WORK PERMIT

Department of Chemical and Biomolecular Engineering

化學工程及生物分子工程學系

Project Title : Persistence Study of S. aureus Towards Antibiotics

Researcher(s) : Chan Shek Nga

Supervisor(s) : Prof Henry H. N. Lam

Work Plan No. : 17035

Date of Approval : 9/6/2017

Date of Revalidation : N/A

Signature of Approval :

Prof. Barford
DSO

Student Working under the Supervision of a M.Phil. / Ph.D. student or HKUST staff under an Existing Approved Work Plan

I have read, understood the approved Work Plan # 17010 and declare that I will follow strictly follow the experimental procedure contained therein.

I will be supervised at, ALL times, by Lee Yik Yeung (Supervising M.Phil./~~PhD~~ student).

I have read and understand and will comply with the feedback from HSEO for this work plan.

I understand that any departure from the contents of this work plan with require an amendment to Work Plan or New Work Plan submission.

I have completed and signed a separate CBME Risk Assessment Audit Declaration and submitted this to the relevant technician.

Name: Chan Shek Nga

Signature: _____

Date: 1/6/2017

15. CBME Risk Assessment Audit Declaration

I, LEE Yik Yeung, have read and understood the relevant sections of the HKUST Safety and Environmental Protection manual. I am aware of my responsibilities with regard to the health and safety of myself and others and state that, to the best of my knowledge, the information provided in this work plan is complete and correct:

I have attended and passed the following safety courses :

- ☑ 1. Hazardous Waste Management (**mandatory**) ___MC03
- ☑ 2. Chemical Safety for Laboratory Users (**mandatory**)__MC07
- ☑ 3. Fire Safety (**mandatory**)
- ☐ 4. Laser Safety___MC04
- ☑ 5. Biological Safety___MC06
- ☐ 6. Pressure Safety___MC05
- ☐ 7. Others : _____

Are any of the following categories relevant to the proposed research?

◻ High voltage power supplies	☐ Yes	☑ No
◻ Biologically active materials	☑ Yes	☐ No
◻ Radioactive materials or ionising radiation sources	☐ Yes	☑ No
◻ Non-ionising radiation (UV, microwaves, lasers)	☑ Yes	☐ No
◻ Highly toxic, carcinogenic or mutagenic materials	☐ Yes	☑ No
◻ Highly flammable or explosive materials	☐ Yes	☑ No
◻ Operation under extreme pressure or temperature	☐ Yes	☑ No

Status of Researcher

1. ☑ HKUST **CBME** ◻ HKUST_____ ◻ Non HKUST_____ ◻ Others_____
2. ◻ Ph.D. ☑ M.Phil. ◻ M.Sc.
   ◻ FYP ◻ UROP ◻ Student Helper
   ◻ Visiting Scholar ◻ Research Assistant ◻ Research Associate
   ◻ Intern-PG ◻ Exchange-PG ◻ Post Doc
   ◻ Intern-UG ◻ Exchange-UG ◻ Faculty
   ◻ UG Research
   ◻ _____

Insurance Coverage

All researchers ***other than undergraduate or postgraduate students*** must provide a copy of their letter of appointment to ensure that they have official status within the Department and that they have appropriate insurance coverage. Any researchers employed by anyone other than the Department of Chemical and Biomolecular Engineering at HKUST, must provide full details of their insurance coverage by their employer.

Assessment checked by Technical staff associated with ALL laboratories in which the planned EXPERIMENTAL work will be undertaken.

Laboratory	Responsible Technician (Name)	Signature	Date
7110	Kam Tim TANG		

Please list ALL laboratories in which ANY ANALYTICAL work will be undertaken:

Bioengineering Laboratory (Room 6104),

BioCRF (Room 6127)

Laboratory	Responsible Technician Name	Equipment	Training Required Y/N	Training Completed Y/N
6104	Inez S M Tsui	BCA assay	Y	N
6127	Joyce Wong	LC-MS	Y	N

* **Note:** After completion of training, a record of training completion will be recorded and kept by the relevant technician

I have had my work plan checked by the relevant technical staff associated will all laboratories in which the planned work will be undertaken and also by my Faculty research supervisor. I have considered their suggestions and feedback and have modified the work plan accordingly.

