Video Article

Total Protein Extraction and 2-D Gel Electrophoresis Methods for *Burkholderia* Species

Billie Velapatiño[1], James E. A. Zlosnik[1], Trevor J. Hird[1], David P. Speert[1]

[1]Department of Pediatrics, Centre for Understanding and Preventing Infection in Children, University of British Columbia

Correspondence to: Billie Velapatiño at bvelap@alumni.ubc.ca

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Abstract

The investigation of the intracellular protein levels of bacterial species is of importance to understanding the pathogenic mechanisms of diseases caused by these organisms. Here we describe a procedure for protein extraction from *Burkholderia* species based on mechanical lysis using glass beads in the presence of ethylenediamine tetraacetic acid and phenylmethylsulfonyl fluoride in phosphate buffered saline. This method can be used for different *Burkholderia* species, for different growth conditions, and it is likely suitable for the use in proteomic studies of other bacteria. Following protein extraction, a two-dimensional (2-D) gel electrophoresis proteomic technique is described to study global changes in the proteomes of these organisms. This method consists of the separation of proteins according to their isoelectric point by isoelectric focusing in the first dimension, followed by separation on the basis of molecular weight by acrylamide gel electrophoresis in the second dimension. Visualization of separated proteins is carried out by silver staining.

Video Link

The video component of this article can be found at https://www.jove.com/video/50730/

Introduction

The genus *Burkholderia* comprises more than 62 species, Gram negative organisms isolated from a wide range of niches, and it is divided in two main clusters[1,2]. The first cluster includes human, animal and phytotrophic organisms, and most studies have focused on the pathogenic species of this group due to their clinical importance. The most pathogenic members are *B. pseudomallei* and *B. mallei* (which causes melioidosis and glanders respectively)[3,4] and opportunistic pathogens (the 17 defined species of the *Burkholderia cepacia* complex, BCC)[5], which cause disease in cystic fibrosis (CF) and chronic granulomatous disease (CGD)[1]. The second cluster, with more than 30 nonpathogenic species, includes bacteria associated with plants or with the environment, and are considered potentially beneficial to the host[2].

Numerous complications emerge from bacterial infection with the pathogenic members of the *Burkholderia* genus, such as transmission of the pathogen between patients, spread of the disease and treatment failure because of the intrinsic or acquired resistance to antibiotics making hard to eradicate in most of the cases[6-9]. Therefore, gaining a clearer understanding of the basis for establishment of bacterial infection is vital to the treatment of diseases caused by these organisms. In order to gain insight into the establishment of infection, extensive investigations on the bacterial components associated with pathogenesis are needed. Studies focusing on the proteomic analysis of *Burkholderia* organisms using proteomic approaches described proteins that have been implicated in bacterial pathogenesis as well as changes in their proteome profiles[10-16].

Protein extraction methods using sonication and freeze-thawed cycles in lysis buffer containing high concentration of urea, thiourea in combination with detergent and ampholytes has been applied in *Burkholderia* proteomic studies[10-13]. Although urea is quite efficient for protein denaturation, it can establish an equilibrium in aqueous solution with ammonium isocyanate, which can react with amino acid groups, thereby forming artifacts (carbamylation reaction)[17]. Therefore, it is recommended to include carrier ampholytes, which act as cyanate scavengers and avoid temperatures above 37 °C[17]. Furthermore, to prevent any chemical interference of lysis buffer with protein quantification, the same lysis buffer can be used to generate the standard curve so that the samples and the standards have the same background[10]. Other methodologies involve the use of alkaline buffers and detergents with heat incubation periods[17,18]; however these conditions might induce changes in the proteome and some detergents are not compatible with proteomics application unless subsequent detergent removal steps are included[17,18].

After adequate extraction and quantification, global protein expression of each individual protein can be studied using proteomic approaches such as two-dimensional (2-D) gel electrophoresis. This technique was first described by O'Farell[19] and consists in the separation of proteins according to their isoelectric point by isoelectric focusing in the first dimension, and then according to their molecular weight by acrylamide gel electrophoresis in the second dimension. Due to its resolution and sensitivity, this technique is a powerful tool for the analysis and detection of proteins from complex biological sources[19,20]. This separation technique is currently available in protein-centric approaches with the great advantage of resolving protein isoforms caused by post-transcriptional modifications or proteolytic processing. Quantitative changes can be detected by comparing the intensity of the corresponding spot after staining of the gel[20]. However, this technique is not suited for the

identification of very large proteins, membrane proteins, extremely basic and acidic or hydrophobic proteins, and is a somewhat laborious and time-consuming technique[20]. New peptide-centric approaches (non gel-based) that are more robust and objective become available and can be used for quantitative comparison by differential stable isotope labeling methods such as cysteine labeling by isotope-coded affinity tagging (ICAT)[21], and amino group labeling by isotope tagging for relative and absolute quantitation (iTRAQ)[22]. The use of a single proteomic technique might give insufficient information; therefore the use of two complementary proteomic approaches is necessary for most fully assessing changes in proteome. Nevertheless, 2-D gel electrophoresis is widely used and can be routinely applied for quantitative expression of several proteins in different organisms.

