LAMPIRAN A
PEMBUATAN REAGEN

Larutan buffer fosfat pH 7

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.

Larutan *Congo-red* 0,1%

Ditimbang dengan teliti 0,1 gram *congo-red* kemudian dilarutkan dengan 100 ml akuades.

Larutan asam 3,5-dinitrosalisilat (DNS)

Sebanyak 1 g DNS dilarutkan dengan 50 ml akuades dalam labu takar 100ml, ditambahkan 12,5 ml NaOH 2N, 10 ml $NaSO_3$ 0,5%, 10 ml Fenol 2% dan ditimbang sebanyak 1 ml garam Rochelle 4% ditambahkan setelah terbentuknya kompleks warna antara DNS dan gula pereduksi hasil hidrolisis.

Substrat CMC 1%

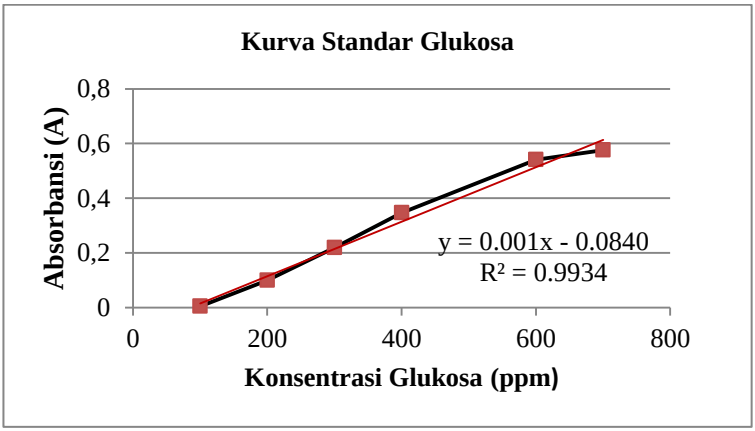
Ditimbang dengan teliti sebanyak 1 gram serbuk *Carboxymethyl Cellulose*dilarutkan dalam 100 ml buffer fosfat sitrat pH 7, kemudian diautoklaf.

LAMPIRAN B

PEMBUATAN KURVA STANDAR GLUKOSA

Tabel L.2.1. Larutan Standar Glukosa

C (ppm)	A^{521}
100	0,005
200	0,100
300	0,219
400	0,347
600	0,541
700	0,576



Gambar L.2.1. Kurva standar glukosa.

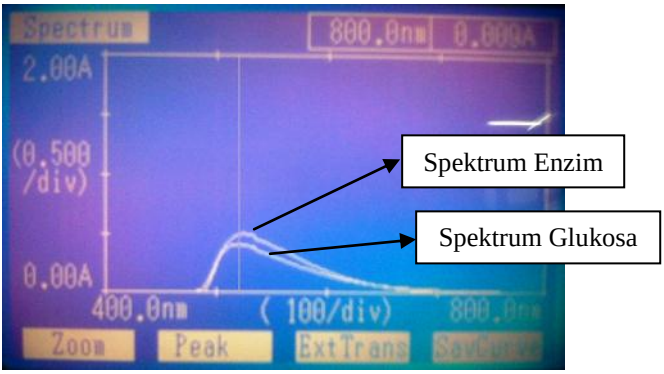
Persamaan regresi linier yang diperoleh adalah:

Y =0,0010x-0,0840

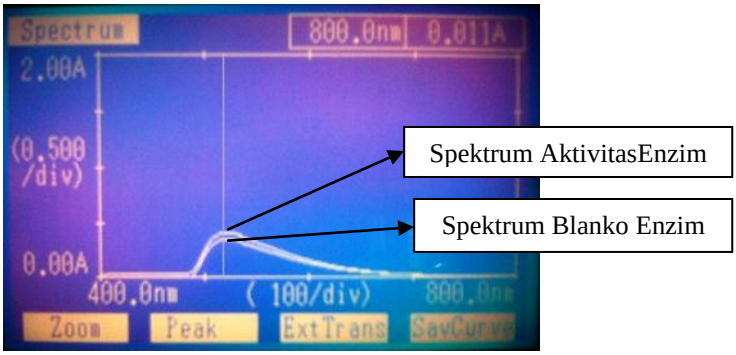
r = 0.9934

LAMPIRAN C

PENENTUAN PANJANG GELOMBANG TERPILIH



Gambar L.3.1. Hasil tumpang tindih spektrum glukosa dengan spektrum aktivitas enzim.



