APPENDIX A

Medium preparation and composition

APPENDIX A

Medium preparation and Composition

A.1 Medium preparation and composition

The medium preparation and composition were used in this study can be seen in the table below:

Table A.1 Medium preparation and composition were used as a substrate for cellulase activity and reducing sugar assay.

CMC broth (g/L)	OPEFB broth (g/L)	Residue broth (g/L)
10 g CMC	10 g OPEFB fiber	10 g Residue fiber
1 g KH_2PO_4	1 g KH_2PO_4	1 g KH_2PO_4
0.5 g $MgS0_4.7H_2O$	0.5 g $MgS0_4.7H_2O$	0.5 g $MgS0_4.7H_2O$
0.5 g NaCl	0.5 g NaCl	0.5 g NaCl
0.01 g $MnSO_4.H_2O$	0.01 g $MnSO_4.H_2O$	0.01 g $MnSO_4.H_2O$
0.01 g $FeSO_4.7H_20$	0.01 g $FeSO_4.7H_20$	0.01 g $FeSO_4.7H_20$
0.5 g $(NH_4)_2SO4$	0.5 g $(NH_4)_2SO4$	0.5 g $(NH_4)_2SO4$
1 l Distilled water	1 l Distilled water	1 l Distilled water
15 g agar*		

***=** if it makes CMC agar medium
All mediums were adjusted at pH 7

Table A.2 Medium preparation and composition were used for growing microbial.

Nutrient broth (g/L)	Potato dextrose broth (g/L)	YPG broth (g/L)
3 g Beef extract	200 g Potato	10 g yeast extract
15 g Peptone	15 g Peptone	20 g peptone
1 l Distilled water	15 g Dextrose	20 g glucose
15 g agar*	1 l Distilled water	1 l Distilled water
	15 g agar*	15 g agar*

***=** if it makes NA, PDA and YPG agar medium

APPENDIX B

Proximate analysis

APPENDIX B

Proximate analysis

B. Proximate analysis of OPEFB and residue

B.1 Moisture (AOAC, 1984)

The cup aluminum was dried in an oven at a temperature of 105^{o}C for 15 minutes, then cooled and weighed (A). Samples were weighed as much as 5 grams (B). After that the cup containing the sample was dried in an oven at temperature of 105^{o}C for 6 hours and then cooled in a desiccator and weighed to obtain final weight (C). Water content was calculated by the formula (B.1) and moisture content was determined by equation (B.2).

$$\text{Moisture ((\%) Wet basis) = = } \frac{[B-(C-A)]}{B} \text{ x } 100\% \quad \text{(B.1)}$$

$$\text{Moisture ((\%) Dry base) = } \frac{[B-(C-A)]}{C-A} \text{ x } 100\% \quad \text{(B.2)}$$

B.2 Ash assay (AOAC, 1984)

Samples were weighed as much as 1-5 grams, and then put in the cup porcelain known weight equipment. Samples were made into ashes on a small Bunsen flame until smoking, then put into a furnace at a temperature of 500-600 ^{o}C until a white ash. The cup was containing the ashes cooled in a desiccator and done weighting to obtain fixed weights. Ash content was determined by equation (B.3).

$$\text{Ash (\%) = } \frac{\text{Weight Of Ash (g)}}{\text{Weight of the sample (g)}} \text{ x } 100\% \quad \text{(B.3)}$$

B.3 Protein assay (AOAC, 1984)

The sample was calculated as 0.5 to 3 g then inserted into the flask Kjeldahl and the sample was destruction using 20 mL of concentrated sulfuric acid with heating to occur the clear colorless solution. Destruction resulting solution diluted and distilled with the addition of 40 ml of 40% NaOH. Distillate accommodated in a 20 mL solution of 4% H_3BO_3. H_3BO_3 solution was titrated with a standard HCL solution by using red as an indicator of metal. Levels of protein samples were calculated by multiplying the total nitrogen and correction factors. Total of nitrogen and protein were determined by equation (B.4) and (B.5).

Where Corection factor = 6.25

$$\text{Total Nitrogen (\%) = } \frac{\text{ml titration x N HCl x CF x 14}}{\text{weight of the sample}} \text{ x } 100\% \quad \text{(B.4)}$$

$$\text{Total Protein (\%) = Total Nitrogen x 6.25} \quad \text{(B.5)}$$

B.4 Lipid assay (AOAC, 1984)

Flasks fat fits into the soxhlet extraction apparatus dried in an oven. Then cooled in a desiccator and weighed until the weight remains. A total of 5 g sample wrapped in paper strain, then covered with cotton wool fat-free.The filter paper containing the sample is placed in a soxhlet extraction apparatus. The solvent diethyl ether or petroleum ether is poured into the flask fat sufficient according to the measure used. Further reflux at least 5 hours until the solvent drop back to fat flask clear colored. Solvent present in the flask and distilled fatty accommodated. Then the flask containing the extracted fat was heated in an oven at a temperature of 105^{o}C. Subsequently cooled in a desiccator and weighing is done to obtain fixed weights. Lipid content was determined by equation (B.6).

$$\text{Lipid (\%)} = \frac{\text{Weight of the fat}}{\text{weight of the sample}} \text{ x } 100\% \qquad \text{(B.6)}$$

B.5 Carbohydrate assay

To determine of the carbohydrate was carried out by formula (B.7).

$$\text{Carbohydrate (\%)} = (100\%) - (\text{Ash (\%)} + \text{Protein (\%)} + \text{Lipid (\%)}) \qquad \text{(B.7)}$$

APPENDIX C

Determination of cellulose, hemicellulose and lignin

APPENDIX C

Determination of cellulose, hemicellulose and lignin

C.1 Determination of acid detergent fiber (ADF) (Van Soest et al., 1991)

Reagents:
Preparation of acid detergent solution; 49.09 g sulfuric acid was added into distilled water and mixed, then the solution was adjusted to 1,000 mL. To estimate the normality of the solution, 0.1 N standard NaOH solution was used to find really normality of acid detergent solution (normality of acid detergent solution should be 1 N). Cetyl trimethyl ammonium bromide was added and mixed completely.

