LAMPIRAN A
PEMBUATAN REAGEN

Larutan buffer fosfat pH 7 50mM

Ditimbang NaH_2PO_4 sebanyak 1,2686 gram dan $Na_2HPO_4.H_2O$ 50 mM sebanyak 2,3072 gram. Kemudian kedua bahan dilarutkan dengan akuades dan ditambahkan akuades hingga 500 ml.

Pati 0,5%

Ditimbang *Starch Soluble* sebanyak 0,5 gram kemudian dilarutkan dalam 100 ml buffer fosfat pH 7 50mM.

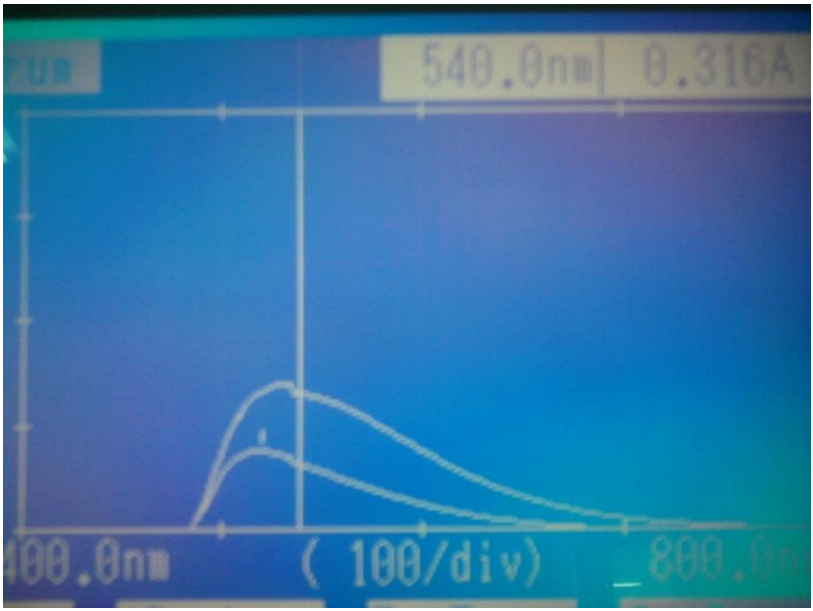
Larutan asam 3,5-dinitrosalisilat (DNS)

Ditimbang sebanyak 1,06 gram DNS dan 1,98 gram NaOH kemudian dilarutkan dengan 141,6 ml akuades. Setelah larut, ditambahkan Natrium Kalium Tartrat sebanyak 30,6 gram, 0,76 ml fenol, dan 0,83 gram Natrium metabisulfit. Diaduk hingga larut.

Larutan kemudian dititrasi dengan 0,1 N menggunakan indikator Phenolptalein (PP).

LAMPIRAN B

SPEKTRUM GLUKOSA DAN BLANKO ENZIM



Gambar L.2.1. Hasil tumpang tindih spektrum glukosa dan blanko enzim

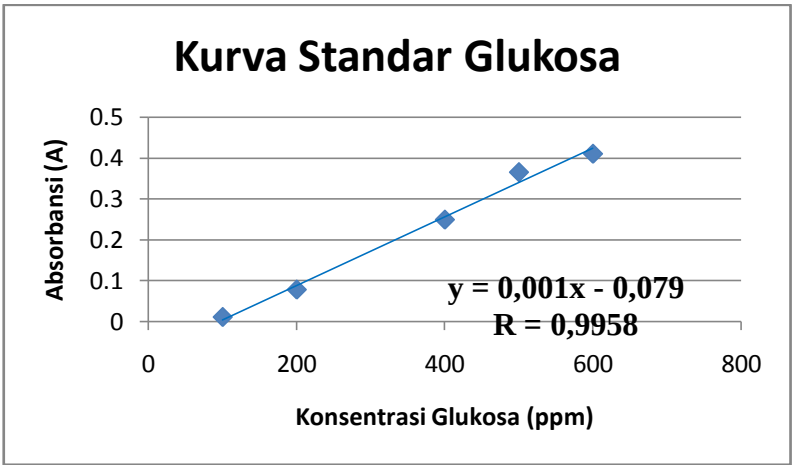
Panjang gelombang pada puncak spektrum glukosa (spektrum bawah) adalah 521 nm dan Panjang gelombang pada puncak spektrum blanko enzim (spektrum atas) adalah 534 nm.

