LAMPIRAN A.

Pembuatan Reagen

Komposisi Pereaksi 1 Metode Lowry, Komposisi Larutan Buffer Fosfat pH 7, Komposisi Media Agar Susu Skim dan Media Produksi

a. Komposisi Pereaksi 1 menurut Pakpahan (2009) : Pereaksi A (2 gr Na_2CO_3 dilarutkan dalam NaOH 0,1 N hingga batas 100 mL dalam labu takar), pereaksi B (0,5 gr $CuSO_4.5H_2O$ dilarutkan dalam Natrium Kalium Tartrat 1% hingga batas 100 mL dalam labu takar) dan pereaksi 1 (50 mL pereaksi A ditambah 1 mL pereaksi B dan dihomogenkan).

b. Komposisi Larutan Buffer Fosfat, yaitu ditimbang NaH_2PO_4 50 mM sebanyak 0,4024 gram dan $Na_2HPO_4.H_2O$ 50 mM sebanyak 0,9228 gram. Lalu kedua bahan dilarutkan dengan akuades dan dituang dalam beaker 500 ml.

c. Komposisi media agar susu skim, yaitu media agar susu skim mengandung 4,9 gr susu skim dan 3 gr agar. 4,9 gr susu skim dilarutkan dalam 200 mL akuades kemudian disterilkan dengan otoklaf dengan tekanan 10 lbs. 3 gr agar dilarutkan dalam 200 mL akuades, disterilkan dengan otoklaf dengan tekanan 15 lbs dan dicampur dengan 20 mL larutan susu waktu masih panas.

d. Komposisi media produksi, yaitu susu skim tanpa agar (4,9 gr susu skim dilarutkan dalam 200 mL akuades kemudian disterilkan

dengan otoklaf dengan tekanan 10 lbs. 200 mL akuades, disterilkan dengan otoklaf dengan tekanan 15 lbs dan dicampur dengan 20 mL larutan susu waktu masih panas.

LAMPIRAN B.

Contoh Perhitungan Aktivitas Enzim Protease

- **Perhitungan kurva baku**

Larutan baku @ 1ml + 5 ml pereaksi 1 + 0,5 ml pereaksi folin → vol_{tot} = 6,5 ml

No	Konsentrasi Albumin µg/ml	Konsentrasi dalam Kuvet µg/ml	Absorbansi
1	200	30,79	0,259
2	600	92,31	0,376
3	1000	153,85	0,523
4	1200	184,62	0,535
5	1500	230,77	0,584

Cara perhitungan :

C dalam kuvet

- 200 ppm → 1/ 6,5 x 200 = 30, 79 µg/ml
- 600 ppm → 1/ 6,5 x 600 = 92,31 µg/ml
- 1000 ppm → 1/ 6,5 x 1000 = 153,85 µg/ml
- 1200 ppm → 1/ 6,5 x 1200 = 184,62 µg/ml
- 1500 ppm → 1/ 6,5 x 1500 = 230,77 µg/ml

RL : C kuvet Vs A

A = 0,2225

B = 1,6820 x 10^{-3}

r = 0,9812

- **<u>Contoh Perhitungan Kadar Protein Enzim dan Substrat Terhidrolisis pada Enzim kasar dengan waktu inkubasi 24 jam (sampel):</u>**

1. 0,5 ml enzim + 1 ml buffer Fosfat + 5 ml pereaksi 1 + 0,5 ml pereaksi folin → V_{tot} = 7ml

   0,5 ml enzim (wkt inkubasi 24 jam_1) dengan absorbansi 0,570

   Y = 0,2225 + 1,6820 x 10^{-3} x

   0,570 = 0,2225 + 1,6820 x 10^{-3} x

   X = 206,60

   0,5 ml enzim (wkt inkubasi 24 jam_2) dengan absorbansi 0,590

   Y = 0,2225 + 1,6820 x 10^{-3} x

   0,590 = 0,2225 + 1,6820 x 10^{-3} x

   X = 218,49 µg/ml

   → rata-rata hasil dari enzim 24 jam dan 48 jam = 212,545µg/ml

   Jadi, protein yang terdapat pada 7 ml larutan = 7 x 212,542 = **1487,815 µg.**

2. Untuk kadar protein 0,5 ml protease kasar + 0,5 ml substrat (BSA 1000 µg/ml) + 5 ml pereaksi 1 + 0,5 ml pereaksi folin → V_{tot} = 7ml