Method:

a. Wight 0.1 g dried sample in a beaker and then add 100 mL acid detergent solution.
b. 2 ml decahphydronapthalene was added.
c. Boiling the solution with reflux apparatus until 60 min.
d. Sample was filtrated into filter crucible (crucible had to know a mass, gram)
e. Washing with boiled water (90^oC-100^oC) for 2 or 3 times and then replacing with acetone for 2 times (Solution is colorless).
f. Dehydrated in oven at 100^oC for 8 hr.
g. ADF was determined as remaining mass of sample in crucible.

$$ADF(\%\ dry\ weight) = \frac{100x[(crucible\ weight + ADF) - crucible\ weight}{sample\ mass\ (dry\ weight)} \qquad (C.1)$$

C.2 Determination of Lignin content (ADL) (Van Soest et al., 1991)

Reagent: 72 % sulfuric acid

Method:

a. Estimated ADF crucible in previous step was put into beaker.
b. 72% sulfuric acid (kept in 15^oC) was added into ADF crucible until ADF was covered with sulfuric acid. And then mixing sample with glass rod.
c. Sulfuric acid was added every a hour until 3 hour.
d. Sample was filtrated and washed with hot water (more one time) until sample was neutralized.
e. Dehydrated in oven at 100^oC for 8 hr.
f. Recording mass (g) as W1
g. Burn in oven at 550^oC for 8 hr.
h. Recording mass (g) as W2

$$\%\ Acid\ detergent\ lignin\ (ADL) = \frac{100\ \text{x}\ (\text{W1} - \text{W2})}{\text{sample mass (dry weight)}} \qquad (C.2)$$

C.3 Determination of cellulose content (Van Soest et al., 1991)

$$\text{Cellulose}\ (\%) = (\%)\text{ADF} - (\%)\ \text{AD}L \qquad \text{(C.3)}$$

C.4 Determination of hemicellulose (Van Soest, 1991)

C.4.1 Neutral dietary fiber (NDF) preparation

Reagents:

a. Sodium borate decahydrate ($Na_2B_4O_7$ - 10 H_2O) 6.81 g
b. Disodium ethylenediaminetetraacetate (EDTA, $C_{10}H_{14}N_2Na_2O_8$) NDF solution – Using a 2 L
c. 18.61 g Sodium lauryl sulfate neutral ($C_{12}H_{25}NaO_4S$)
d. 30 g 2-ethoxyethanol (Ethylene glycol monoethyl ether, Cellosolve, $C_4H_{10}O_2$)
e. 10 mL Disodium phosphate anhydrous (Na_2HPO_4) 4.56 g
f. Distilled water 1,000 mL. Pour Sodium borate and disodium EDTA in a beaker and dissolve with a part of distilled water while heating. Add lauryl sulfate and 2-ethoxyethanol. Separately dissolve disodium phosphate in part of distilled water while heating until complete solution is obtained. Mix the two solutions and the remaining distilled water and control the pH which must be between 6.9 and 7.1

2. decahydronaphthalene
3. Sodium sulfite anhydrous (Na_2SO_3)
4. Acetone

Method:

1. Grind the air dried sample to pass 1 mm screen.
2. Weigh in a crucible 1 g of grinded sample with 1 mg approximation.
3. Add 100 mL of neutral detergent solution at room temperature into crucible with 0.5 g of sodium sulfite and 2 mL decahydronaphthalene.
4. Heat to boiling and reflux 60 minutes from onset of boiling.
5. Filter and wash 3 times with boiling water, then twice with cold acetone.
6. Dry 8 hours at 105 °C and let cool in a desiccator.
7. Weigh.
8. Calculate neutral detergent fiber:

$$\text{NDF \%} = (\text{weight of crucible} + \text{weight of residue}) - \text{weight} \qquad \text{(C.4)}$$

$$\text{Hemicellulose}\ (\%) = (\%)\text{NDF} - (\%)\text{ADF} \qquad \text{(C.5)}$$

APPENDIX D

Slide culture preparation for fungi and gram staining for bacteria

APPENDIX D

Slide culture preparation for fungi and gram staining for bacteria

D. Slide culture preparation for fungi and Gram staining for bacteria

D.1 Slide culture preparation for fungi

One plate of nutrient agar; potato dextrose is recommended, however, some fastidious fungi may require harsher media to induce sporulation like cornmeal agar or Czapek Dox agar.

1. Using a sterile blade cut out an agar block (7 x 7 mm) small enough to fit under a coverslip.
2. Flip the block up onto the surface of the agar plate.
3. Inoculate the four sides of the agar block with spores or mycelial fragments of the fungus to be grown.
4. Place a flamed coverslip centrally upon the agar block.
5. Incubate the plate at 30^{o}C until growth and sporulation have occurred.
6. Remove the cover slip from the agar block.
7. Apply a drop of 95% alcohol as a wetting agent.
8. Gently lower the coverslip onto a small drop of Lactophenol cotton blue on a clean glass slide.
9. The slide can be left overnight to dry and later sealed with fingernail polish.
10. When sealing with nail polish use a coat of clear polish followed by one coat of red colored polish.

D.2 Gram staining for bacteria

Transfer a loopful of the liquid culture to the surface of a clean glass slide, and spread over a small area. Two to four cultures may be stained on the same slide, which can be divided into 2-4 sections with vertical red wax pencil lines. To stain material from a culture growing solid media, place a loopful of tap water on a slide; using a sterile cool loop transfer a small sample of the colony to the drop, and emulsify. Allow the film to air dry. Fix the dried film by passing it briefly through the Bunsen flame two or three times without exposing the dried film directly to the flame. The slide should not be so hot as to be uncomfortable to the touch.

1. Flood the slide with crystal violet solution for up to one minute. Wash off briefly with tap water (not over 5 seconds). Drain.
2. Flood slide with Gram's Iodine solution, and allow to act (as a mordant) for about one minute. Wash off with tap water. Drain.
3. Remove excess water from the slide and blot, so that alcohol used for decolorization is not diluted. Flood slide with 95% alcohol for 10 seconds and wash off with tap water. (Smears that are excessively thick may require longer decolorization. This is the most sensitive and variable step of the procedure, and requires experience to know just how much to decolorize). Drain the slide.
4. Flood slide with safranin solution and allow to counterstain for 30 seconds. Wash off with tap water. Drain and blot dry with bibulous paper. Do not rub.
5. All slides of bacteria must be examined under the oil immersion lens.