LAMPIRAN C

KURVA STANDAR GLUKOSA

Tabel L.3.1. Data Pengukuran Standar Glukosa

C (ppm)	Absorbansi (A)
100	0,011
200	0,078
400	0,249
500	0,365
600	0,410



Gambar L.3.1. Kurva standar glukosa

Persamaan regresi linier yang diperoleh adalah:

Y = 0,001x-0,079

R = 0,9958

LAMPIRAN D

HASIL PENGUKURAN BLANKO ENZIM

Tabel L.4.1. Hasil Pengukuran Absorbansi Blanko Enzim

Inkubasi	Rep	A	Inkubasi	Rep	A
24 jam	1	0,528	48 jam	1	0,424
24 jam	2	0,567	48 jam	2	0,491
Rata-rata		0,548	Rata-rata		0,458

LAMPIRAN E

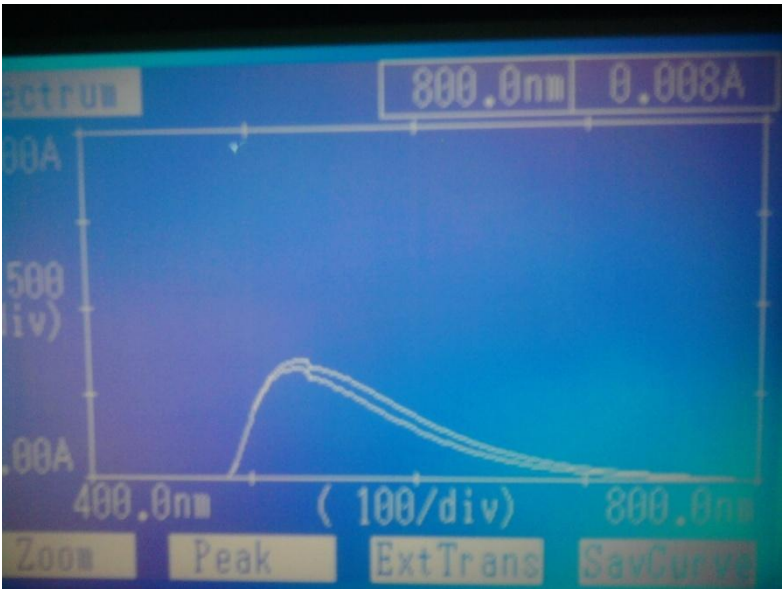
HASIL PENGUKURAN AKTIVITAS ENZIM AMILASE

Tabel L.5.1. Hasil Pengukuran Aktivitas Ekstrak Kasar Enzim Amilase

Inkubasi (jam)	Rep	A	ΔA	Cs (ppm)	mg Glukosa	AE (U)	AErata2 ± SD
24	1	0,678	0,131	250,083	0,500	0,278	0,290 ± 0,049
24	2	0,655	0,108	222,634	0,445	0,247	
24	3	0,728	0,181	309,756	0,620	0,344	
48	1	0,742	0,285	433,875	0,868	0,482	0,458 ± 0,025
48	2	0,704	0,247	388,523	0,777	0,432	
48	3	0,725	0,268	413,586	0,827	0,460	

LAMPIRAN F

SPEKTRUM SAMPEL DAN BLANKO ENZIM



Gambar L.6.1. Hasil tumpang tindih spektrum sampel dan blanko enzim

Panjang gelombang yang digunakan adalah 521 nm. Spektrum atas merupakan spektrum dari sampel dan spektrum bawah merupakan spektrum dari blanko enzim. Aktivitas enzim ditentukan dari selisih absorbansi antara sampel dan blanko enzim.