Gambar L.3.2. Hasil tumpang tindih spektrumblanko enzim dengan spektrum aktivitas enzim.

LAMPIRAN D

HASIL PENGUKURAN BLANKO ENZIM

Inkubasi	Rep	A^{521}	Inkubasi	Rep	A^{521}
24 jam	1	0,300	48 jam	1	0,325
24 jam	2	0,314	48 jam	2	0,333
24 jam	3	0,308	48 jam	3	0,342
Rata-rata		0,307	Rata-rata		0,333

Tabel L.4.1. Hasil Pengukuran Blanko Enzim

LAMPIRAN E
PENENTUAN AKTIVITAS ENZIM SELULASE DENGAN METODE DNS

Persamaan regresi Linier Y = bx + a

Aktivitas enzim = Konsentrasi produk (µg/ml) x 1000 : $BM_{glukosa}$ x Waktu inkubasi

Delta A = A sampel - A rata-rata blanko enzim

(Ket: A sampel diambil dari data inkubasi 24 jam replikasi 1)

Misalnya:

(Data diambil dari hasil uji aktivitas enzim inkubasi 24 jam replikasi 1)

Delta A = 0,411 – 0,037

= 0,104

Persamaan regresi linier Y =0,0010x-0,0840

Absorbansi yang diperoleh dari delta A : 0.104

Waktu inkubasi : 30 menit

Berat molekul glukosa :180

Konsentrasi glukosa :

Y = 0,0010x-0,0840

Y= absorbansi

X= Konsentrasi glukosa

0.104 = 0,0010x-0,0840

X = 188.322 µg/ml = 0,188 mg

mg glukosa = 0,188

Aktivitas Selulase = (0,188 x 1000) : (180 x 30)

= 0,035 unit/ml

LAMPIRAN F

DATA AKTIVITAS ENZIM SELULASE

Tabel L.6.1. Data Hasil Uji Aktivitas Ekstrak Kasar Enzim Selulase pada 24 Jam

Inkubasi	Rep	Absorbansi (A)	ΔA (A)	Cs (ppm)	mg Glukosa	AE (U/ml)
24 jam	1	0,411	0,104	188,322	0,188	0,035
24 jam	2	0,330	0,023	107,039	0,107	0,020
24 jam	3	0,361	0,054	138,147	0,138	0,026

Rata – rata aktivitas enzim = $\frac{0,035+0,020+0,026}{3}$ = 0,027 unit/ml

Standar Deviasi aktivitas enzim= 0,007

Tabel L.6.2. Data Hasil Uji Aktivitas Ekstrak Kasar Enzim Selulase pada 48 Jam

Inkubasi	Rep	Absorbansi (A)	ΔA (A)	Cs (ppm)	mg Glukosa	AE (U/ml)
48 jam	1	0,354	0,021	105,032	0,105	0,019
48 jam	2	0,392	0,059	143,165	0,178	0,033
48 jam	3	0,404	0,071	155,207	0,194	0,036

