH = 7,4	

Cara Pembuatan

Reagen-reagen di atas dilarutkan dalam aquadestillata sebanyak 1000 mL, dipanaskan sampai larut sempurna, kemudian disterilkan dengan autoklaf pada suhu 121ºC selama 15 menit dan dituangkan dalam cawan petri (Bridson 1998).

2. Nutrient Agar (NA)

Komposisi : Beef extract 3 g
Pepton 5 g
Agar 15 g
Akuades ad 1000 ml

Cara Pembuatan

Akuades sebanyak 100 ml dibagi menjadi dua, satu bagian untuk melarutkan *Beef extract* dan peptone dan sebagian lagi untuk melarutkan agar. Agar dilarutkan pada sebagian air kemudian diaduk. Akuades sebagian digunakan untuk melarutkan pepton dan *beef extract*. Bahan yang sudah larut dituangkan ke larutan agar dan diaduk sampai homogen, kemudian dilakukan pengukuran pH media dengan mencelupkan kertas

pH indikator. pH yang belum netral dapat ditambahkan HCl/NaOH sampai netral. Media yang larut dimasukkan ke dalam labu Erlenmeyer dan disterilisasi dengan autoklaf dengan suhu 121˚C selama 15 menit. Media steril dituangkan ke cawan petri steril secara aseptis.

Lampiran 15. Informasi PCR 16S rDNA dari Macrogen

Humanizing Genomics
macrogen

16S rDNA region Sequencing Analysis

PCR machine: DNA Engine Tetrad 2 Peltier Thermal Cycler (BIO-RAD)
PCR product purification: multiscreen filter plate (Millipore Corp.)
Sequencing Kit : BigDye(R) Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems)
Sequencer: ABI PRISM 3730XL Analyzer (96 capillary type)

---------- ***Information*** ----------

Primer Information

PCR Primer Name Primer Sequences
27F 5' (AGA GTT TGA TCM TGG CTC AG) 3'
1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3'

Sequencing Primer Name Primer Sequences
785F 5' (GGA TTA GAT ACC CTG GTA) 3'
907R 5' (CCG TCA ATT CMT TTR AGT TT) 3'

Method

The primers 27F 5' (AGA GTT TGA TCM TGG CTC AG) 3' and 1492R 5' (TAC GGY TAC CTT GTT ACG ACT T) 3' were used for the PCR. The PCR reaction was performed with 20 ng of genomic DNA as the template in a 30μl reaction mixture by using a *EF-Taq*(SolGent, Korea) as follows: activation of Taq polymerase at 95 °C for 2minutes, 35 cycles of 95 °C for 1minutes, 55°C, and 72 °C for 1minutes each were performed, finishing with a 10-minute step at 72 °C. The amplification products were purified with a multiscreen filter plate (Millipore Corp., Bedford, MA, USA). Sequencing reaction was performed using a PRISM BigDye Terminator v3.1 Cycle sequencing Kit. The DNA samples containing the extension products were added to Hi-Di formamide (Applied Biosystems, Foster City, CA). The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice and then analyzed by ABI Prism 3730XL DNA analyzer (Applied Biosystems, Foster City, CA).