LAMPIRAN G

PERHITUNGAN AKTIVITAS ENZIM AMILASE

Absorbansi (ΔA) = $Abs_{sampel} - Abs_{enzim}$

Persamaan regresi Linier $Y = bx + a$

Aktivitas enzim = $\frac{\text{jumlah glukosa (mg) x 1000}}{BM_{glukosa} \text{ x Masa inkubasi (menit)}}$

Contoh:

(Sampel 24 jam replikasi 1)

Persamaan regresi linier Y = 0,001x-0,0079

Abs_{sampel} : 0,678

Abs_{enzim} : 0,548

ΔA : 0,131

Waktu inkubasi : 10 menit

Berat molekul glukosa :180

Konsentrasi produk :

Y = 0,001x-0,0079

Y= ΔA

x= Konsentrasi glukosa

0,131 = 0,001x-0,0079

x= 250,083 ppm

jumlah glukosa (mg) = (250,083 / 1000) x 2 = 0,500

(enzim yang digunakan 0,5 ml sehingga jumlah glukosa dikalikan 2 untuk mendapatkan aktivitas enzim per ml)

$$\text{Aktivitas amilase} = \frac{0{,}500 \times 1000}{180 \times 30}$$

$$= 0{,}278 \text{ U/ml}$$

LAMPIRAN H

KARAKTERISTIK FISIOLOGIS SPESIES *BACILLUS*

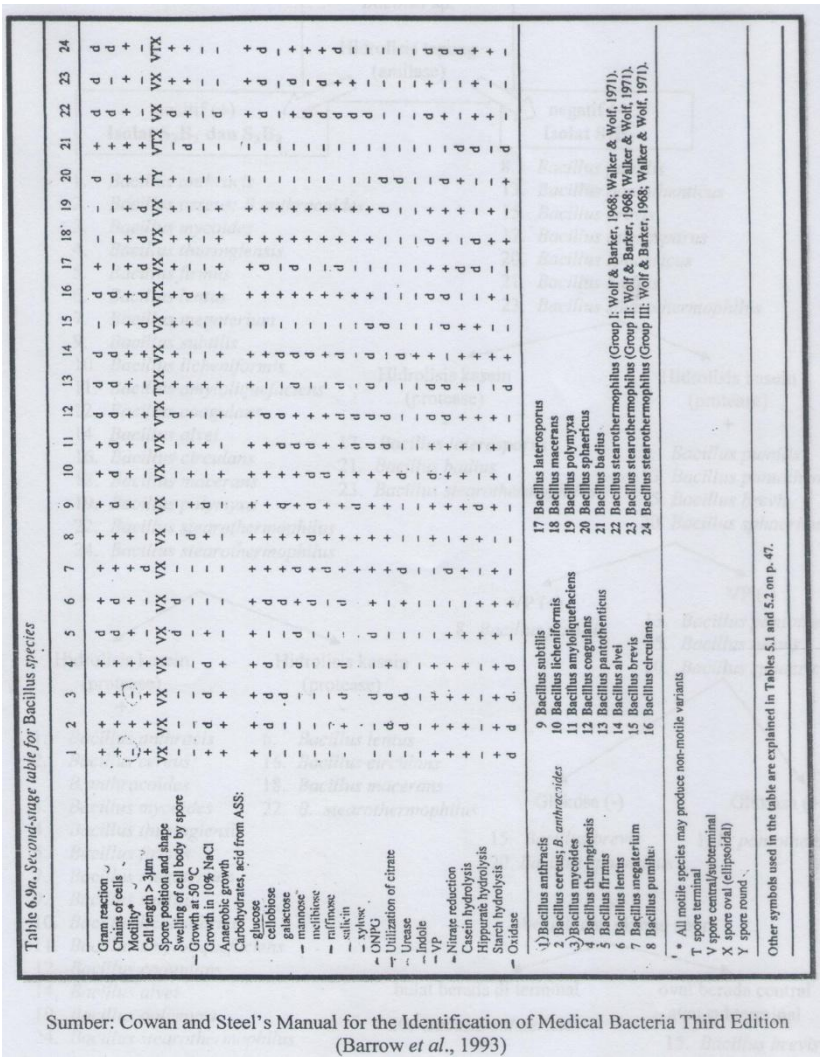


Table 6.9*n*. *Second-stage table for* Bacillus *species*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Gram reaction	+	+	+	+	d	+	+	+	+	+	+	d	d	d	–	d	+	–	–	d	+	d	d	d
Chains of cells	+	+	+	+	d	d	+	+	d	d	d	d	d	–	d	–	+	–	–	–	+	d	–	d
Motility*	(–)	+	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	–
Cell length > 3µm	+	+	+	+	–	d	–	–	–	–	–	+	–	+	d	d	d	d	d	+	+	+	–	–
Spore position and shape	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VTX	TVX	VX	VTX	VX	VX	VX	TV	VTX	VX	VX	VTX
Swelling of cell body by spore	–	–	–	–	d	d	–	–	–	–	–	d	+	+	+	+	+	+	+	+	–	d	+	+
Growth at 50 °C	–	–	–	–	–	–	–	d	+	+	+	+	+	–	+	d	–	+	–	–	d	+	+	+
Growth in 10% NaCl	+	d	d	d	+	–	–	+	d	–	+	–	+	–	–	–	–	+	–	–	–	–	–	–
Anaerobic growth	+	+	+	+	–	–	–	–	–	+	–	+	+	+	–	+	+	+	+	–	–	d	–	–
Carbohydrates, acid from ASS:																								
– glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+