Rata – rata aktivitas enzim = $\frac{0,019 + 0,033 + 0,036}{3}$ = 0,029 unit/ml

Standar Deviasi aktivitas enzim= 0,009

LAMPIRAN G

KARAKTERISTIK FISIOLOGIS SPESIES *BACILLUS*

Karakteristik fisiologis spesies bakteri dari genus *Bacillus*

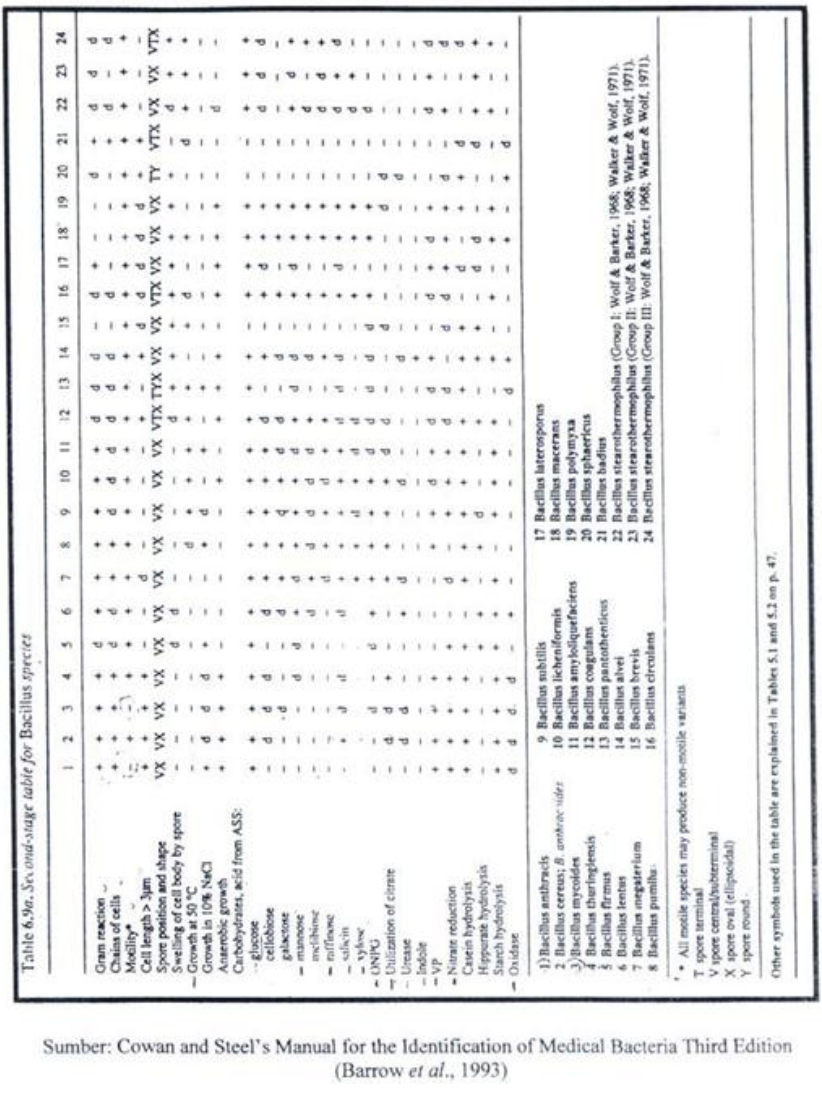


Table 6.9a. *Second-stage table for* Bacillus *species*

	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	d
Motility*	–	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cell length > 3μm	–	+	+	+	–	–	d	–	–	–	–	+	–	+	d	d	d	d	d	d	+	–	–	–
Spore position and shape	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VX	VTX	TYX	VX	VX	VTX	VX	VX	VX	TY	VTX	VX	VX	VTX
Swelling of cell body by spore	–	–	–	–	d	–	–	–	–	–	–	d	+	+	+	+	+	+	+	+	–	d	+	+
– Growth at 50 °C	–	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	d	+	–	–
Growth in 10% NaCl	+	d	d	d	+	–	–	+	d	–	+	–	+	–	–	–	–	–	–	–	–	–	–	–
Anaerobic growth	+	+	+	+	–	–	–	–	–	–	–	+	+	+	–	+	+	+	+	+	–	d	–	–
Carbohydrates, acid from ASS:																								
– glucose	+	+	+	+	+	+	+	+	+	+	+	d	–	+	–	+	+	+	+	–	–	–	+	+
cellobiose	–	d	d	–	d	+	+	+	d	+	d	d	–	d	–	d	d	+	+	–	–	d	d	d