APPENDIX E

Microorganisms and substrate

APPENDIX E

Microorganisms and substrate

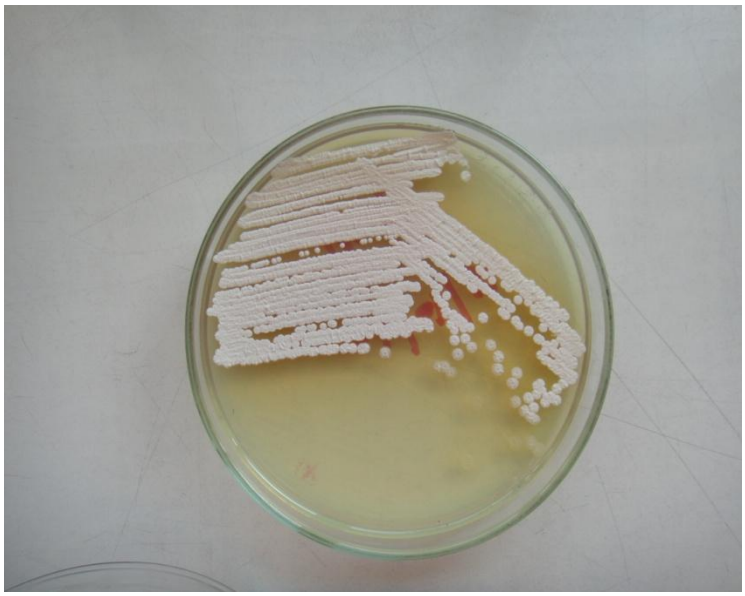
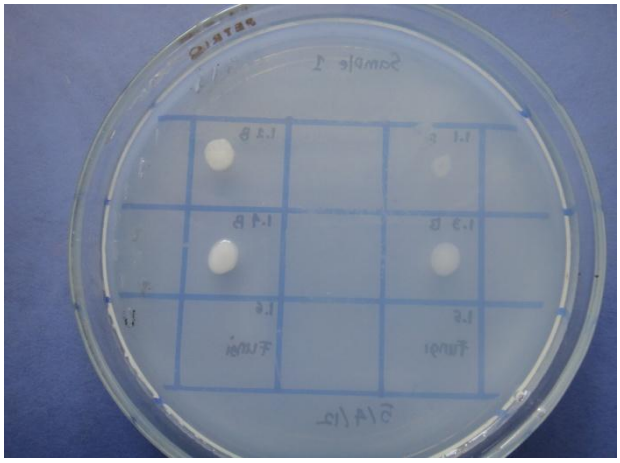


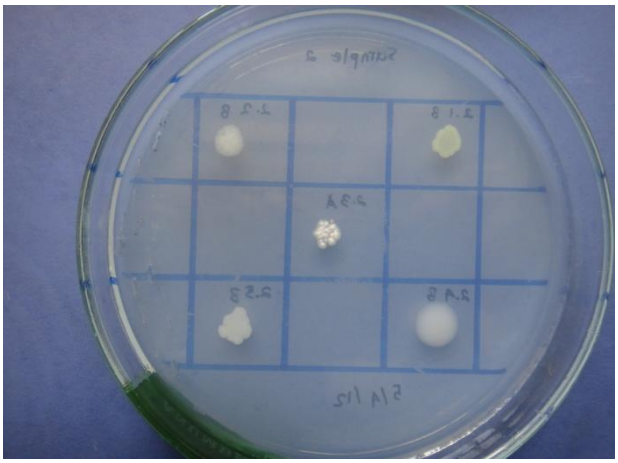
Figure E.1 The isolates 12.3.A.

(A) (B)

Figure E.2 OPEFB (A) and the residue of acid hydrolysis of OPEFB fiber (B).



Sample 1



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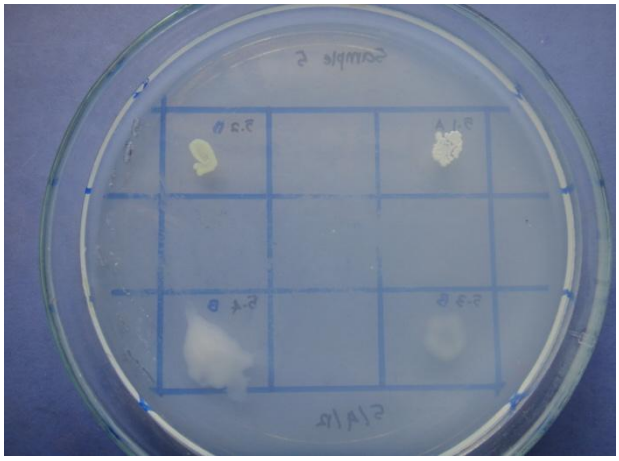
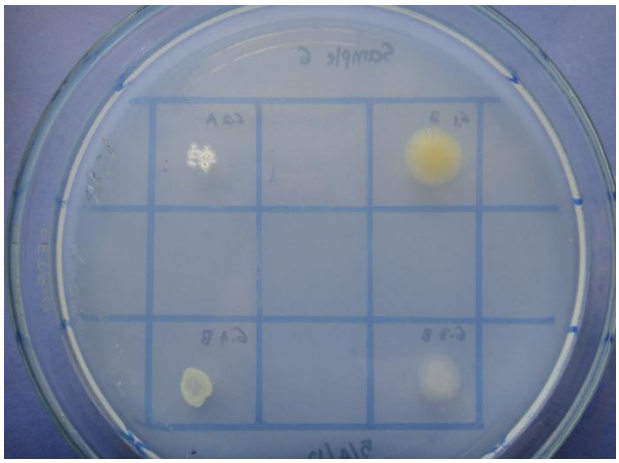
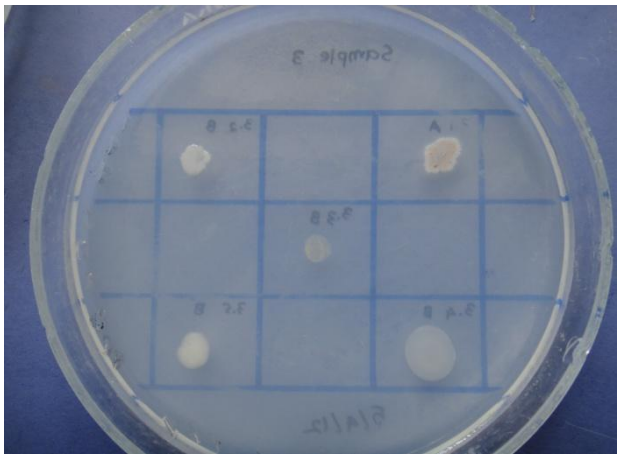