cellobiose		d	d	d	–	d	+	+	+	+	+	d	–	+	–	+	+	+	+	–	–	d	d	d
galactose	–	–	d	–	d	d	+	+	d	+	d	d	d	d	–	d	–	+	+	–	–	–	–	–
– mannose	–	–	–	–	d	+	d	+	d	+	d	+	–	–	–	+	–	+	+	–	–	+	d	+
melibiose	–	–	–	–	–	d	+	d	d	d	d	+	–	d	–	+	–	+	+	–	–	d	–	+
– raffinose	–	–	–	–	–	d	+	d	+	+	+	+	–	+	–	+	–	+	+	–	–	d	d	+
– salicin	–	+	d	d	–	d	+	+	+	+	+	d	d	d	–	d	+	+	+	–	–	d	+	d
– xylose	–	–	d	–	d	–	+	+	d	+	+	d	d	–	–	+	–	+	+	–	–	d	–	–
+ ONPG	–	–	–	–	–	+	+	+	+	+	+	d	d	d	d	+	–	+	+	–	–	d	–	–
+ Utilization of citrate	–	d	d	+	–	+	+	+	+	+	+	d	–	–	d	–	–	–	d	–	–	–	–	–
– Urease	–	d	d	–	–	+	d	–	–	d	–	–	–	d	–	–	–	–	–	d	–	–	–	–
– Indole	+	+	+	+	–	–	–	–	–	–	–	–	d	+	–	d	d	–	–	–	–	–	+	d
– VP	+	+	+	+	–	–	–	+	+	d	+	d	d	+	–	d	+	d	+	–	–	d	–	d
+ Nitrate reduction	+	+	+	+	+	–	d	–	+	+	+	d	d	–	d	d	+	+	+	d	–	+	–	d
Casein hydrolysis	+	+	+	–	+	–	+	+	+	+	+	+	+	+	+	–	d	–	+	+	d	+	+	+
Hippurate hydrolysis	–	–	–	+	+	+	–	+	d	–	–	+	–	+	+	–	+	d	–	–	d	+	–	+
Starch hydrolysis	+	+	+	+	+	+	–	–	+	+	+	+	–	+	–	+	–	+	+	–	–	+	–	+
– Oxidase	d	d	d.	d	–	+	–	–	–	–	–	–	d	+	–	–	–	+	–	+	d	–	–	–

1) Bacillus anthracis
2 Bacillus cereus; *B. anthracoides*
3) Bacillus mycoides
4 Bacillus thuringiensis
5 Bacillus firmus
6 Bacillus lentus
7 Bacillus megaterium
8 Bacillus pumilus
9 Bacillus subtilis
10 Bacillus licheniformis
11 Bacillus amyloliquefaciens
12 Bacillus coagulans
13 Bacillus pantothenticus
14 Bacillus alvei
15 Bacillus brevis
16 Bacillus circulans
17 Bacillus laterosporus
18 Bacillus macerans
19 Bacillus polymyxa
20 Bacillus sphaericus
21 Bacillus badius
22 Bacillus stearothermophilus (Group I: Wolf & Barker, 1968; Walker & Wolf, 1971).
23 Bacillus stearothermophilus (Group II: Wolf & Barker, 1968; Walker & Wolf, 1971).
24 Bacillus stearothermophilus (Group III: Wolf & Barker, 1968; Walker & Wolf, 1971).