   Diperoleh absorbansi 0,574 nm

   Y = 0,2225 + 1,6820 x 10^{-3} x

   0,574 = 0,2225 + 1,6820 x 10^{-3} x

   X = 208, 98 µg/ml

Jadi, jumlah protein dalam 7 ml larutan hasil reaksi E+S = 7 x 208,98 = **1462,86 µg.**

3. Kadar protein dalam 0,5 ml substrat (BSA 1000 µg/ml) = 500 µg.

   S = (a + b) – c

   Keterangan :

   S = Banyaknya substrat yang terhidrolisis

   a = kadar protein dalam substrat

   b = kadar protein dalam enzim

   c = kadar protein hasil reaksi Enzim dan substrat

   S = (a+b) – c

   = 500 + 1487,815 - 1462,86

   = **524,955 µg.**

Jadi, Banyaknya substrat yang terhidrolisis sebesar **524,955 µg.**

4. Aktivitas enzim (V) = Δ [s] / Δs

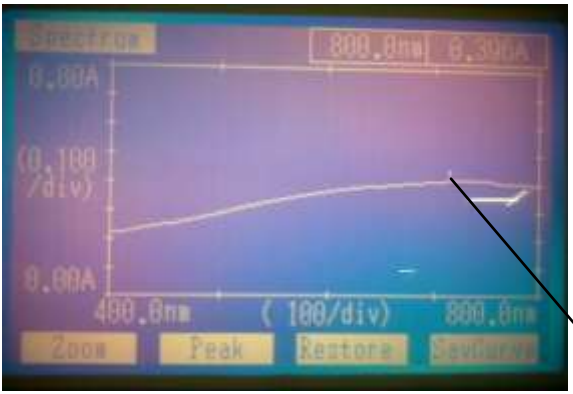
   = 524,955 / 10

   = **52,4955 Unit** (untuk 0,5 ml enzim)

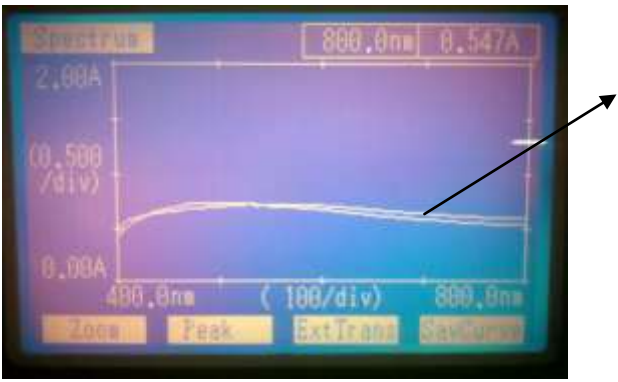
   = 104,991 U/ml

LAMPIRAN C.

Pemilihan Panjang Gelombang



Gambar L3.1. Hasil serapan yang diberikan oleh baku Albumin (C5)



λ pada puncak spektrum adalah 715 nm

Gambar L3.2. Hasil tumpang tindih antara spektrum blanko enzim dengan spektrum sistem enzim+substrat

LAMPIRAN D.

Alat Inkubasi Bakteri Disertai Pengocokan 150 rpm

LAMPIRAN E.

Alat Sentrifugasi dengan Kecepatan 3000 rpm dan suhu 4°C

LAMPIRAN F.