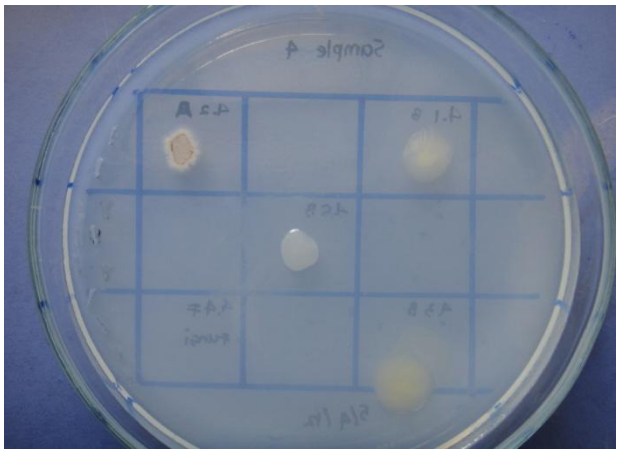
Sample 3



Sample 4

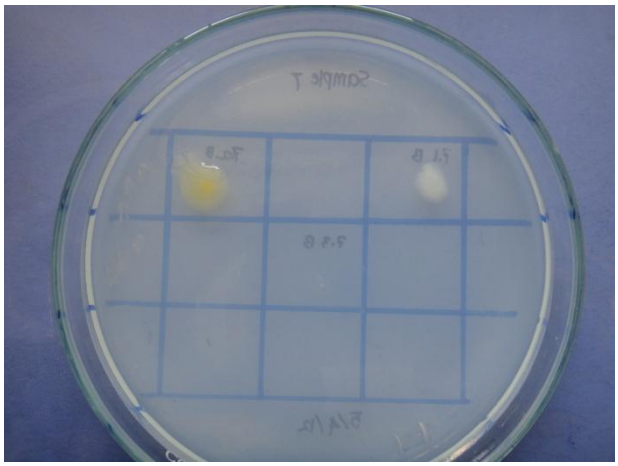


Sample 5

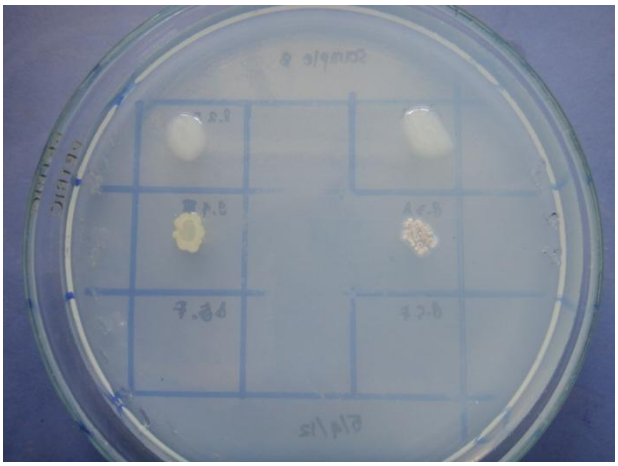


Sample 6

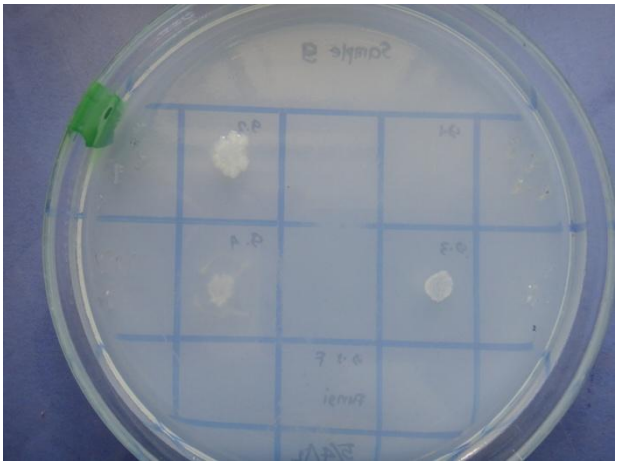
Figure E.3 Isolates of microorganisms from the samples 1– 6.



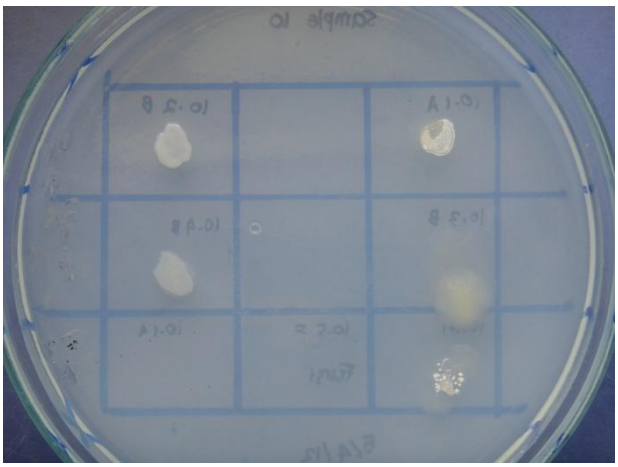
Sample 7



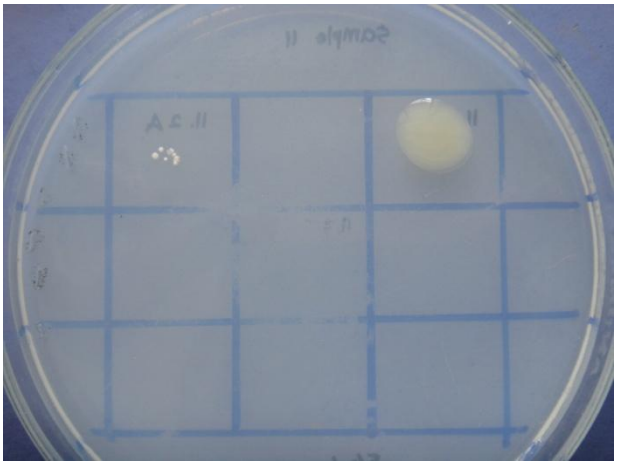
Sample 8



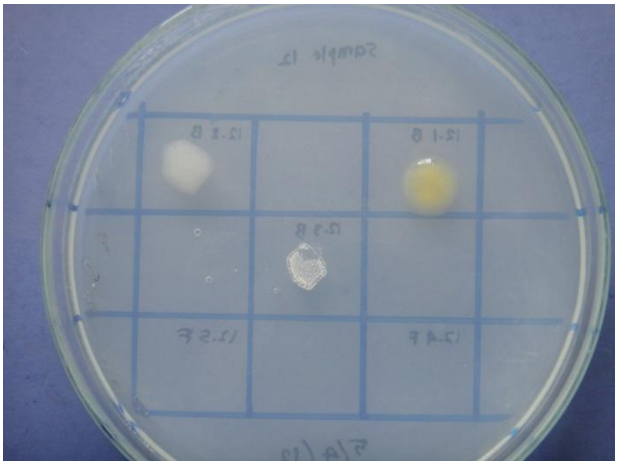
Sample 9



Sample 10

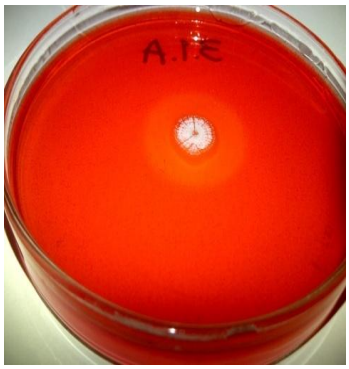


Sample 11



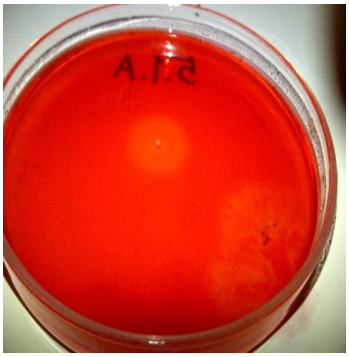
Sample 12

Figure E.4 Isolates of microorganisms from the samples 7 – 12.



Actinomyces 3.1.A

Actinomyces 4.2.A



Actinomyces 5.1.A

Actinomyces 8.3.A

Bacteria 8.4.B

Bacteria 9.3.B

Actinomyces 10.1.A

Actinomyces 11.2.A

Actinomyces 12.3.A

Figure E.5 Clear zone from isolates of microorganisms.