* All motile species may produce non-motile variants

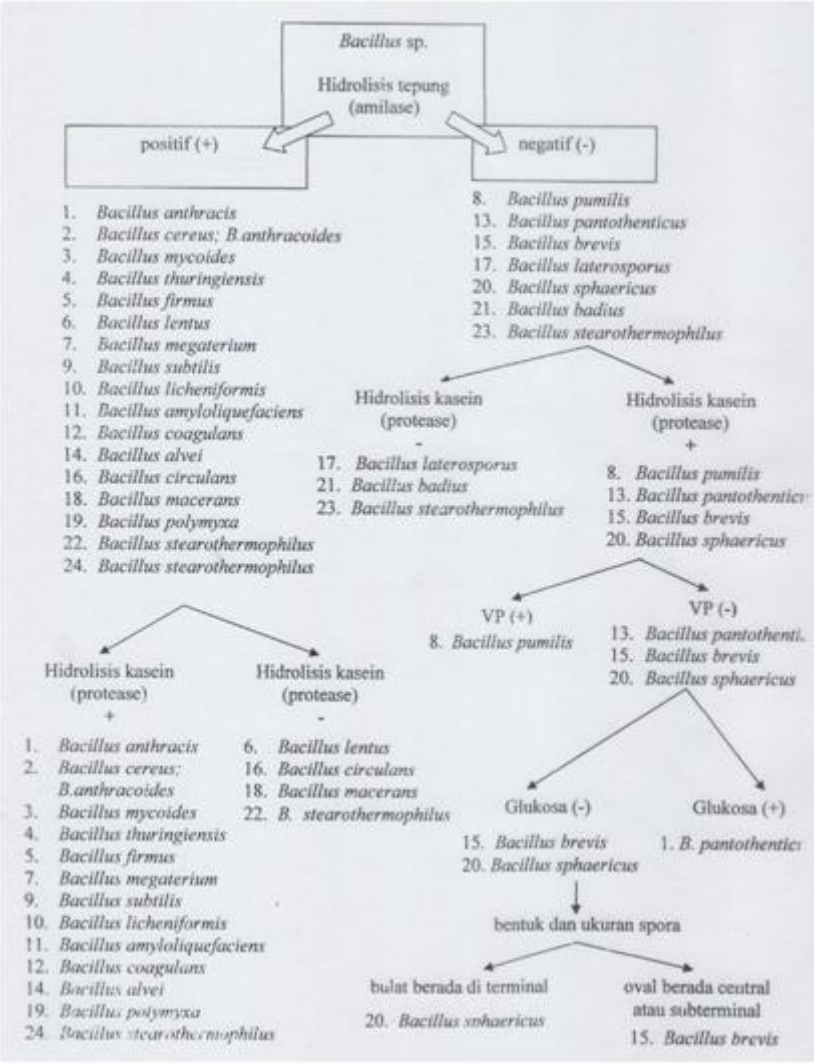
T spore terminal
V spore central/subterminal
X spore oval (ellipsoidal)
Y spore round