Karakteristik Fisiologis Spesies *Bacillus*

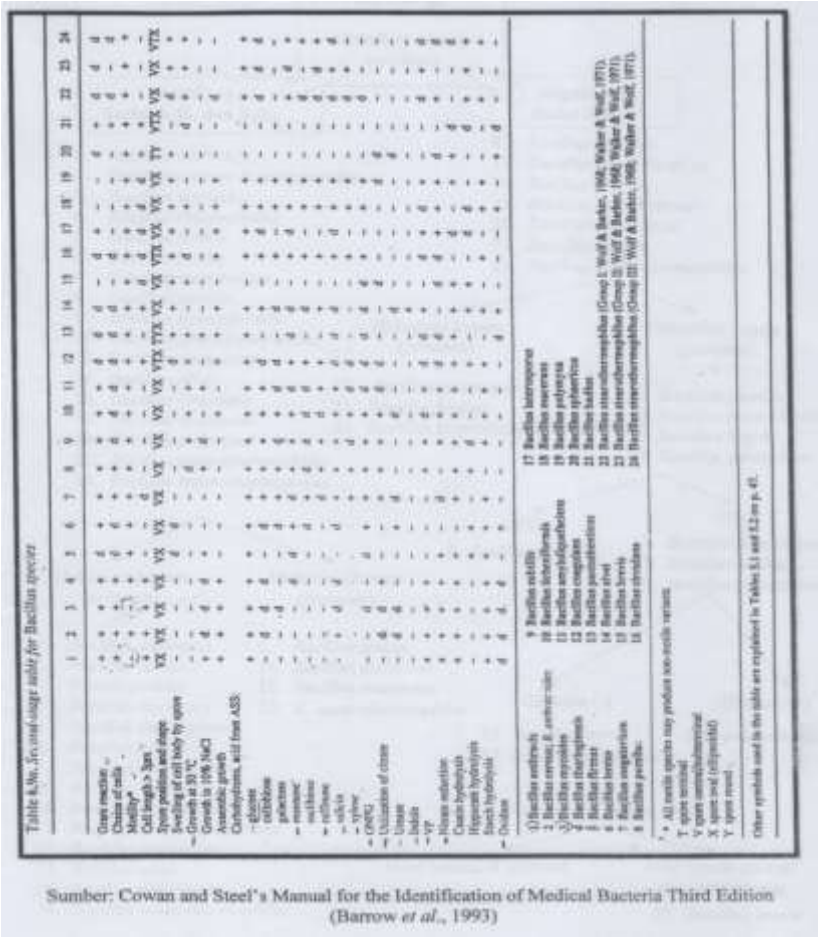


Table 8. Wo. Sporulation table for Bacillus species

	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	+	+	+	+	+	+	+	+
Motility*	+	+	+	+	-	+	+	+	+	+	d	+	-	+	+	d	+	+	+	d	+	+	+	-
Cell length > 3µm	+	+	+	+	-	d	-	-	-	-	-	-	d	d	d	d	d	d	d	d	+	+	+	-
Spore position and shape	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VTX	VX	VX	VX	VTX	VX	VX	VX	VX	TV	VTX	VX	VTX
Swelling of cell body by spore	-	-	-	-	d	-	-	-	-	-	-	d	+	+	+	+	+	+	+	+	-	d	+	+
+ Growth at 30 °C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	d	+	+	+	+	d	+	+	+
Growth in 10% NaCl	-	-	d	+	-	-	-	+	+	+	+	+	-	-	-	d	+	+	+	-	-	-	-	-
Anaerobic growth	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-	d	-	-
Carbohydrates, acid from ASS:																								
+ glucose	+	+	d	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+ arabinose	-	-	d	+	-	+	-	+	+	+	+	d	-	+	+	+	+	+	+	-	-	+	+	+
+ mannose	-	-	d	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ xylose	-	-	-	+	-	d	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ mannitol	-	-	-	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ salicin	-	-	-	+	-	d	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ xylose	-	-	-	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ ONPG	-	-	d	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
+ Utilization of citrate	-	-	d	+	-	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	+	+
- Urease	-	-	d	-	-	-	-	-	-	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
+ VP	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
+ Nitrate reduction	+	+	+	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
Casein hydrolysis	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Hippurate hydrolysis	-	-	-	+	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
+ Oxidase	d	d	d	+	+	+	+	+	+	+	+	+	d	+	+	+	+	+	+	+	d	+	+	+

1) Bacillus anthracis
2. Bacillus cereus, B. anthracis-like
3) Bacillus mycoides
4. Bacillus thuringiensis
5. Bacillus firmus
6. Bacillus larvae
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 lentus
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).

