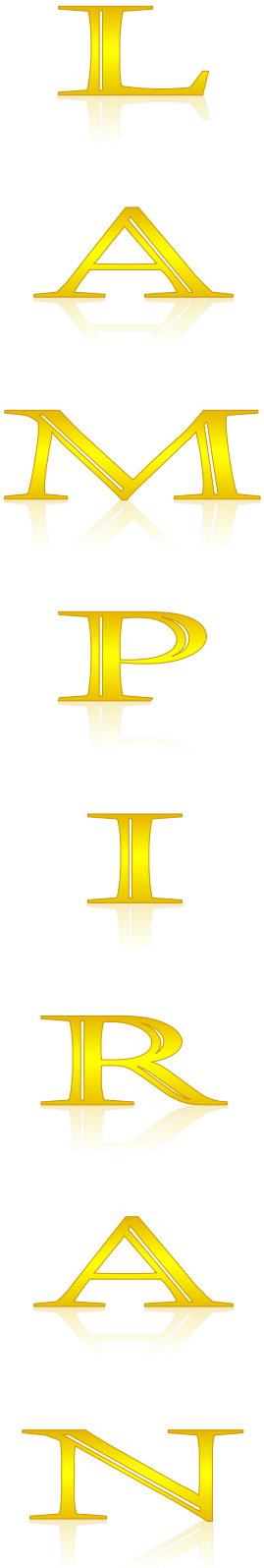
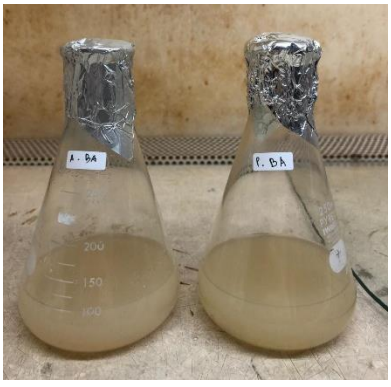

LAMPIRAN

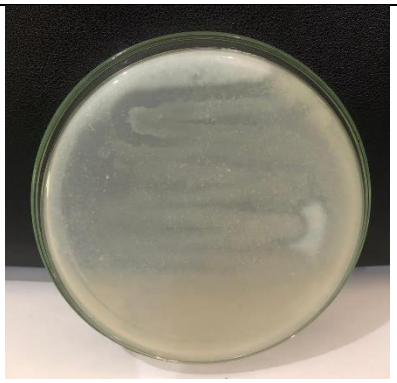
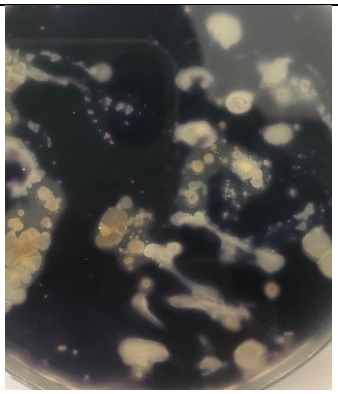
Lampiran 1. Isolat bakteri *Bacillus altitudinis* pada media NA

Lampiran 2. Starter Bakteri

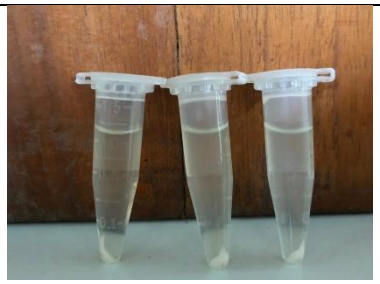
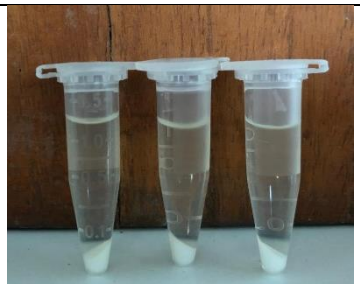
Lampiran 3. Media produksi

	
Sebelum di *incubator shaker*	**Sebelum di *incubator shaker***

Lampiran 4. Hasil skrining aktivitas ekstrak enzim

	
Pada media *Skim Milk Agar* (SMA)	**Pada media *Starch Agar* (SA)**

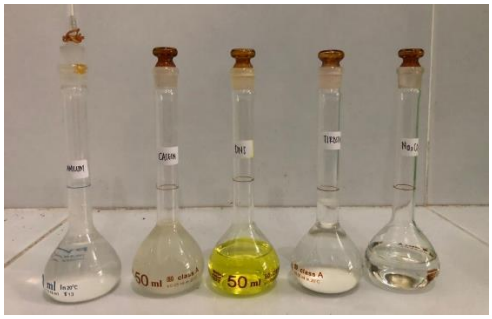
Lampiran 5. Hasil sentrifugasi ekstrak enzim