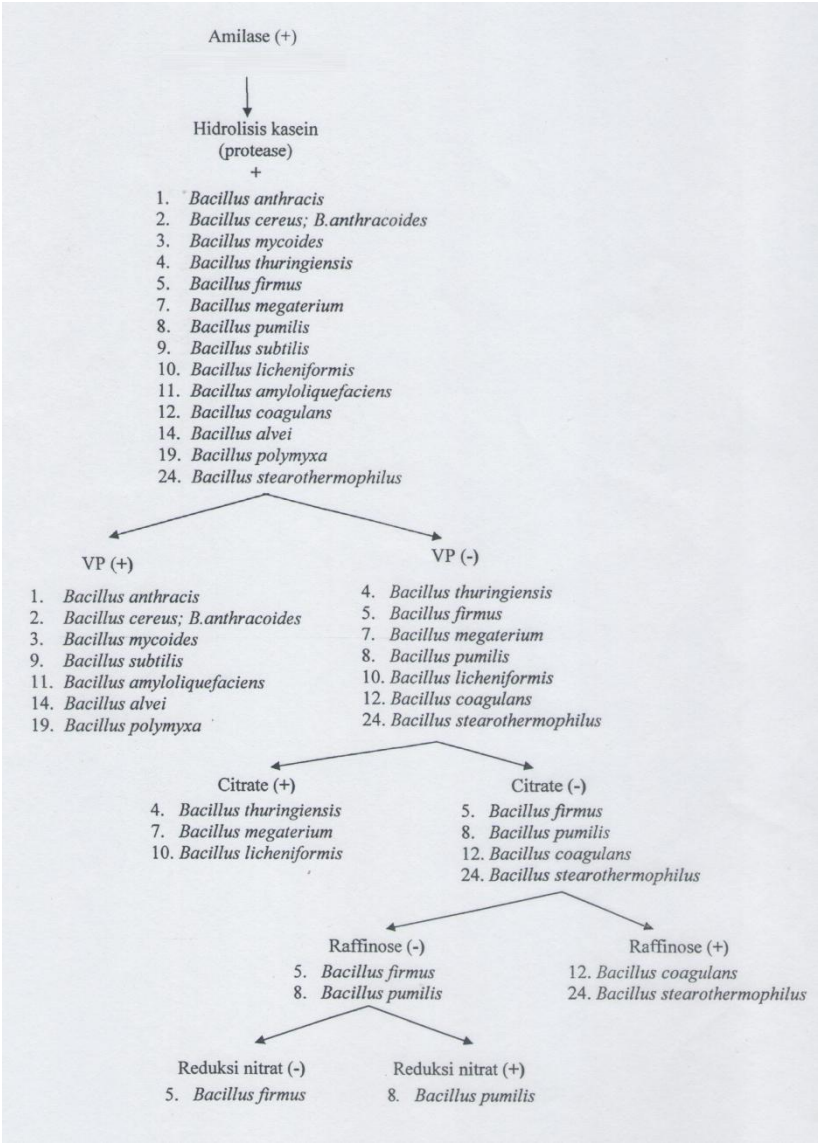
Other symbols used in the table are explained in Tables 5.1 and 5.2 on p. 47.

Sumber: Cowan and Steel's Manual for the Identification of Medical Bacteria Third Edition (Barrow *et al*., 1993)

LAMPIRAN I

PENGELOMPOKKAN SPESIES *BACILLUS*




Amilase (+)
Hidrolisis kasein
(protease)
+
1. Bacillus anthracis
2. Bacillus cereus; B.anthracoides
3. Bacillus mycoides
4. Bacillus thuringiensis
5. Bacillus firmus
7. Bacillus megaterium
8. Bacillus pumilis
9. Bacillus subtilis
10. Bacillus licheniformis
11. Bacillus amyloliquefaciens
12. Bacillus coagulans
14. Bacillus alvei
19. Bacillus polymyxa
24. Bacillus stearothermophilus
VP (+)
1. Bacillus anthracis
2. Bacillus cereus; B.anthracoides
3. Bacillus mycoides
9. Bacillus subtilis
11. Bacillus amyloliquefaciens
14. Bacillus alvei
19. Bacillus polymyxa
VP (-)
4. Bacillus thuringiensis
5. Bacillus firmus
7. Bacillus megaterium
8. Bacillus pumilis
10. Bacillus licheniformis
12. Bacillus coagulans
24. Bacillus stearothermophilus
Citrate (+)
4. Bacillus thuringiensis
7. Bacillus megaterium
10. Bacillus licheniformis
Citrate (-)
5. Bacillus firmus
8. Bacillus pumilis
12. Bacillus coagulans
24. Bacillus stearothermophilus
Raffinose (-)
5. Bacillus firmus
8. Bacillus pumilis
Raffinose (+)
12. Bacillus coagulans
24. Bacillus stearothermophilus
Reduksi nitrat (-)
5. Bacillus firmus
Reduksi nitrat (+)
8. Bacillus pumilis

LAMPIRAN J

PEMBACAAN MICROBACT KIT 12A DAN 12B

TABLE OF SUBSTRATES AND REACTIONS (12A/12F/24E):