APPENDIX F

Plate count

APPENDIX F

Plate count

The standard plate count method consists of diluting a sample with sterile saline or until the bacteria are dilute enough to count accurately. The final plates in the series should have between 30 and 300 colonies. Fewer than 30 colonies are not acceptable for statistical reasons (too few may not be representative of the sample), and more than 300 colonies on a plate are likely to produce colonies too close to each other to be distinguished as distinct colony-forming units (CFUs). The assumption is that each viable bacterial cell is separate from all others and will develop into a single discrete colony (CFU). Thus, the number of colonies should give the number of bacteria that can grow under the incubation conditions employed. A wide series of dilutions (e.g., 10-4 to 10-100) is normally plated because the exact number of bacteria is usually unknown. Greater accuracy is achieved by plating duplicates or triplicates of each dilution, although we will not be doing that in this exercise.

Standard plate count procedures:

1. Label the bottom of eight petri plates 1-8. Label eight tubes of saline 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}. Using aseptic technique, the initial dilution is made by transferring 1 ml of bacteria sample to a 9 ml sterile saline blank is called 10^{-1} dilution.
2. The 10^{-1} dilution is then shaken by grasping the tube between the palms of both hands and rotating quickly to create a vortex. This serves to distribute the bacteria and break up any clumps. Immediately after the 10^{-1} dilution has been shaken, uncap it and aseptically transfer 1 ml to a second 9 ml saline blank. Since this is a 10^{-1} dilution, this first blank represents a 10^{-1} dilution of the original sample.
3. Shake the 10^{-2} dilution vigorously and transfer 1mL to the third 9 ml blank. This third dilution represents a 10^{-3} dilution of the original sample. Repeat the process once more to produce a 10^{-8} dilution.
4. Shake the 10^{-1} dilution again and aseptically transfer 1.0 mL to one petri plate and 0.1 mL to another petri plate. Do the same for the 10^{-2} and the 10^{-8} dilutions.
5. Remove one agar pour tube from the 48 to 50^{o}C water bath. Carefully remove the cover from the 10^{-1} petri plate and aseptically pour the agar into it. The agar and sample are immediately mixed gently moving the plate in a figure-eight motion or a circular motion while it rests on the tabletop. Repeat this process for the remaining seven plates. After the pour plates have cooled and the agar has hardened, they are inverted and incubated at 25^{o}C for 48 hours or 37^{o}C for 24 hours.
6. At the end of the incubation period, select all of the petri plates containing between 30 and 300 colonies. Plates with more than 300 colonies cannot be counted and are designated too many to count (TMTC). Plates with fewer than 30 colonies are designated too few to count (TFTC). Count the colonies on each plate. A Quebec colony counter should be used.
7. Calculate the number of bacteria (CFU) per milliliter or gram of sample by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar. Record your result

$$\frac{\text{Bacteria}}{\text{mL}} = \frac{\text{number of colonies (CFUs)}}{\text{dilution X amount plated}} \qquad \text{(F.1)}$$

APPENDIX G

Cellulase activity

APPENDIX G

Cellulase activity

G. Cellulase activity determination by DNS method (Ghose, 1987)

Substrate:
1% Carboxymethyl cellulose CMC) in 0.05 M phosphate buffer, pH 7.

Method:
1. Add 0.5 ml enzyme, diluted in phosphate buffer, to a test tube of volume at least 25 mL. At least two dilutions must be made of each enzyme sample investigated. One dilution should release slightly more and one slightly less than 0.5 mg (absolute amount) of glucose (= reducing sugars as glucose) in the reaction conditions.
2. Add 0.5 mL substrate solution, mix well and incubate at 50°C for 60 minutes.
3. Add 3.0 mL DNS, mix. Transfer to a rack on the table.
4. Boil for exactly 5.0 mm in a vigorously boiling water bath containing sufficient water. All samples, enzyme blanks, glucose standards and the spectro zero should be boiled together. After boiling, transfer immediately to a cold water bath.
5. Add 20 mL deionized or distilled water. Mix by completely inverting the tube several times so that the solution separates from the bottom of the tube at each inversion.
6. Measure the color formed against the spectro zero at 540 nm.
7. Translate the absorbance of the sample tube (corrected if necessary by subtraction of the enzyme blank) into glucose production during the reaction using a glucose standard curve.

APPENDIX H

Reducing sugar

APPENDIX H

Reducing sugar

H. Reducing sugar determination by DNS method (Miller, 1959)

1. Chemical reagent :
   - 3, 5-dinitrosalicylic acid (Aldrich) 10. g
   - Na_2SO_3 (Ajax Finechem) 0.5 g
   - Na-K tratrate (APS Finechem) 182.0 g
   - NaOH (Merck) 10.0 g
   - Phenol (Merck) 2.0 g
   - Deionized water 1000 ml

NaOH 10 g are added into 700 mL of deionized water and mixed in order to add the 300 g Na-K tratrate. When the solution dissolved, 3,5-dinitrosalicylic acid 10 g is then added and continuously stired. After that, the 0.5 g of Na_2SO_3 and 2.0 g of phenol are dissolved, respectively. Finally, the volume is adjusted to 1,000 mL by deionized water and kept away from light.

2. Reducing sugar determination procedure :
   a. The samples 0.5 mL are mixed with 0.5 mL of DNS solution. The mixture is boiled for 10 min.
   b. The sample is cooled down by immersing the sample tube into cold water immediately. Five ml of water is added. The mixture was mixed well, and measured at absorbance 540 nm.

3. Absorbance 540 nm is converted to glucose concentration with standard curve.

APPENDIX I

Standard curve of glucose

APPENDIX I

Standard curve of glucose

I. Standard curve of glucose

Sock solution of glucose were prepared glucose standard is 20 mg glucose and dissolved in 10 mL of distilled water. The consentration of glucose is 20 mg/10 mL or 2 mg/mL.