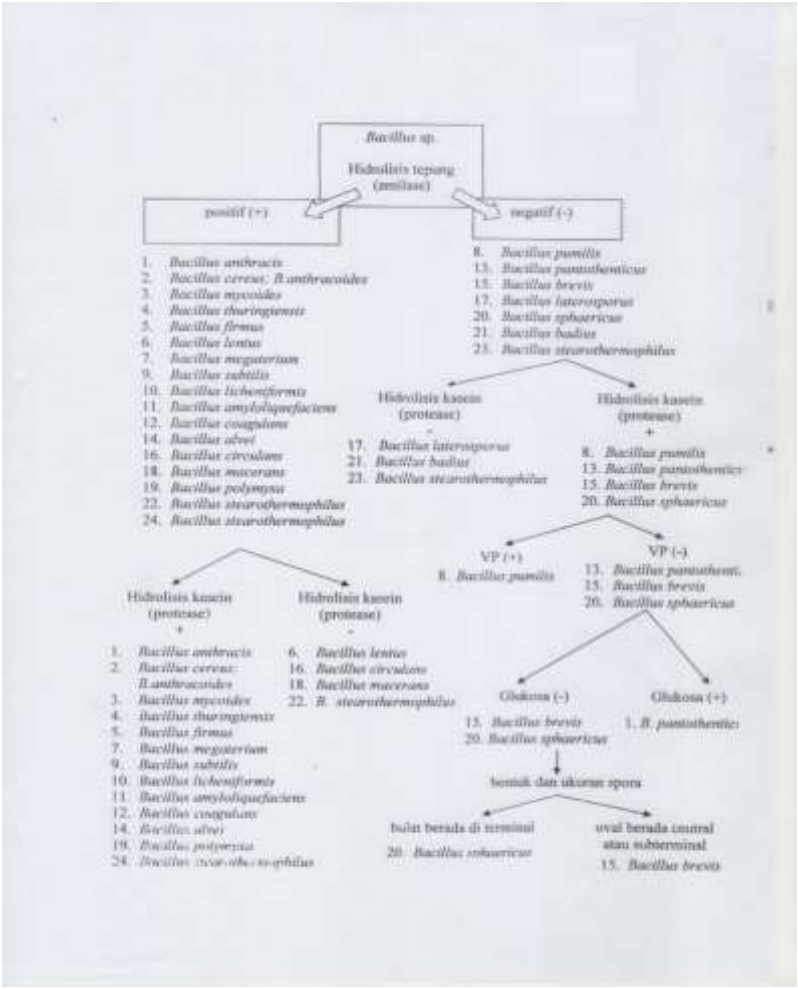
* = All motile species may produce non-motile variant.
T= spore terminal
V= spore central/paracentral
X= spore oval (ellipsoidal)
Y= spore round

Other symbols used in the table are explained in Tables 8.1 and 8.2 on p. 45.

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

LAMPIRAN G.

Pengelompokan Spesies *Bacillus*




Lanjutan diagram Hidrolisis kasein (protease) positif (+)
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 H.

Pembacaan Microbact Kit 12A dan 12B

TABLE OF SUBSTRATES AND REACTIONS (12A/12E/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 &-galactosidase	Colourless	Yellow	o-nitrophenol 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-Proskauer 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. 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.
3/15	Inositol	Inositol fermentation	Blue-green	Yellow	

4/16	Sorbitol	Sorbitol fermentation	Blue-green	Yellow	Bromothymol blue indicator changes from blue to yellow when the carbohydrate is fermented.
5/17	Rhamnose	Rhamnose fermentation	Blue-green	Yellow	
6/18	Sucrose	Sucrose fermentation	Blue-green	Yellow	
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			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
		24 hours	Yellow	Green-blue	
		48 hours	Yellow-green	Blue	

LAMPIRAN I.

Hasil Uji Fisiologis Isolat

Tabel 1. Isolat bakteri A pada uji amilase positif (+)

No	Jenis uji	A	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	Bentuk 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 :																		
10	Glucose	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
11	Cellubiose (Hydrolysis selulase)	-	-	d	d	d	-	d	+	+	+	+	d	+	+	+	+	d	d
12	Galactose (Gelatin)	-	-	-	d	-	-	d	+	d	+	d	d	d	+	+	+	-	-
13	Mannose (Mannitol)	-	-	-	-	d	d	+	d	+	+	d	+	d	+	+	+	+	+
14	Raffinose	-	-	-	-	-	-	-	d	+	d	+	+	+	+	+	+	d	+
15	Salicin	-	-	+	d	d	-	d	+	+	+	+	d	d	+	+	+	d	d
16	Xylose	-	-	-	-	-	-	-	+	d	+	d	d	-	+	+	+	d	-
17	ONPG	+	-	-	d	-	d	+	+	+	+	d	d	d	+	+	+	d	-
18	Citrate	-	-	d	d	+	-	-	+	+	+	d	d	-	-	-	d	-	-
19	Urease	d	-	d	d	-	-	+	d	-	d	-	-	d	-	-	-	-	-
20	Indole	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
21	VP (*Voges Proskauer*)	+	+	+	+	+	-	-	-	+	d	+	d	+	d	d	+	d	d
22	Reduksi nitrate	+	+	+	+	+	+	-	d	+	+	+	d	-	d	+	+	+	d
23	Oksidase	+	d	d	d	d	-	+	-	-	-	-	-	+	-	+	-	-	-
23	Hidrolisis kasein (protein)	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	d
24	Hidrolisis tepung (amilum)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	Koefisien sebanding (%)		**78**	74	70	65	65	52	48	48	48	48	43	57	43	48	48	43	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 J.

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:

$$Ss = \frac{a+d}{b+c+d} \times 100\%$$

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 = 3

c = 2

d = 9

$$Ss = \frac{9+9}{3+2+9} \times 100\%$$

= 78%