	
Protease	**Amilase**

Lampiran 6. Hasil supernatan ekstraksi ekstrak enzim

Lampiran 7. Hasil pemurnian enzim

Lampiran 8. Reagen uji aktivitas ekstrak enzim secara kuantitatif



Lampiran 9. Alat yang digunakan untuk praktikum

	
Jarum ose	***Incubator shaker***

Sentrifugator

Autoklaf

Magnetic stirrer

Spektrofotometer UV-Vis

Lampiran 10. Perhitungan aktivitas ekstrak enzim

a. Ekstrak enzim amilase

$$Unit\ Aktivitas\ = \frac{C}{BM\ produk\ x\ t} + \frac{H}{E}$$

Keterangan : C = Konsentrasi gulareduksi (ppm)
BM = Berat molekul glukosa
t = Waktu inkubasi (menit)
H = Volume emzim-substrat (mL)
E = Volume enzim (mL)

Sampel (%)	**Absorbansi (λ = 540 nm)**	**Rata- rata absorbansi**
1 %	0.1073	0.1173
	0.1228	
	0.1218	
1,25 %	0.2479	0.2614

	0.2650	
	0.2713	
1,5 %	0.3620	0.3952
	0.3941	
	0.4295	

1. Konsentrasi substrat 1 %
   y = 0,0044 x + 0,0393
   0, 1773 = 0,0044 x + 0,0393
   x = 31,36 ppm

$$Unit\ Aktivitas\ = \frac{31{,}36\ ppm}{180\ x\ 20} + \frac{2}{1}$$

= 0,0174 U/mL

2. Konsentrasi substrat 1,25%
   0,2614 = 0,0044 x + 0,0393
   x = 50,47 ppm

$$Unit\ Aktivitas\ = \frac{50{,}47\ ppm}{180\ x\ 20} + \frac{2}{1}$$

= 0,0280 U/mL

3. Konsentrasi substrat 1,5%
   0,3953 = 0,0044 x + 0,0393
   x = 80,88 ppm

$$Unit\ Aktivitas\ = \frac{80{,}88\ ppm}{180\ x\ 20} + \frac{2}{1}$$

= 0,0449 U/mL

b. Ekstrak enzim protease

$$\text{PU} = \frac{Asp - Abl}{Ast - Abl} x \frac{1}{T}\ x\ fp$$

Keterangan : PU = unit aktivitas protease (U/mL)
Asp = nilai absorbansi sampel
Ast = nilai absorbansi standar
Abl = nilai absorbansi blanko
T = waktu inkubasi (menit)
Fp = faktor pengenceran

Sampel	**Absorbansi (λ = 578 nm)**	**Rata- rata absorbansi**
1%	0.274	0.2753
	0.276	
	0.276	

1,5%	0.334	0.3440
	0.351	
	0.347	
2%	0.479	0.4827
	0.483	
	0.486	

a. Konsentrasi substrat 1 %

$$\text{PU} = \frac{0{,}2753 - 0{,}1713}{0{,}5360 - 0{,}1713} x \frac{1}{10}\ x\ 10$$

$$= 0{,}2851 \text{ U/mL}$$

b. Konsentrasi substrat 1,5%

$$\text{PU} = \frac{0{,}3440 - 0{,}1713}{0{,}5360 - 0{,}1713} x \frac{1}{10}\ x\ 10$$

$$= 0{,}4735 \text{ U/mL}$$

c. Konsentrasi substrat 2%

$$\text{PU} = \frac{0{,}4827 - 0{,}1713}{0{,}5360 - 0{,}1713} x \frac{1}{10}\ x\ 10$$

$$= 0{,}8538 \text{ U/mL}$$

Lampiran 11. Perhitungan statistik

a. Ekstrak enzim amilase

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Absorbansi	9	,257967	,1217679	,1073	,4295

One-Sample Kolmogorov-Smirnov Test

		Absorbansi
N		9
Normal Parameters[a,b]	Mean	,257967
	Std. Deviation	,1217679
Most Extreme Differences	Absolute	,200
	Positive	,200
	Negative	-,137

Kolmogorov-Smirnov Z	,600
Asymp. Sig. (2-tailed)	,865

a. Test distribution is Normal.

b. Calculated from data.