Table I.1 The different concentration of glucose standard

Glucose (mg/mL)	Distilled Water (mL)	Concentration of glucose (mg/mL)
0	0	0
0.5	4.5	0.2
1.0	4.0	0.4
1.5	3.5	0.6
2.0	3.0	0.8
2.5	2.5	1

Table I.2 Absorbances of glucose

Concentration (mg/mL)	Absorbance 1	Absorbance 2	Average of Absorbances
0	0	0	0
0.2	0.089	0.085	0.087
0.4	0.146	0.152	0.149
0.6	0.229	0.225	0.227
0.8	0.331	0.337	0.334
1	0.389	0.389	0.389

The relationship between reducing sugar and absorbance at 540 nm is illustrated in Figure I.3

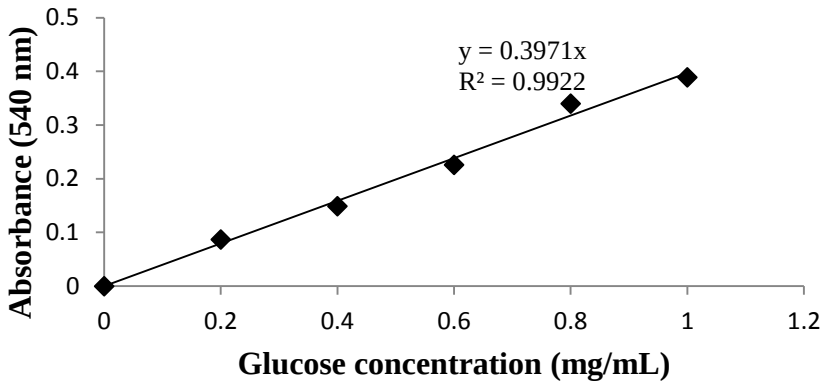


Figure I.3 Standard curve of glucose

This, therefore, provides the equation below for the calculation of sugar concentration from the absorbance at 540 nm.

Reducing sugar concentration (mg/ml) = $\frac{A\ 540\ nm}{0.9922}$

APPENDIX J

Protein content determination

APPENDIX J

Protein content determination

J.1 Protein content determination by Lowry method (1951)

Reagent:
A: 2% Na_2CO_3 in 0.1 N NaOH
B: 0.5% $CuSO_4.5H_2O$ in 1% sodium tartrate
C: 1 N folin phenol reagent

Method:

1. Mix reagent A with reagent B (50:1) is called reagent D, use as soon as possible because it creates sediment after keeping long time
2. 3 ml reagent D and 0.6 ml of the sample are added into test tube
3. Mixing and keeping at room temperature
4. 0.3 reagent C is added and mix immediately
5. Keeping at room temperature for 30 minutes
6. Measuring at 750 nm
7. Calculating the protein content compared to standard curve

Standard curve of protein

1. By using BSA, prepare the protein solution of 30 – 330 µg/mL
2. 3 ml reagent D and 0.6 ml 0f BSA solution are added into test tube
3. Mixing and keeping at room temperature
4. 0.3 reagent C is added and mix immediately
5. Keeping at room temperature for 30 minutes
6. Measuring at 750 nm

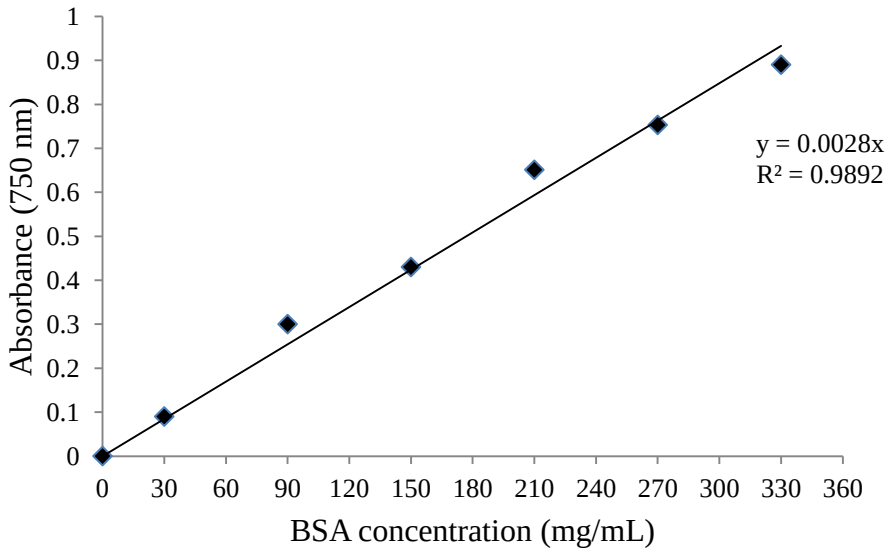


Figure J.1. Standard curve of protein

APPENDIX K

Clear zone of cellulase by zymogram

APPENDIX K

Clear zone of cellulase by zymogram

K.1 Preparation of glass plate

Assembling to the glass plate with a distance ± 1 mm. Gel made 2 layers where the gel as the sample (stacking gel) and the gel as separation media for protein separation (separating gel).

K.2 Separating gel (12%) with 0.1 % CMC

Created by preparing glass beaker 100 mL, 8 mL of stock acrylamide was inserted and added 5 mL 1.5 M Tris-HCl pH 8.8 and whipped. Added 4 mL 0.1% CMC solution and 2.7 mL of distillate water, 200 µl of SDS 10%, 100 µL of 10% APS and also added 10 µl of TEMED and shaken until all solution mixed evenly, then inserted all solution has been prepared using 1 mL micro pipette to glass plate. Do with carefully not to form air bubbles, slowly add ethanol solution above the gel the agar plate surface of the gel is not wavy, let the gel solidify for about 45 minutes.

K.3 Stacking gel (3%)

To prepare gel staking is the same with separating gel preparation and composition materials used were 0.67 mL of 30% stock acrylamide, 1.25 mL of 0.5 M Tris-HCl pH 6.8, 3.075 mL of distillate water, 50 µL 10% SDS, 50 µL of APS 10 %, and 10 µL of TEMED. Put of comb on top of the separating gel. Staking gel solution is poured between the hole of comb until the gel solidifies and sinks formed. Plate mounted on the electrophoresis set and running buffer was poured on the tool.

K.4 Sample injection

A total of 15 mL of sample plus 5 ml zymogram dye was inserted in the gel wells with a volume of 18 mL for each well. Insert a protein marker as standard of molecular weight. Then running of the sample was used a power supply that connected to the mains with an electrical current of 120 volts for 60 minutes. The separation process was stopped after the blue marker ± 0.5 cm from the lower limit of the gel plate.

APPENDIX L

Staining process in zymogram method

APPENDIX L

Staining process in zymogram method

Procedure (Her et al., 1999):

1. After finishing preparing gel and running by electrophoresys advice, the gel was removed careful from the glass.
2. The gel then was rinsed in Tris buffer (50 mmol/L, pH 7) containing 1% Triton X-100 for 1 h at room temperature to remove SDS.
3. Then gel was also incubated by Tris buffer (50 mmol/L, pH 7) to allow for enzyme activity against the substrate for 1.5 hours.
4. The gel was stained with 0.1% Congo red for 30 minutes
5. After the gel was by congo red then the gel was also destaind by 1 mol/L NaCl to reveal a clear zone.

APPENDIX M

Residue preparation after enzymatic saccharification by evaporator

APPENDIX M

Residue preparation after enzymatic saccharification by evaporator

Procedur evaporation:

1. Sample measured before it put in the bowl glass
2. Put small ice in the big tube and covered
3. All devices of evaporator (water jet pump, vacum control and heating bath) were on
4. Setup vacuum control were at 4 Mbars, heating bath 60^{o}C and rotary vacuum at 60 rpm.
5. Stopped all devices after the sample was approximately desired.
6. Samples evaporated here were OPEFB and residue that the result final from enzymatic saccharification process prior to ethanol fermentation.