Researcher

Name LEE, Yik Yeung Signature _____ Date _____

Assessment checked by research supervisor(s)

Name Prof. Henry H. N. Lam Signature _____ Date _____

Authorized by CBME Departmental Safety Officer (DSO)

Name Prof. John Barford Signature _____ Date _____

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Laboratory	Responsible Technician (Name)	Signature	Date

Please list ALL laboratories in which ANY ANALYTICAL work will be undertaken:
Analytical Lab (7101),
Spectroscopy and Surface Characterization (7106)
Material Processing and Characterisation (7119)

Laboratory	Responsible Technician Name	Equipment	Training Required Y/N	Training Completed Y/N

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Researcher

Name	Signature	Date
Chan Shek Nga		2/6/2017

Assessment checked by research supervisor(s)

Name HENRY LAM Signature _____ Date 2/6/2017

Name _____ Signature _____ Date _____

Authorized by CBME Departmental Safety Officer (DSO)

Name Professor John Barford Signature _____ Date 2/6/2017

THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY
DIVISION OF BIOMEDICAL ENGINEERING

Persistence Study of *S. aureus* Towards Antibiotics

Work Plan #17035

Researcher: Chan Shek Nga
Supervisor: Prof. Henry H. N. Lam

2 June, 2017

1. General Information

Name of Researcher:	Chan Shek Nga
Name of Project Supervisor:	Prof. Henry H. N. Lam
Project Title:	Persistence Study of *S. aureus* Towards Antibiotics
Research Area:	Proteomics
Location:	Rm 7110 and Bioengineering Laboratory
Proposed Start Date:	2 June 2017

2. Experiment/Project Description

This project is to analyze the proteins in S. aureus after which is treated by antibiotics by proteomics. The bacteria will be extracted from medium and lysed to extract the proteins and the proteomes will be analyzed by mass spectrometry (MS). The method has been used in the research field for investigating the proteome changes of cells. Proteins will be extracted from S. aureus. The extracted protein will be desalinated and then analyzed by MS.

After the mass spectrum is successfully obtained, it will be compared with the protein spectrum existed in the Spectra Library. Comparing spectra with Spectra Library will require computational calculation. By this comparison, identifications and structures of the proteins can be revealed so that the changes of proteomes can be determined.

3. Equipment List

1. Biochemical Incubator
2. Incubator shaker
3. Biological Safety Cabinet
4. Chemical Fume Hood
5. Centrifuges
6. SpeedVac
7. Micro-plate Reader
8. Ultrasonic Homogenizer
9. High Performance Liquid Chromatography (HPLC)
10. Linear Trap Quadrupole (LTQ) Mass Spectrometry
11. Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometry

4. Experimental Procedures

4.1 Cell Culture

4.1.1 Preparation of Mueller-Hinton liquid media

Reagent	Amount to add (for 100 mL)
Acid hydrolysate of casein	1.75 g
Beef extract	0.3 g
Starch	0.15 g
H_2O	100 mL

4.1.2 *S. aureus* cell culture

Pick a single *S. aureus* colony from a normal agar plate and inoculate a starter culture of 2-5 mL Mueller-Hinton liquid media with different antibiotics in different test-tubes. Incubate for approximately 8-12 h at 37 °C with vigorous shaking in nearly 300rpm.

4.2 Protein Extractions from bacteria

4.2.1 Harvest the *S. aureus* cells by centrifugation at 6000 × *g* for 15 minutes under 4°C and aspirate the supernatant.

4.2.2 Resuspend the pellet in cold PBS buffer and centrifuge at 6000 × *g* for 10 minutes under 4°C and aspirate the supernatant. Repeat 2 times.

4.2.3 Add ice-cold cell lysis buffer (8 M urea) and resuspend the pellet. Incubate on ice for 10 minutes.

4.2.4 Vortex tubes briefly and proceeds to sonication for 5 min at 4°C.

4.2.5 Transfer the supernatant to a new tube and centrifuge samples at 6000 × *g* for 15 minutes at 4°C to remove any insoluble material.

4.2.6 Add ice-cold acetone to precipitate the proteins.

4.2.7 Aspirate the supernatant and redissolve the proteins in buffer (4 M urea and 30 mM Tris-HCl, pH 6.5).

4.2.8 Take an aliquot for the protein quantification.

4.2.9 Repeat step 4.2.1 to 4.2.8 for other antibiotic samples.

4.3 Sample preparation

4.3.1 Reduce the proteins with dithiothreitol (DTT, 10 mM final concentration) at 37°C for 3 hours.

4.3.2 Alkylate the proteins with iodoacetamide (IAA, 20 mM final concentration) in dark for 1 hr. The alkylation reaction is quenched by adding DTT (10 mM final concentration) again.