Here we describe a whole-cell protein extraction and 2-D gel electrophoresis procedures for *Burkholderia* species that were adapted and optimized from the GE Healthcare 2-D Electrophoresis Principles and methods Handbook 80-6429-60AC 2004 (www.amershambiosciences.com). The protein extraction was carried out using a bead beater with glass beads in the presence of PBS containing 5 mM EDTA (ethylenediamine tetraacetic acid) and 1 mM PMSF (phenylmethylsulfonyl fluoride). This procedure allows quantification of proteins with minimal degradation and is amendable for proteomic approaches as previously reported[15,23,24]. 2-D gel electrophoresis was carried out using 24 cm long immobilized pH 4-7 gradient and proteins were separated according to their isoelectric point. Then proteins were separated according to their molecular weight by SDS-polyacrylamide gel. Additionally, we described a silver staining method for visualization of spot proteins, and a silver stain method that is compatible for mass spectrometry analysis. Together these procedures can allow the identification of important proteins from *Burkholderia* species that could be involved in pathogenesis.

Protocol

1. Culture Growth (Days 1+2)

1. Grow a starter culture: 3 ml of Luria Bertani (LB) broth in a snap top 15 ml tube, at 37 °C rotator overnight. Dilute overnight growth to an OD_{600} of 0.6. Add 1 ml of this dilution to 100 ml of LB broth in a 250 ml Erlenmeyer flask. Incubate at 37 °C with shaking at 250 rpm for 16 hr into stationary phase (SP), to better approximate, in batch culture, infection conditions and to assure that bacterial virulence factors under the control of stationary phase signal factors such as rpoS and quorum sensing, are expressed. Alternatively, bacteria grown at early SP or mid- or late-logarithmic phase can be used to gain information on the bacterial adaptation phase.
2. After 16 hr measure the OD_{600} and ensure the culture has reached SP by comparison to a previously constructed growth curve under identical conditions.

2. Protein Extraction (Day 3)

1. Chill all media and solutions beforehand in fridge overnight. All steps should be carried out with tubes in an ice bucket. Prechill a microcentrifuge and a Beckman Coulter High Performance centrifuge (or equivalent) to 4 °C and the rotor inside (rotor=JA-20, centrifuge=J2-HS).
2. Prepare fresh 0.1 M phenylmethanesulfonyl fluoride (PMSF, 174.19 g/mol) solution in acetone by dissolving 0.0174 g of PMSF ('CAUTION' PMSF is toxic and corrosive, use face shields and gloves) in 1 ml of acetone ('CAUTION' acetone is toxic and flammable, use face shields and gloves).
3. Make up a PBS/EDTA/PMSF solution: 0.1 ml of 0.1 M PMSF, 0.1 ml of 0.5 M EDTA, and 9.8 ml of PBS. Check the OD of the 16 hr culture is approximately where it should be in SP.
4. Take 35 ml of the bacterial culture and transfer to an Oakridge centrifuge tube and centrifuge the culture at 4,500 × *g* for 20 min at 4 °C.
5. Remove supernatant to a waste container and add 35 ml of cooled PBS and resuspend the pellet. Centrifuge again at 4,500 × g for 20 min at 4 °C.
6. Remove supernatant and resuspend in 1 ml of cold PBS. Transfer to sterile 2 ml Eppendorf and centrifuge 1 min on high in the microcentrifuge at 14,000 × g at 4 °C. Remove and discard supernatant.
7. Add 1 ml of PBS/EDTA/PMSF solution, resuspend the pellet and transfer to a 2 ml screw cap tube (with o-ring) containing ~0.5 ml glass beads (presterilized). Add the re-suspended pellet to the tube containing the glass beads and bead bash them at 4 °C for 1 min in cold-room. Do this 3x, putting the sample on ice after each bash.
8. Centrifuge at 14,000 x g for 1 min in the microcentrifuge. Remove the supernatant and put it in a sterile 5 ml polystyrene tube.
9. To increase the yield add 1 ml of PBS/EDTA/PMSF solution to same tube contain glass beads again. Bead bash the tube at 4 °C for 1 min. Do this 2x putting the sample on ice after bash. Centrifuge at 14,000 x g for one minute on the centrifuge and remove the supernatant and add to supernatant from step 2.8. Use a 1 ml syringe and a 0.20 µm filter the supernatant from the polystyrene tube to a new sterile 2 ml Eppendorf tube.
10. At this point the samples should be assayed for protein quantity, using the Pierce MicroBCA protein extraction kit and aliquots of 200 µg should be frozen at -80 °C until required.