APPENDIX N

Analysis of alcohol by gas chromatography (GC)

APPENDIX N

Analysis of alcohol by gas chromatography (GC)

Equipment:
PerkinElmer Autosystem XL Gas Chromatography
Compressed gases; air, nitrogen, hydrogen
Computer
Pipettes
60/80 Chromosorb Paw 20 m capillary column or equivalent

Reagents: Ethanol

Preparation:
1. Prepare ethanol standards with ethanol concentration such as 0.1, 0.2, 0.4, 0.6, 0.8 and 1%.
2. Ethanol standards were injected in GC equipment and the results were calibrated.
3. 2.5 µL of the samples were injected in GC equipment and The record data of ethanol were obtanied.

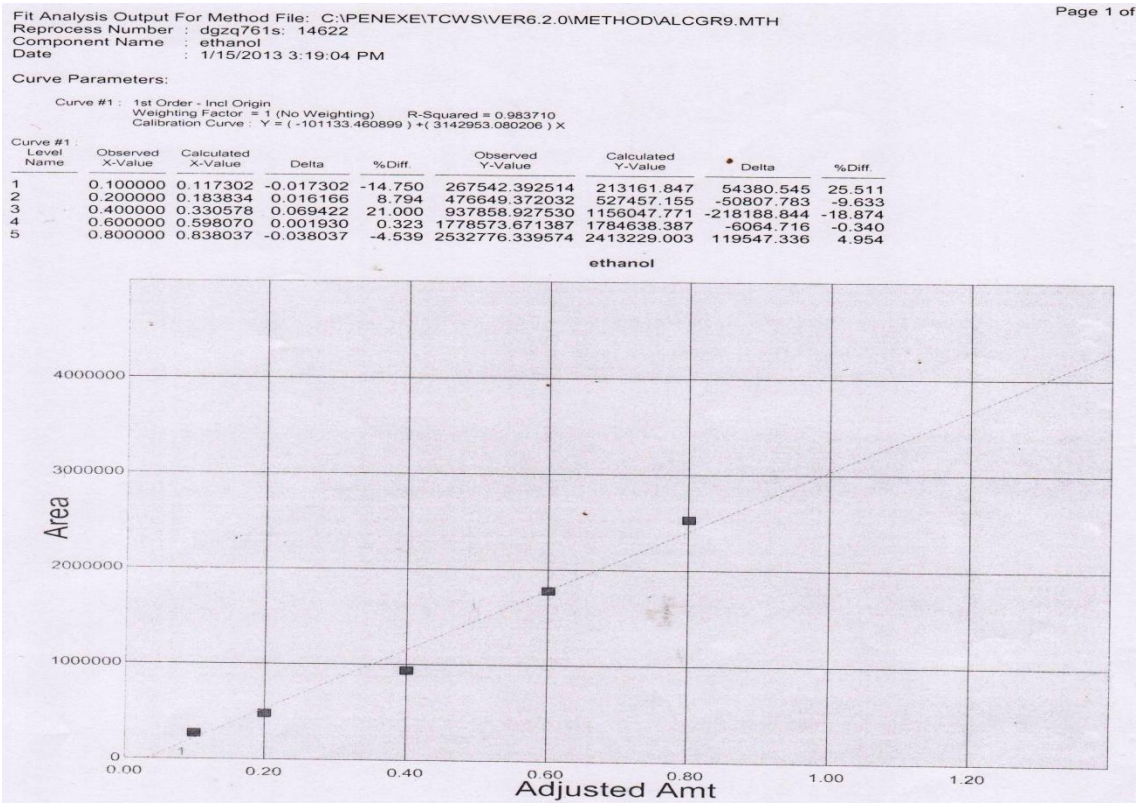


Figure N.1 Ethanol standard graph for Gas chromatography analysis

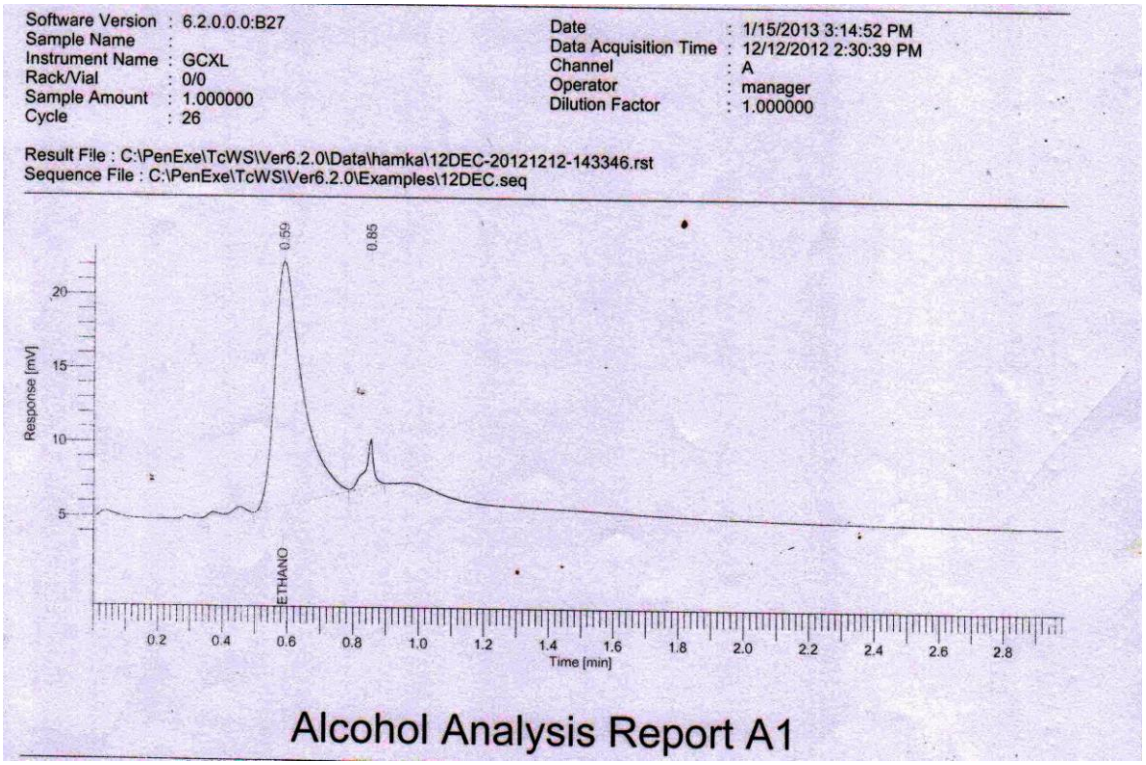


Figure N.2 Ethanol analysis report by Gas Chromatography (GC) autosystem XL

APPENDIX O

16S rDNA

APPENDIX O

16S rDNA

The base sequences of the isolate 12.3.A from forward primer 518F and Reverse primer 800 R.