4.3.3 Dilute the samples to a concentration of urea less than 1 M.

4.3.4 Digest the proteins with sequencing grade modified typsin (1: 50, w/w) at 37°C overnight.

4.3.5 Acidify samples with 10% formic acid to a final concentration of 0.5% (v/v).

4.3.6 Desalt the sample with C18 reverse-phase ZipTip.

4.3.7 Dry the sample with SpeedVac and store it at -20°C before use.

4.4 Analysis by mass spectrometry

4.4.1 Run the mass spectrometry and obtain the spectrum so as to do matching with database.

5. Procedure Template

Experimental Procedure No.	Experimental Procedure Description	Scale (Mass/Volume)	Location (Fumehood, benchtop, etc.)	Method (New or Existing)
4.1.1	Preparation of Mueller-Hinton liquid media	Acid hydrolysate of casein = 1.75 g Beef extract = 0.3 g Starch = 0.15 g H_2O = 100 mL	Room 6104, benchtop	Existing
4.1.2	*S. aureus* cell culturing	2~5 mL	Room 6104, BSC	Existing
4.2	Protein extraction	PBS buffer-5 mL Lysis buffer-2 mL Actone-5 mL	Room 7110, benchtop	Existing
4.3	Sample preparation	DTT-2 µL IAA-2 µL Sequencing grade modified typsin-2 µL Formic acid-2 µL	Room 7110, benchtop	Existing
4.4	Analysis by mass spectrometry	N/A	Room 7101 and BioCRF	Existing

6. HAZOP Template

Hazards and Operability Analysis							
4.1 Cell Culture							
No.	Hazards	Hazards Effect	Severity	Probability	Risk	Minimize Risk By	Residual Risk
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L
2	Autoclave	High pressure steam burns	H	M	H	Strictly follow the safety requests and do not overload the autoclave	L
3	Contact with bacteria	Causes illness and infection	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the biosafety cabinet	L
4.2 Protein Extraction							
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L

2	Centrifuge	Rotor Failure: the rotor that breaks can spin out of control and hit laboratory personnel. Spillage: the tubes may spray over the machine parts.	H	M	H	Strictly follow the safety requests. Balance all samples as closely as possible Set the rotors under the maximum speed.	L
4.3 Sample Preparation							
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L
2	SpeedVac	Rotor Failure: the rotor that breaks can spin out of control and hit laboratory personnel. Spillage: the tubes may spray over the machine parts.	H	M	H	Strictly follow the safety requests. Balance all samples as closely as possible Set the rotors under the maximum speed.	L

Severity: L=Low (Minor injuries, first aid), M=Meddle (Hospitalization, medical leave), H=High (Serious injuries, fatality).
Probability: L=Low (Unlikely), M=Middle (Possible), H=High (Very likely).
Risk=Severity × Probability, the product follows the higher severity or probability

7. Operating Conditions

- Autoclave: 121°C, 103.4 kPa, 20 min
- Cell Culture: 37°C, shaking, 8~12 hr
- Pellet Cells: 6000 × *g*, 4°C, 10~15min
- Reduce Proteins: Dithiothreitol (DTT) (10 mM final concentration), 37°C, 3 hours
- Alkylate Proteins: Iodoacetamide (IAA) (20 mM final concentration), in dark, 1 hr
- Digest Proteins: 37°C, overnight

8. Services List

- Electricity (AC 220V, 50Hz)
- Tap water
- Double deionized Water
- Chemical Fume Hood
- Biological Safety Cabinet (Level 2)
- Linear Trap Quadrupole (LTQ) Mass Spectrometry

9. Chemicals List

Chemical	Purity	Quantity per Experiment
Acid hydrolysate of casein	99.999%	1.75 g
Beef extract	99%	0.3 g
Starch	99.99%	0.15 g
Urea	95%	50 µL (4M solution)
Tris-HCl	99%	10 mL (30 mM solution)
Dithiothreitol	99.5%	1 mg
Iodoacetamide	99%	1 mg
Formic Acid (CH_2O_2)	98%	1 mL

Acetone (C_3H_6O)	99.9%	5 mL
Trypsin	2,500 USP units/mg	3 mg
Phosphate Buffered Saline (PBS)	99.9%	10 mL
Oxacillin Sodium Salt ($C_{19}H_{18}N_3NaO_5S$)	99%	200 µg
Ampicillin sodium salt ($C_{16}H_{18}N_3NaO_4S$)	99%	200 µg
Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$)	99%	200 µg
Cephalexin Hydrate ($C_{16}H_{17}N_3O_4S \cdot xH_2O$)	99%	200 µg
Sulfathiazole Sodium salt ($C_9H_8N_3NaO_2S_2$)	99%	200 µg
Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$)	99%	200 µg
Erythromycin ($C_{37}H_{67}NO_{13}$)	99%	200 µg
Levofloxacin ($C_{18}H_{20}FN_3O_4$)	99%	200 µg