galactose	–	–	d	–	d	+	d	+	d	+	d	d	d	d	–	+	d	+	+	–	–	d	–	–
– mannose	–	–	–	–	–	d	+	d	d	+	d	+	–	d	–	+	–	+	+	–	–	–	d	+
melibiose	–	–	–	–	–	–	d	+	d	d	+	+	d	+	–	+	–	+	+	–	–	+	–	–
– raffinose	–	+	d	d	–	d	+	+	+	d	+	d	d	+	d	+	d	+	+	–	–	d	d	d
– silicin	–	+	d	d	–	d	+	+	+	d	+	d	d	+	d	+	d	+	+	–	–	d	+	+
– xylose	–	–	d	–	d	+	+	+	d	d	+	d	d	–	–	+	–	+	+	–	–	d	+	d
+ ONPG	–	–	d	–	d	+	+	+	+	+	+	d	d	d	d	+	–	+	d	d	–	d	–	–
– Utilization of citrate	–	d	d	+	–	+	+	+	+	+	d	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	–	–	–	d
+ Nitrate reduction	+	+	+	+	+	–	d	–	+	+	+	d	d	+	d	+	+	+	+	d	–	+	–	d
Casein hydrolysis	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	d	–	+	+	d	–	–	d
Hippurate hydrolysis	–	–	–	+	+	+	–	+	d	–	+	+	–	+	+	–	d	d	–	–	d	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	–	+	+	+	+	–	+	–	+	–	+	+	–	–	+	–	+
– Oxidase	d	d	d	d	–	+	–	–	–	–	–	–	d	+	–	–	–	+	–	+	d	–	–	–

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

* + All motile species may produce non-motile variants

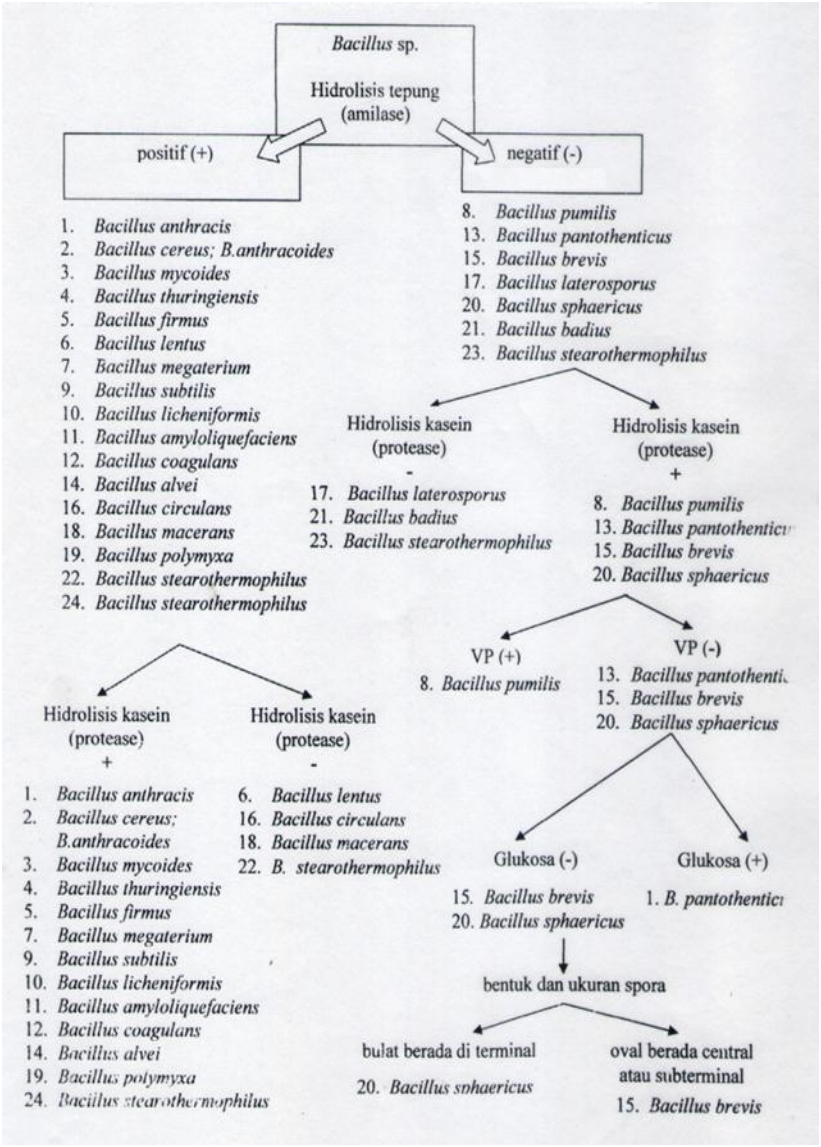
T spore terminal
Y spore central/subterminal
X spore oval (ellipsoidal)
Y spore round