Descriptives

Absorbansi

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
substrat amilum 1%	3	,117300	,0086747	,0050083	,095751	,138849	,1073	,1228
substrat amilum 1,25%	3	,261400	,0121083	,0069907	,231321	,291479	,2479	,2713
substrat amilum 1,5%	3	,395200	,0337634	,0194933	,311327	,479073	,3620	,4295
Total	9	,257967	,1217679	,0405893	,164368	,351566	,1073	,4295

Test of Homogeneity of Variances

Absorbansi

Levene Statistic	df1	df2	Sig.
1,773	2	6	,248

ANOVA

Absorbansi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,116	2	,058	127,654	,000
Within Groups	,003	6	,000		
Total	,119	8			

Multiple Comparisons

Dependent Variable: Absorbansi

Tukey HSD

(I) Bacillusaltitudinis	(J) Bacillusaltitudinis	Mean	Std.	Sig.	95% Confidence Interval

		Difference (I-J)	Error		Lower Bound	Upper Bound
substrat amilum 1%	substrat amilum 1,25%	-,1441000*	,0173962	,000	-,197476	-,090724
	substrat amilum 1,5%	-,2779000*	,0173962	,000	-,331276	-,224524
substrat amilum 1,25%	substrat amilum 1%	,1441000*	,0173962	,000	,090724	,197476
	substrat amilum 1,5%	-,1338000*	,0173962	,001	-,187176	-,080424
substrat amilum 1,5%	substrat amilum 1%	,2779000*	,0173962	,000	,224524	,331276
	substrat amilum 1,25%	,1338000*	,0173962	,001	,080424	,187176

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Absorbansi

Tukey HSD[a]

Bacillusaltitudinis	N	Subset for alpha = 0.05		
		1	2	3
substrat amilum 1%	3	,117300		
substrat amilum 1,25%	3		,261400	
substrat amilum 1,5%	3			,395200
Sig.		1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

b. Ekstrak enzim protease

NPar Tests

Descriptive Statistics

	N	Mean	Std. Deviation	Minimum	Maximum
Absorbansi	15	,361867	,1381675	,1710	,5370

One-Sample Kolmogorov-Smirnov Test

		Absorbansi
N		15
Normal Parameters[a,b]	Mean	,361867

	Std. Deviation	,1381675
	Absolute	,202
Most Extreme Differences	Positive	,133
	Negative	-,202
Kolmogorov-Smirnov Z		,781
Asymp. Sig. (2-tailed)		,575

a. Test distribution is Normal.

b. Calculated from data.

Oneway

Descriptives

Absorbansi

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
blanko	3	,171333	,0005774	,000333	,169899	,172768	,1710	,1720
standar	3	,536000	,0010000	,000577	,533516	,538484	,5350	,5370
substrat kasein 1%	3	,275333	,0011547	,000667	,272465	,278202	,2740	,2760
substrat kasein 1,5%	3	,344000	,0088882	,005131	,321921	,366079	,3340	,3510
substrat kasein 2%	3	,482667	,0035119	,002027	,473943	,491391	,4790	,4860
Total	15	,361867	,1381675	,035674	,285352	,438381	,1710	,5370

Test of Homogeneity of Variances

Absorbansi

Levene Statistic	df1	df2	Sig.
6,279	4	10	,009

ANOVA

Absorbansi

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	,267	4	,067	3551,539	,000

Within Groups	,000	10	,000		
Total	,267	14			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Absorbansi

Tukey HSD

(I) Bacillusaltitudinis	(J) Bacillusaltitudinis	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
blanko	standar	-,3646667*	,0035402	,000	-,376318	-,353015
	substrat kasein 1%	-,1040000*	,0035402	,000	-,115651	-,092349
	substrat kasein 1,5%	-,1726667*	,0035402	,000	-,184318	-,161015
	substrat kasein 2%	-,3113333*	,0035402	,000	-,322985	-,299682
standar	blanko	,3646667*	,0035402	,000	,353015	,376318
	substrat kasein 1%	,2606667*	,0035402	,000	,249015	,272318
	substrat kasein 1,5%	,1920000*	,0035402	,000	,180349	,203651
	substrat kasein 2%	,0533333*	,0035402	,000	,041682	,064985
substrat kasein 1%	blanko	,1040000*	,0035402	,000	,092349	,115651
	standar	-,2606667*	,0035402	,000	-,272318	-,249015
	substrat kasein 1,5%	-,0686667*	,0035402	,000	-,080318	-,057015
	substrat kasein 2%	-,2073333*	,0035402	,000	-,218985	-,195682
substrat kasein 1,5%	blanko	,1726667*	,0035402	,000	,161015	,184318
	standar	-,1920000*	,0035402	,000	-,203651	-,180349
	substrat kasein 1%	,0686667*	,0035402	,000	,057015	,080318
	substrat kasein 2%	-,1386667*	,0035402	,000	-,150318	-,127015
substrat kasein 2%	blanko	,3113333*	,0035402	,000	,299682	,322985
	standar	-,0533333*	,0035402	,000	-,064985	-,041682
	substrat kasein 1%	,2073333*	,0035402	,000	,195682	,218985
	substrat kasein 1,5%	,1386667*	,0035402	,000	,127015	,150318