Well No.	Designation	Reaction Principle	Reaction colours		Comments
			Negative	Positive	
1	Lysine	Lysine decarboxylase	Yellow	Blue - green	Green or blue is positive reaction. Bromothymol blue indicates formation of the specific amine cadaverine
2	Ornithine	Ornithine decarboxylase	Yellow-green	Blue	Green should be regarded as a negative reaction. The pH shift indicated by bromothymol blue caused by formation of the specific amine putrescine is greater than that caused by lysine decarboxylation
3	H_2S	H_2S production	Straw colour	Black	H_2S is produced from thiosulphate. H_2S reacts with ferric salts in the medium to form a black precipitate
4	Glucose	Glucose fermentation	Blue-green	Yellow	Bromothymol blue indicator changes from blue to yellow when the carbohydrate is utilised to form acid
5	Mannitol	Mannitol fermentation	Blue-green	Yellow	
6	Xylose	Xylose fermentation	Blue-green	Yellow	
		Hydrolysis of o-nitrophenyl-ß-d-			ß-galactosidase hydrolysis of the

7	ONPG	galactopyranoside (ONPG)by action of ß-galactosidase	Colourless	Yellow	ONPG releases yellow orth-onitrophenol.
8	Indole	Indole production from tryptophan	Colourless	Pink-red	Indole is formed from metabolism of tryptophan. Indole Kovacs reagent forms a pink-red complex with indole.
9	Urease	Urea hydrolysis	Straw colour	Pink-red	Ammonium released from splitting of urea causes the pH to rise - indicated by phenol red changing from yellow to pink-red
10	VP	Acetoin production (Voges-Proskaüer reaction)	Straw colour	Pink-red	Acetoin is produced from glucose indicated by the formation of a pink-red complex after the addition of alpha-naphthol and creatine.
11	Citrate	Citrate utilization (citrate is the only source of carbon)	Green	Blue	Citrate is the sole carbon source, which if utilized results in a pH rise, indicated by bromothymol blue, with a colour change from green to blue.
12	TDA	Production of indolepyruvate by deamination of tryptophan	Straw colour	Cherry red	Tryptophan deaminase forms indolepyruvic acid from tryptophan which produces a brown colour in the presence of ferric ions. Indole positive organisms may produce a brown colour. This is a negative reaction.

Well No. 12B/24E.	Designation	Reaction Principle	Reaction colours Negative	Positive	Comments
1/13	Gelatin	Gelatin liquefaction	Colourless	Black	Liquefaction of gelatin by proteolytic enzymes diffuses the black pigment Solid gelatin particles which may drift across the well after rehydration should be considered as a negative reaction.
2/14	Malonate	Malonate inhibition	Green	Blue	Sodium malonate is the sole carbon source and this inhibits the conversion of succinic acid to fumaric acid An organism unable to utilize this substrate results in the accumulation of succinic acid and the organism cannot grow. Bromothymol blue is the indicator. Yellow-green is indicative of a negative result.
3/15	Inositol	Inositol fermentation	Blue-green	Yellow	Utilisation of Na malonate at the same time that ammonium sulphate is utilised as the nitrogen source produces sodium hydroxide resulting in increased alkalinity and a blue colouration

4/16	Sorbitol	Sorbitol fermentation	Blue-green	Yellow	
5/17	Rhamnose	Rhamnose fermentation	Blue-green	Yellow	
6/18	Sucrose	Sucrose fermentation	Blue-green	Yellow	Bromothymol blue indicator changes from blue to yellow when the carbohydrate is fermented.
7/19	Lactose	Lactose fermentation	Blue-green	Yellow	
8/20	Arabinose	Arabinose fermentation	Blue-green	Yellow	
9/21	Adonitol	Adonitol fermentation	Blue-green	Yellow	
10/22	Raffinose	Raffinose fermentation	Blue-green	Yellow	
11/23	Salicin	Salicin fermentation	Blue-green	Yellow	
12/24	Arginine	Arginine dihydrolase 24 hours	Yellow	Green-blue	Argine dihydrolase converts arginine into ornithine, ammonia and carbon dioxide. This causes a pH rise as indicated by bromothymol blue. Green reactions occurring at 48 hours should be interpreted as negative
		48 hours	Yellow-green	Blue	

LAMPIRAN K
HASIL UJI FISIOLOGIS ISOLAT

Tabel 2. Isolat bakteri B pada uji amilase positif (+)