10. Summary of Relevant Hazards and Incompatibilities

Chemical	Summary of Hazards	Incompatibilities
Acid hydrolysate of casein	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Beef extract	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Starch	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents

Urea	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Tris-HCl	This substance is not classified as dangerous according to Directive 67/548/EEC.	Bases, Oxidizing agents
Dithiothreitol	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Bases, Oxidizing agents, Reducing agents, Alkali metals
Iodoacetamide	Toxic if swallowed. May cause sensitization by inhalation and skin contact.	Strong acids, Strong bases, Strong oxidizing agents, Strong reducing agents
Formic Acid (CH_2O_2)	Flammable liquid and vapor. Causes severe skin burns and eye damage.	Strong oxidizing agents, Strong bases, Powered metals
Acetone (C_3H6O)	Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness.	Bases, Oxidizing agents, Reducing agents, Acetone reacts violently with phosphorous oxychloride
Trypsin	May cause allergy or asthma symptoms or breathing difficulties if inhaled. Irritating to skin and eyes	N/A
Phosphate Buffered Saline (PBS)	Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.	Strong oxidizing agents, Strong acids
Oxacillin Sodium Salt ($C_{19}H_{18}N_3NaO_5S$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Ampicillin sodium salt ($C_{16}H_{18}N_3NaO_4S$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Oxidizing agents
Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Cephalexin Hydrate ($C_{16}H_{17}N_3O_4S \cdot xH_2O$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents

Sulfathiazole Sodium salt ($C_9H_8N_3NaO_2S_2$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Erythromycin ($C_{37}H_{67}NO_{13}$)	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Levofloxacin ($C_{18}H_{20}FN_3O_4$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents

11. Waste List

- Cell culture (liquid) containing Mueller-Hinton liquid medium, autoclaved and dispose to the sink.
- Used PBS (liquid), autoclaved and disposed in the sink.
- Used acetone (liquid), disposed in the organic waste container of non-halogenated solvent.
- Used pipette and microcentrifuge tubes (solid), disposed in the waste container of biological waste and disposed as domestic waste after autoclave

12. Assessment of Significant Risks

May exposed to toxic or irritating chemicals. Goggles and gloves should be always put on.

13. Safety Precautions

- Personal Protective Equipment
  - A lab coat, safety glasses and respirator must be worn throughout the experiment. Rubber glove should be worn according to the nature of chemicals being handled.
- Fume Extraction System is required and in Place
  - The reaction system is run in the fume cupboard throughout the course of the experiment.
- Warning sign required
  - Chemicals have to be clearly labeled, e.g. harmful, toxic, flammable, and irritant.

- Safety Training Required
  - Safety training courses, included Chemical Safety (I), (II) and Biological Safety organized by the HSEO should be attended.
- Specific training required
  - None
- Emergency shutdown procedures
  - Follow the emergency shutdown procedures, absorb any liquid spillage using the laboratory caustic spill kit, transfer used materials to an appropriately labeled container and inform the person designated responsible for laboratory.
- If connected with chemicals and reagents
  - Immediately flush eyes or skin with top water at least 15 minutes and take off contaminated clothing as far as possible.

14. Action in Case of Abnormal or Emergency Situations

14.1 Service Failure

Close the main valve on the air cylinder and reactant inlet.

14.2 Action in case of fire or explosion

(1) When hear the fire alarm

• Remain calm and check if there is any sign of fire in the vicinity.

• If you see fire or smoke, or hear the announcement asking you to evacuate, follow the evacuation procedures below.

• If there is no sign of a fire, stay alert and pay attention to announcement until the fire alarm is silenced.

• Evacuate if the alarm has sounded for more than two minutes.

• If you hear the buzzer sound which indicates fire alarm is activated in an adjacent fire zone, stay alert and pay attention to announcement.

• If you hear both the buzzer and the fire alarm, you are near the boundary of fire zones, treat as if you hear the fire alarm.

(2) If discover a fire

• Activate the fire alarm by pressing the break glass fire alarm button.

• Report to Security Control Centre by dialing 8999.

• Alert other people. If SAFE to do so, try to put out the fire by firefighting equipment.

• DO NOT take any personal risk. If the fire gets beyond your control, evacuate immediately by following the procedures below.

• Close the door of the room on fire.

(3) When there is a fire and need to evacuate

• Remain calm. Walk, do not run, especially when travelling on staircases.

• Immediately leave the building and go to the assembly point using the nearest exit.