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

Absorbansi

Tukey HSD[a]

Bacillusaltitudinis	N	Subset for alpha = 0.05				
		1	2	3	4	5
blanko	3	,171333				
substrat kasein 1%	3		,275333			
substrat kasein 1,5%	3			,344000		
substrat kasein 2%	3				,482667	
standar	3					,536000
Sig.		1,000	1,000	1,000	1,000	1,000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3,000.

Lampiran 12. Komposisi dan pembuatan media

1. Media produksi amilase

   Komposisi : 5 mL starter bakteri
   1,3 gram *Nutrient Broth* (NB)
   2 gram amilum
   100 mL aquades

   Cara Pembuatan :

   Melarutkan semua bahan dalam 100 mL aquades dan homogenkan menggunakan *magnetic stirrer*. Kemudian di sterilkan dengan autoklaf pada suhu 121℃ selama 15 menit. Starter bakteri yang telah tumbuh di tambahkan lalu inkubasi pada suhu 37℃ selama 24 jam.

2. Media produksi protease

   Komposisi : 5 mL starter bakteri
   1,3 gram *Nutrient Broth* (NB)
   1,5 gram susu skim bubuk
   100 mL aquades

   Cara Pembuatan :

   Melarutkan semua bahan dalam 100 mL aquades dan homogenkan menggunakan *magnetic stirrer*. Kemudian di sterilkan dengan autoklaf

pada suhu 121℃ selama 15 menit. Starter bakteri yang telah tumbuh di tambahkan lalu inkubasi pada suhu 37℃ selama 24 jam.

3. *Skim Milk Agar* (SMA)

   Komposisi : 3 gram susu skim bubuk
   1 gram agar
   150 mL aquades

   Cara pembuatan :

   Susu skim bubuk dilarutkan dalam aquades dan agar juga dilarutkan dalam aquades pada masing-masing erlenmeyer, diaduk menggunakan *magnetic stirrer* sampai larut sempurna, kemudian di sterilkan dengan autoklaf pada suhu 121℃ selama 15 menit. Mencampurkan susu skim dan agar pada saat panas, lalu tuangkan pada cawan petri.

4. *Strach Agar* (SA)

   Komposisi : 2,5 gram *Nutrient Agar* (NA)
   1 gram amilum
   100 mL aquades

   Cara pembuatan :

   Reagen-reagen di atas dilarutkan dalam aquades sebanyak 150 mL, diaduk menggunakan *magnetic stirrer* sampai larut sempurna, kemudian di sterilkan dengan autoklaf pada suhu 121℃ selama 15 menit dan dituangkan dalam cawan petri.

Lampiran 13. Pembuatan larutan standar glukosa

Cara membuat larutan stok glukosa standar 1000 ppm adalah :

1000 ppm = $\frac{100\ mg}{100\ ml} = \frac{0{,}1\ g}{100\ ml}$

Menimbang 0,1 g glukosa anhidrat lalu dilarutkan dengan aquades dan di tanda bataskan dalam labu takar 100 mL. Kemudian larutan glukosa dibuat seri konsentrasi 25, 50, 75, 100, 125 dan 150 ppm sebanyak 100 mL. pembuatan larutan sesuai dengan menggunakan rumus sebagai berikut :

a. Konsentrasi 25 ppm

V1. C1 = V2. C2
V1. 1000 ppm = 100 mL. 25 ppm
V1 = 2,5 mL

b. Konsentrasi 50 ppm
V1. 1000 ppm = 100 mL. 50 ppm
V1 = 5 mL

c. Konsentrasi 75 ppm
V1. 1000 ppm = 100 mL. 75 ppm
V1 = 7,5 mL

d. Konsentrasi 100 ppm
V1. 1000 ppm = 100 mL. 100 ppm
V1 = 10 mL

e. Konsentrasi 125 ppm
V1. 1000 ppm = 100 mL. 125 ppm
V1 = 12,5 mL

f. Konsentrasi 150 ppm
V1. 1000 ppm = 100 mL. 150 ppm
V1 = 15 mL