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 H

PENGELOMPOKAN SPESIES *BACILLUS*



LAMPIRAN I

PEMBACAAN MICROBACT KIT 12A DAN 12B

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	

Lanjutan Lampiran I

7	ONPG	galactopyranoside (ONPG)by action of ß-galactosidase	Colodriess	Yellow	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.

Lanjutan Lampiran I

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.
					Sodium malonate is the sole carbon source and this inhibits the conversion of succinic acid to fumaric acid.

Lanjutan Lampiran I

2/14	Malonate	Malonate inhibition	Green	Blue	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	
5/17	Rhamnose	Rhamnose fermentation	Blue-green	Yellow	

Lanjutan Lampiran I

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			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 J

HASIL UJI FISIOLOGIS ISOLAT

Tabel 3 Isolat bakteri C pada uji amilase negatif (-)

No.	Jenis uji	C	8	13	15	17	20	21	23
1	Pewarnaan Gram	+	+	d	-	+	d	+	d
2	Bentuk sel	bt	bt	bt	bt	bt	bt	bt	bt
3	Sel yang membentuk rantai	+	+	d	-	-	-	+	-
4	Motilitas	+	+	+	+	+	+	+	+
5	Panjang sel > 3 µm	+	-	-	d	d	+	+	-
6	Bentuk dan ukuran spora	VX	VX	TYX	VX	VX	TY	VTX	VX
7	Pembengkakan sel spora	-	-	+	+	+	+	-	+
	Uji produksi asam dari fermentasi karbohidrat :								
8	Glucose	+	+	+	-	+	-	-	+
9	Cellubiose (Hydrolysis selulase)	+	+	-	-	d	-	-	d
10	Galactose (Gelatin)	-	+	-	-	-	-	-	-
11	Mannose (Mannitol)	+	+	d	-	d	-	-	d
12	Raffinose	+	+	-	-	-	-	-	d
13	Salicin	+	+	d	-	d	-	-	+
14	Xylose	+	+	-	-	-	-	-	+
15	ONPG (Ortho-Nitrophenyl-ß-D-Galactopyranoside)	+	+	d	d	-	-	-	-
16	Citrate	+	+	-	d	-	d	-	-
17	Urease	+	-	-	-	-	d	-	-
18	Indole	-	-	-	-	-	-	-	-
19	VP (*Voges Proskauer*)	+	+	d	-	+	-	-	+
20	Reduksi nitrate	+	-	d	d	+	d	+	-
21	Oksidase	+	-	-	-	-	+	-	-
22	Hidrolisis kasein (protein)	+	+	+	+	d	+	d	-
23	Hidrolisis tepung (amilum)	-	-	-	-	-	-	-	-
	Koefisien sebanding (%)		78	61	43	61	47	47	61

Keterangan:

(T) spora terminal , (V) spora sentral atau subterminal; (X) spora oval; (Y) spora bulat.

(8) *Bacillus pumilis*; (13) *Bacillus pantothenticus*; (15) *Bacillus brevis*; (17) *Bacillus laterosporus*; (20) *Bacillus sphaericus*; (21) *Bacillus badius*; (23) *Bacillus stearothermophilus*

LAMPIRAN K

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:

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

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 pumiluss*)

a = 15

b = 4

c = 1

d = 3

Ss = (15 + 3) / (15 + 4 + 1 + 3)x 100%

= (18 / 23) x 100%

= 78 %