• Try to help those who may have difficulties travelling such as disabled and pregnant persons.

• DO NOT USE THE LIFTS.

• Report to your Fire & Safety Officer at the assembly point as far as practicable.

• Do not return to the building until permission is given by the Fire Services Department Officer in charge of the scene.

(4) Fire Fighting Equipment

• Water from the hose reels is good for wood, paper and structural fire, but NOT for oil, electrical or metal fire.

• The most common fire extinguishers on campus are the carbon dioxide types (black containers) which are good for general purposes, including oil and/or electrical fire.

• Some laboratories have dry powder fire extinguishers (blue container), which are good for chemical and/or metal fire.

• Sand (lab buckets) can be used to contain flammable liquid as well as put out a fire, including metal fire.

• Fire blanket can be used when someone's clothing catches fire.

14.3 Action in case of hazardous chemical spill in a research laboratory

• Alert co-workers.

• If safe to do so, (1). Confine the spill with appropriate materials. (2). Turn off remotely all heat/ignition sources if flammable vapor is involved.

• Ask for assistance if necessary.

• Press the Emergency Ventilation button (do not activate this button in case of fire).

• Inform the Security Control Centre by dialing 8999 from a safe location.

• Evacuate everyone in the affected area. Leave contaminated clothing and close the door.

• Activate local warning system to prevent others from entering the room.

• If possible, maintain a safe distance from the scene, keep the entrance or access routes in sight and help to prevent entry to the affected room.

• If conditions allow, remain to assist the emergency response team.

14.4 Action in case of other abnormal situations

In case of accidental contact with chemicals, flush eyes or skin with copious amount of water immediately for at least 15 minutes while removing contaminated clothing and shoes. For inhalation, remove to fresh air. If swallowed, wash mouth with water. Contact nearest physician.

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Are any of the following categories relevant to the proposed research?

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▫ Biologically active materials	☑ Yes	☐ No
▫ Radioactive materials or ionising radiation sources	☐ Yes	☑ No
▫ Non-ionising radiation (UV, microwaves, lasers)	☑ Yes	☐ No
▫ Highly toxic, carcinogenic or mutagenic materials	☐ Yes	☑ No
▫ Highly flammable or explosive materials	☐ Yes	☑ No
▫ Operation under extreme pressure or temperature	☐ Yes	☑ No

Status of Researcher

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   ▫ FYP ▫ UROP ▫ Student Helper
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Researcher

Name LEE, Yik Yeung Signature _____ Date _____

Assessment checked by research supervisor(s)

Name Prof. Henry H. N. Lam Signature _____ Date _____

Authorized by CBME Departmental Safety Officer (DSO)

Name Prof. John Barford Signature _____ Date _____

THE HONG KONG UNIVERSITY OF SCIENCE AND TECHNOLOGY
DIVISION OF BIOMEDICAL ENGINEERING

Persistence Study of *S. aureus* Towards Antibiotics

Work Plan #17010

Researcher: LEE Yik Yeung
Supervisor: Prof. Henry H. N. Lam

6 Feb, 2017

1. General Information

Name of Researcher:	LEE Yik Yeung
Name of Project Supervisor:	Prof. Henry H. N. Lam
Project Title:	Persistence Study of *S. aureus* Towards Antibiotics
Research Area:	Proteomics
Location:	Rm 7110 and Bioengineering Laboratory
Proposed Start Date:	15 February 2017

2. Experiment/Project Description

This project is to analyze the proteins in S. aureus after which is treated by antibiotics by proteomics. The bacteria will be extracted from medium and lysed to extract the proteins and the proteomes will be analyzed by mass spectrometry (MS). The method has been used in the research field for investigating the proteome changes of cells. Proteins will be extracted from S. aureus. The extracted protein will be desalinated and then analyzed by MS.

After the mass spectrum is successfully obtained, it will be compared with the protein spectrum existed in the Spectra Library. Comparing spectra with Spectra Library will require computational calculation. By this comparison, identifications and structures of the proteins can be revealed so that the changes of proteomes can be determined.

3. Equipment List

1. Biochemical Incubator
2. Incubator shaker
3. Biological Safety Cabinet
4. Chemical Fume Hood
5. Centrifuges
6. SpeedVac
7. Micro-plate Reader
8. Ultrasonic Homogenizer
9. High Performance Liquid Chromatography (HPLC)
10. Linear Trap Quadrupole (LTQ) Mass Spectrometry
11. Quadrupole Time-of-Flight (Q-TOF) Mass Spectrometry

4. Experimental Procedures

4.1 Cell Culture

4.1.1 Preparation of Mueller-Hinton liquid media

Reagent	Amount to add (for 100 mL)
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Beef extract	0.3 g
Starch	0.15 g
H_2O	100 mL

4.1.2 *S. aureus* cell culture

Pick a single *S. aureus* colony from a normal agar plate and inoculate a starter culture of 2-5 mL Mueller-Hinton liquid media with different antibiotics in different test-tubes. Incubate for approximately 8-12 h at 37 °C with vigorous shaking in nearly 300rpm.