>121130-12_M21_ACT1_518F.
GGGGACGTTGGTCGGGATATTGTGGCGTAAGAGCTCGTAGGCGGCTTGTCACGTCGGGTGTG
AAAGCCCGGGGCTTAACCCCTGGGTCTGCATCCGATACGGGCAGGCTAGAGTGTGGTAGGG
GAGATCGGAATTCCTGGTGTAGCGGTGAAATGCGCAGATATCAGGAGGAACACCGGTGGCG
AAGGCGGATCTCTGGGCCATTACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGAT
TAGATACCCTGGTAGTCCACGCCGTAAACGTTGGGAACTAGGTGTTGGCGACATTCCACGTC
GTCGGTGCCGCAGCTAACGCATTAAGTTCCCCGCCTGGGGAGTACGGCCGCAAGGCTAAAA
CTCAAAGGAATTGACGGGGGCCCGCACAAGCAGCGGAGCATGTGGCTTAATTCGACGCAAC
GCGAAGAACCTTACCAAGGCTTGACATATACCGGAAAGCATTAGAGATAGTGCCCCCCTTGT
GGTCGGTATACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTC
CCGCAACGAGCGCAACCCTTGTCCTGTGTTGCCAGCATGCCCTTCGGGGTGATGGGGACTCA
CAGGAGACCGCCGGGGTCAACTCGGAGGAAGGTGGGGACGACGTCAAGTCATCATGCCCCT
TATGTCTTGGGCTGCACACGTGCTACAATGGCCGGTACAAAGAGCTGCGATGCCGCGAGGC
GGAGCGAATCTCAAAAAGCCGGTCTCAGTTCGGATTGGGGTCTGCAACTCGACCCCATGAA
GTCGGAGTTGCTAGTAATCGCAGATCAGCATTGCTGCGGTGAATACGTTCCCGGGCCTTGTA
CACACCGCCCGTCACGTCACGAAAGTCGGTAACACCCGAAGCCGGTGGCCCAACCCCTTGTG
GGAGGGAGCTGTCGAAGGTGGGACCAGCGATTGGACGATCTACAGAGAGGGACCCCCATAT
AATAAAAGGGGGGTAAGAGGGGGGGCT

>121130-12_O21_ACT1_800R.ab1 1403
GGCGGTCTTTCGCTCTCAGCGTCAGTAATGGCCCAGAGATCCGCCTTCGCCACCGGTGTTCCT
CCTGATATCTGCGCATTTCACCGCTACACCAGGAATTCCGATCTCCCCTACCACACTCTAGCC
TGCCCGTATCGGATGCAGACCCGGGGTTAAGCCCCGGGCTTTCACACCCGACGTGACAAGCC
GCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTTGCGCCCTACGTATTACCGCGG
CTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCACTTTCGCTTCTTCCCTGC
TGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAGGCTTTC
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CAACAAGCTGATAGGCCGCGGGCTCATCCTTCACCGCCGGAGCTTTCCAGCCCGGGAGATGC
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CNCCCCNCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCNNCNCCCCNCCCCCCCCCNCCCCC
CCCCCCCCCCCCCCNCCNCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCGGGGNNNNNNNNNNNNNNN
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NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
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CCNNNCCNCCCCCCCCCCNNCCNCNNCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCNNNCN
NNCCCCCCCCCCCCCCCCCCCCCCCNCNCCCCCCCCCCCCCCCCCCCCCNCNCCCCCCCCCCC
CCCNCNCCCCCCCCCCCCCCNNCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCNCCCCCNNNNCC
CCCCCCCCCCCCCCCCCCCNCCCCNNNNNCCCNNCCCCCCCCCNN

APPENDIX P

Reagent and chemical preparation

APPENDIX P

Reagent and chemical preparation

Table P.1 Reagent and chemical preparation

No.	Reagens
1.	Saline solution (0.85%) To make a 0.85% solution by weighing 0.85 g NaCl and then dissolved in 70 mL distillate water then put in a 100 mL measuring flask and added distillated water to mark boundaries.
2.	0.1 M KH_2PO4 solution To make a 500 mL solution of KH_2PO4, then weighed 6.8045 g KH_2PO4 dissolved solids with distilled water in a 100 ml glass beaker, poured into a 500 mL flask and then diluted with distilled water to mark boundaries.
3.	0.1 M K_2HPO_4 solution To make 500 ml solution of K_2HPO_4, then weighed 8.709 g K_2HPO_4 dissolved solids with distilled water in a 100 mL glass beaker, poured into a 500 mL flask and then diluted with distilled water to mark boundaries.
4.	Phosphate buffer pH 6.5 Taken 30.1 mL of 0.1 M K_2HPO_4 solution into a 100 mL beaker which was placed electrode pH meter then added slowly to a solution of 0.1 M KH_2PO4 to pH 6.5. (Comparison of the volume of K_2HPO_4 and KH_2PO4 to 0.1 M, respectively: pH 5.5 (4.15: 95.85), pH 6 (13.2: 86.8), pH 6.5 (30.1: 69.9), pH 7 (61.5: 38.5), pH 7.5 (81.36: 18.64), pH 8 (94: 6)
5	APS (Ammonium persulphate) Solution Weighed 0.1 g Ammonium persulfate and diluted with 1 mL of distilled water by then shaken using a vortex and stored at 4 ^{o} C.
6.	30%Polyacrylamide solution (T-Acrylamide) Weighed 2.92 g Acrylamide and 0.08 g of bis-Acrylamide, diluted with 70 mL distilled water using a magnetic stirrer, and then added water until 100 mL.
7.	Solution of Tris-HCl pH 8.8 Weighed 1.2114 g Tris and dissolved in sterile water as much 7 mL and added HCl to pH 8.8. Then put in a flask added 10 mL of sterile distilled water to mark boundaries.
8.	Solution of Tris-HCl pH 6.8 Weighed 1.2114 g Tris and dissolved in sterile water as much 7 mL and added HCl to pH 6.8. Then put in a flask added 10 mL of sterile distilled water to mark boundaries.
9.	10% SDS stock solution Created by weighing 0.1 g SDS and distilled water added to 1 mL
10.	Running Buffer Weighed 3.03 g Tris base, 14.4 g glycine and 1 g SDS, and then dissolved in 1 liter.
11.	Reducing Sample Buffer Solution (RSB). Taken with a micropipette 60 µL of 1 M Tris-HCl pH 6.8, 500 µL 50% (v/v) glycerol, 200 µl SDS 10% (w / v), 50 µL 2-merkaptoetanol and 100 µL of 1% bromophenol blue later added distilled water of 90 µL.

CURRICULUM VITAE

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