No.	Jenis uji	B	1	2	3	4	5	6	7	9	10	11	12	14	16	18	19	22	24
1	Pewarnaan Gram	+	+	+	+	+	d	+	+	+	+	+	d	d	d	-	-	d	d
2	Bentuk sel	bt	bt	bt	bt	bt	bt	bt	bt	bt	bt	Bt	bt	bt	bt	bt	bt	bt	bt
3	Sel yang membentuk rantai	-	+	+	+	+	d	d	+	d	d	d	d	d	d	-	-	d	d
4	Motilitas	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Panjang sel > 3 µm	+	+	+	+	+	-	-	d	-	-	-	+	+	d	d	d	-	-
6	Betuk dan ukuran spora	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VTX	VX	VTX	VX	VX	VX	VTX
7	Pembengkakan sel spora	-	-	-	-	-	d	d	-	-	-	-	d	+	+	+	+	d	+
	Uji produksi asam dari fermentasi karbohidrat :																		
8	Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	Cellubiose (Hydrolysis selulase)	-	-	d	d	d	-	d	+	+	+	+	d	+	+	+	+	d	d
10	Galactose (Gelatin)	-	-	-	d	-	d	d	+	d	+	d	d	d	+	+	+	-	-
11	Mannose (Mannitol)	-	-	-	-	d	d	+	d	+	+	d	+	d	+	+	+	+	+
12	Raffinose	-	-	-	-	-	-	-	d	+	d	+	+	+	+	+	+	d	+
13	Salicin	-	-	+	d	d	-	d	+	+	+	+	d	d	+	+	+	d	d
14	Xylose	+	-	-	-	-	-	-	+	d	+	d	d	-	+	+	+	d	-
15	ONPG	+	-	-	d	-	d	+	+	+	+	d	d	d	+	+	+	d	-
16	Citrate	-	-	d	d	+	-	-	+	+	+	d	d	-	-	-	d	-	-
17	Urease	-	-	d	d	-	-	+	d	-	d	-	-	d	-	-	-	-	-
18	Indole	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
19	VP (*Voges Proskauer*)	d	+	+	+	+	-	-	-	+	d	+	d	+	d	d	+	d	d
20	Reduksi nitrate	+	+	+	+	+	+	-	d	+	+	+	d	-	d	+	+	+	d
21	Oksidase	+	d	d	d	d	-	+	-	-	-	-	-	+	-	+	-	-	-
22	Hidrolisis kasein (protein)	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	d
23	Hidrolisis tepung (amilum)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Koefisien sebanding (%)		**82**	61	61	61	61	40	48	52	48	52	48	43	48	56	52	52	48

Keterangan:
(T) spora terminal , (V) spora sentral atau subterminal, (X) spora oval , (Y) spora bulat
(1) *Bacillus anthracis*; (2) *Bacillus cereus*, *Bacillus anthracoide*; (3) *Bacillus mycoides*; (4) *Bacillus thuringiensis* ; (5) *Bacillus firmus*;
(6) *Bacillus lentus*; (7) *Bacillus megaterium*; (9) *Bacillus subtilis*; (10) *Bacillus licheniformis*; (11) *Bacillus amyloliquefaciens*;
(12) *Bacillus coagulans*; (14) *Bacillus alvei*; (16) *Bacillus circulans*; (18) *Bacillus macerans*; (19) *Bacillus polymyxa*;
(22) *Bacillus stearothermophilus*; (24) *Bacillus stearothermophilus*

LAMPIRAN L
PERHITUNGAN ANGKA KOEFISIEN SEBANDING *BACILLUS*

Perhitungan persentase indeks kesamaan menggunakan koefisien sebanding (Ss) mencakup kesamaan positif dan negatif (Stainer *et al*, 1986). Perhitungan menggunakan rumus:

$$Ss = \left(\frac{a + d}{a + b + c + d}\right) \times 100\%$$

Keterangan :

Ss = Koefisien sebanding
a = Jumlah ciri positif pada kedua galur bakteri
b = Jumlah ciri positif pada galur 1 dan negatif pada galur 2
c = Jumlah ciri negatif pada galur 1 dan positif pada galur 2
d = Jumlah ciri negatif pada kedua galur bakteri

Contoh :

(koefisien sebanding dengan *Bacillus anthracis*)
a = 9
b = 2
c = 2
d = 10

Ss = (9 + 10 / (9 + 2 + 2 + 10)) x 100%
= (19 / 23) x 100%
= 82 %