4.2 Protein Extractions from bacteria

4.2.1 Harvest the *S. aureus* cells by centrifugation at 6000 × *g* for 15 minutes under 4°C and aspirate the supernatant.

4.2.2 Resuspend the pellet in cold PBS buffer and centrifuge at 6000 × *g* for 10 minutes under 4°C and aspirate the supernatant. Repeat 2 times.

4.2.3 Add ice-cold cell lysis buffer (8 M urea) and resuspend the pellet. Incubate on ice for 10 minutes.

4.2.4 Vortex tubes briefly and proceeds to sonication for 5 min at 4°C.

4.2.5 Transfer the supernatant to a new tube and centrifuge samples at 6000 × *g* for 15 minutes at 4°C to remove any insoluble material.

4.2.6 Add ice-cold acetone to precipitate the proteins.

4.2.7 Aspirate the supernatant and redissolve the proteins in buffer (4 M urea and 30 mM Tris-HCl, pH 6.5).

4.2.8 Take an aliquot for the protein quantification.

4.2.9 Repeat step 4.2.1 to 4.2.8 for other antibiotic samples.

4.3 Sample preparation

4.3.1 Reduce the proteins with dithiothreitol (DTT, 10 mM final concentration) at 37°C for 3 hours.

4.3.2 Alkylate the proteins with iodoacetamide (IAA, 20 mM final concentration) in dark for 1 hr. The alkylation reaction is quenched by adding DTT (10 mM final concentration) again.

4.3.3 Dilute the samples to a concentration of urea less than 1 M.

4.3.4 Digest the proteins with sequencing grade modified typsin (1: 50, w/w) at 37°C overnight.

4.3.5 Acidify samples with 10% formic acid to a final concentration of 0.5% (v/v).

4.3.6 Desalt the sample with C18 reverse-phase ZipTip.

4.3.7 Dry the sample with SpeedVac and store it at -20°C before use.

4.4 Analysis by mass spectrometry

4.4.1 Run the mass spectrometry and obtain the spectrum so as to do matching with database.

5. Procedure Template

Experimental Procedure No.	Experimental Procedure Description	Scale (Mass/Volume)	Location (Fumehood, benchtop, etc.)	Method (New or Existing)
4.1.1	Preparation of Mueller-Hinton liquid media	Acid hydrolysate of casein = 1.75 g Beef extract = 0.3 g Starch = 0.15 g H_2O = 100 mL	Room 6104, benchtop	Existing
4.1.2	*S. aureus* cell culturing	2~5 mL	Room 6104, BSC	Existing
4.2	Protein extraction	PBS buffer-5 mL Lysis buffer-2 mL Actone-5 mL	Room 7110, benchtop	Existing
4.3	Sample preparation	DTT-2 μL IAA-2 μL Sequencing grade modified typsin-2 μL Formic acid-2 μL	Room 7110, benchtop	Existing
4.4	Analysis by mass spectrometry	N/A	Room 7101 and BioCRF	Existing

6. HAZOP Template

Hazards and Operability Analysis							
4.1 Cell Culture							
No.	Hazards	Hazards Effect	Severity	Probability	Risk	Minimize Risk By	Residual Risk
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L
2	Autoclave	High pressure steam burns	H	M	H	Strictly follow the safety requests and do not overload the autoclave	L
3	Contact with bacteria	Causes illness and infection	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the biosafety cabinet	L
4.2 Protein Extraction							
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L

2	Centrifuge	Rotor Failure: the rotor that breaks can spin out of control and hit laboratory personnel. Spillage: the tubes may spray over the machine parts.	H	M	H	Strictly follow the safety requests. Balance all samples as closely as possible Set the rotors under the maximum speed.	L
4.3 Sample Preparation							
1	Contact with chemicals	Causes sever skin and eye burns	H	M	H	Wear protective gloves, face shield and lab coats; Conduct experiments in the fume hood	L
2	SpeedVac	Rotor Failure: the rotor that breaks can spin out of control and hit laboratory personnel. Spillage: the tubes may spray over the machine parts.	H	M	H	Strictly follow the safety requests. Balance all samples as closely as possible Set the rotors under the maximum speed.	L

Severity: L=Low (Minor injuries, first aid), M=Meddle (Hospitalization, medical leave), H=High (Serious injuries, fatality).
Probability: L=Low (Unlikely), M=Middle (Possible), H=High (Very likely).
Risk=Severity × Probability, the product follows the higher severity or probability

7. Operating Conditions

- Autoclave: 121°C, 103.4 kPa, 20 min
- Cell Culture: 37°C, shaking, 8~12 hr
- Pellet Cells: 6000 × *g*, 4°C, 10~15min
- Reduce Proteins: Dithiothreitol (DTT) (10 mM final concentration), 37°C, 3 hours
- Alkylate Proteins: Iodoacetamide (IAA) (20 mM final concentration), in dark, 1 hr
- Digest Proteins: 37°C, overnight

8. Services List

- Electricity (AC 220V, 50Hz)
- Tap water
- Double deionized Water
- Chemical Fume Hood
- Biological Safety Cabinet (Level 2)
- Linear Trap Quadrupole (LTQ) Mass Spectrometry

9. Chemicals List

Chemical	Purity	Quantity per Experiment
Acid hydrolysate of casein	99.999%	1.75 g
Beef extract	99%	0.3 g
Starch	99.99%	0.15 g
Urea	95%	50 µL (4M solution)
Tris-HCl	99%	10 mL (30 mM solution)
Dithiothreitol	99.5%	1 mg
Iodoacetamide	99%	1 mg
Formic Acid (CH_2O_2)	98%	1 mL

Acetone (C_3H_6O)	99.9%	5 mL
Trypsin	2,500 USP units/mg	3 mg
Phosphate Buffered Saline (PBS)	99.9%	10 mL
Oxacillin Sodium Salt ($C_{19}H_{18}N_3NaO_5S$)	99%	200 µg
Ampicillin sodium salt ($C_{16}H_{18}N_3NaO_4S$)	99%	200 µg
Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$)	99%	200 µg
Cephalexin Hydrate ($C_{16}H_{17}N_3O_4S \cdot xH_2O$)	99%	200 µg
Sulfathiazole Sodium salt ($C_9H_8N_3NaO_2S_2$)	99%	200 µg
Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$)	99%	200 µg
Erythromycin ($C_{37}H_{67}NO_{13}$)	99%	200 µg
Levofloxacin ($C_{18}H_{20}FN_3O_4$)	99%	200 µg

10. Summary of Relevant Hazards and Incompatibilities

Chemical	Summary of Hazards	Incompatibilities
Acid hydrolysate of casein	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Beef extract	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Starch	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents

Urea	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Tris-HCl	This substance is not classified as dangerous according to Directive 67/548/EEC.	Bases, Oxidizing agents
Dithiothreitol	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Bases, Oxidizing agents, Reducing agents, Alkali metals
Iodoacetamide	Toxic if swallowed. May cause sensitization by inhalation and skin contact.	Strong acids, Strong bases, Strong oxidizing agents, Strong reducing agents
Formic Acid (CH_2O_2)	Flammable liquid and vapor. Causes severe skin burns and eye damage.	Strong oxidizing agents, Strong bases, Powered metals
Acetone (C_3H6O)	Highly flammable. Irritating to eyes. Repeated exposure may cause skin dryness or cracking. Vapors may cause drowsiness and dizziness.	Bases, Oxidizing agents, Reducing agents, Acetone reacts violently with phosphorous oxychloride
Trypsin	May cause allergy or asthma symptoms or breathing difficulties if inhaled. Irritating to skin and eyes	N/A
Phosphate Buffered Saline (PBS)	Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.	Strong oxidizing agents, Strong acids
Oxacillin Sodium Salt ($C_{19}H_{18}N_3NaO_5S$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Ampicillin sodium salt ($C_{16}H_{18}N_3NaO_4S$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Oxidizing agents
Amoxicillin trihydrate ($C_{16}H_{19}N_3O_5S \cdot 3H_2O$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Cephalexin Hydrate ($C_{16}H_{17}N_3O_4S \cdot xH_2O$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents

Sulfathiazole Sodium salt ($C_9H_8N_3NaO_2S_2$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Tetracycline hydrochloride ($C_{22}H_{24}N_2O_8 \cdot HCl$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents
Erythromycin ($C_{37}H_{67}NO_{13}$)	This substance is not classified as dangerous according to Directive 67/548/EEC.	Strong oxidizing agents
Levofloxacin ($C_{18}H_{20}FN_3O_4$)	Harmful if swallowed. Irritating to eyes, respiratory system and skin.	Strong oxidizing agents

11. Waste List

- Cell culture (liquid) containing Mueller-Hinton liquid medium, autoclaved and dispose to the sink.
- Used PBS (liquid), autoclaved and disposed in the sink.
- Used acetone (liquid), disposed in the organic waste container of non-halogenated solvent.
- Used pipette and microcentrifuge tubes (solid), disposed in the waste container of biological waste and disposed as domestic waste after autoclave

12. Assessment of Significant Risks

May exposed to toxic or irritating chemicals. Goggles and gloves should be always put on.

13. Safety Precautions

- Personal Protective Equipment
  - A lab coat, safety glasses and respirator must be worn throughout the experiment. Rubber glove should be worn according to the nature of chemicals being handled.
- Fume Extraction System is required and in Place
  - The reaction system is run in the fume cupboard throughout the course of the experiment.
- Warning sign required
  - Chemicals have to be clearly labeled, e.g. harmful, toxic, flammable, and irritant.

- Safety Training Required
  - Safety training courses, included Chemical Safety (I), (II) and Biological Safety organized by the HSEO should be attended.
- Specific training required
  - None
- Emergency shutdown procedures
  - Follow the emergency shutdown procedures, absorb any liquid spillage using the laboratory caustic spill kit, transfer used materials to an appropriately labeled container and inform the person designated responsible for laboratory.
- If connected with chemicals and reagents
  - Immediately flush eyes or skin with top water at least 15 minutes and take off contaminated clothing as far as possible.

14. Action in Case of Abnormal or Emergency Situations

14.1 Service Failure

Close the main valve on the air cylinder and reactant inlet.

14.2 Action in case of fire or explosion

(1) When hear the fire alarm

• Remain calm and check if there is any sign of fire in the vicinity.

• If you see fire or smoke, or hear the announcement asking you to evacuate, follow the evacuation procedures below.

• If there is no sign of a fire, stay alert and pay attention to announcement until the fire alarm is silenced.

• Evacuate if the alarm has sounded for more than two minutes.

• If you hear the buzzer sound which indicates fire alarm is activated in an adjacent fire zone, stay alert and pay attention to announcement.

• If you hear both the buzzer and the fire alarm, you are near the boundary of fire zones, treat as if you hear the fire alarm.

(2) If discover a fire

• Activate the fire alarm by pressing the break glass fire alarm button.

• Report to Security Control Centre by dialing 8999.

• Alert other people. If SAFE to do so, try to put out the fire by firefighting equipment.

• DO NOT take any personal risk. If the fire gets beyond your control, evacuate immediately by following the procedures below.

• Close the door of the room on fire.

(3) When there is a fire and need to evacuate

• Remain calm. Walk, do not run, especially when travelling on staircases.

• Immediately leave the building and go to the assembly point using the nearest exit.

• Try to help those who may have difficulties travelling such as disabled and pregnant persons.

• DO NOT USE THE LIFTS.

• Report to your Fire & Safety Officer at the assembly point as far as practicable.

• Do not return to the building until permission is given by the Fire Services Department Officer in charge of the scene.

(4) Fire Fighting Equipment

• Water from the hose reels is good for wood, paper and structural fire, but NOT for oil, electrical or metal fire.

• The most common fire extinguishers on campus are the carbon dioxide types (black containers) which are good for general purposes, including oil and/or electrical fire.

• Some laboratories have dry powder fire extinguishers (blue container), which are good for chemical and/or metal fire.

• Sand (lab buckets) can be used to contain flammable liquid as well as put out a fire, including metal fire.

• Fire blanket can be used when someone's clothing catches fire.

14.3 Action in case of hazardous chemical spill in a research laboratory

• Alert co-workers.

• If safe to do so, (1). Confine the spill with appropriate materials. (2). Turn off remotely all heat/ignition sources if flammable vapor is involved.

• Ask for assistance if necessary.

• Press the Emergency Ventilation button (do not activate this button in case of fire).

• Inform the Security Control Centre by dialing 8999 from a safe location.

• Evacuate everyone in the affected area. Leave contaminated clothing and close the door.

• Activate local warning system to prevent others from entering the room.

• If possible, maintain a safe distance from the scene, keep the entrance or access routes in sight and help to prevent entry to the affected room.

• If conditions allow, remain to assist the emergency response team.

14.4 Action in case of other abnormal situations

In case of accidental contact with chemicals, flush eyes or skin with copious amount of water immediately for at least 15 minutes while removing contaminated clothing and shoes. For inhalation, remove to fresh air. If swallowed, wash mouth with water. Contact nearest physician.