3. Sample Preparation (Day 4)

1. Prepare 25 ml rehydration stock solution (8 M urea, 2% CHAPS, 2% IPG Buffer, 0.002% bromophenol blue) and store in 2.5 ml aliquots at -20 °C. Add 7 mg dithiothreitol (DTT, 154.2 g/mol) just prior to use to a 2.5 ml aliquot of rehydration stock solution.
2. Process thawed samples from step 2.10 using a 2-D Clean up kit with final resuspension in 450 µl of rehydration stock solution prepared in step 3.1.

4. First-dimension Isoelectric Focusing (IEF) (Day 4)

All steps in this section are using the Ettan IGPhor IEF System.

1. Set up the IPG first dimension strips: clean strip holders with strip cleaning solution and then wash with Milli-Q water, leave upside down to dry. Assign each sample to a gel strip holder number.
2. Load the sample from rehydration step in the second wider area from the (-) end. Use clean forceps to pull gel strips out of packaging. Take out protective layer from gel strips. Line strips rough side down in a slow, sliding motion to get the strips wet along the way from (-) end to (+) end.
3. Put damp blotting paper with sterilized water on top of electrode and under the gel strips to prevent burnout. Rinse tweezers in-between rounds with purified water and ethanol. Remove all bubbles, overlay thinly with mineral oil to prevent dry outs. Line the gel strip holders on the machine.
4. Run 12 hr at 20 °C for rehydration, 1 hr at 200 V, 1 hr at 500 V, 1 hr at 1,000 V, 0.5 hr at 4,000 V, 12 hr at 8,000 V, 24 hr hold at 300 V and can be taken out at this time.
5. After IEF is finished, it is recommended to perform the second-dimension SDS-polyacrylamide gel electrophoresis, unless the IPG strip are being frozen at -80 °C in Saran Wrap coated with mineral oil for future analysis. Alternatively, in order to avoid point streaking due to DTT access it is possible to include a second equilibration step of the IPG strips prior to the second dimension with a buffer that contains iodoacetamide.

5. Second-dimension SDS-polyacrylamide Gel Electrophoresis (Day 5)

All steps in this section are using the Ettan DALTsix electrophoresis apparatus.

1. To assemble the gel caster apparatus, thoroughly clean all components and ensure that gel caster is level and the grey rubber binding is lubricated with silicon gel. Put the black rubber triangular stopper on the bottom. Then, alternately place the separator and the glass plates with the shorter plate of glass facing front. Make sure the separators are on the back and the front. Fill the remaining space with the fillers (about 5 sheets) so the top is flat. Put the front panel on with the red tip on the outside. Use clamps to tighten each side and tighten the bottom screws.
2. Preparing the gel: Add 297.3 ml of Duracryl, ('CAUTION' Duracryl is toxic use face shields and gloves), 147 ml of Tris Buffer 1.5 M pH 8.8, and 143.3 ml of Milli-Q water into a flask with vacuum tip containing a stir bar. Put lid on top and mix solution at moderate speed with the vacuum on for 20 min. The vacuum is used to de-gas the solution. Make a fresh 10% of ammonium persulfate solution (APS, 218.8 g/mol): 0.2 g of APS to 2 ml of Milli-Q water.
3. Adding the gel mixture to the gel caster: When vacuum is done add 6 ml of 10% sodium dodecyl sulfate (SDS, 228.38 g/mol) to the side of the flask to avoid putting more bubbles in the solution. Add APS, stir, add 0.25 ml N,N,N',N'-tetramethylethylenediamine (TEMED, 116.20 g/mol) ('CAUTION' TEMED is flammable use face shields and gloves) and stir.
4. Pour gel mixture into gel caster through a funnel inserted at the back of the apparatus. Pour up to an inch below the short plate. Add 1.5 ml of water-saturated butanol to the top of the gel and wrap top with saran wrap to avoid dehydration. Wait for 1 hr to ensure full polymerization.
5. Checking the gel: When the gel is done (check the flask), disassemble the gel caster by a sink. Check for gel integrity while rinsing the glass plates with warm water; do not allow water to enter the plate. Pour out the butanol and rinse the top of gel twice with water to get rid of butanol. Fill the top with water again and let sit if the gels are not used right away.
6. Preparing the strips: Prepare equilibration buffer solution from the freezer (6 M Urea, 75 mM Tris-HCl pH 8.8, 29.9% SDS, 0.002% bromophenol blue). This can be preprepared and stored in 10 ml aliquots at -20 °C. Dissolve 0.1 g of DTT to a 10 ml of equilibration buffer solution. One tube can be used for two IPG strips. Place the gels of the strip face down onto the buffer and let sit for 30 min. Remember which end is which.
7. Preparing the Electrophoresis unit (2-D separation apparatus): Add 4.5 L of 1x electrophoresis buffer (25 mM Tris Base [121.1 g/mol], 192 mM Glycine [288.38 g/mol], and 0.1% w/v SDS [288.38 g/mol]) to the running tank. This buffer can be used for up to 3x. Turn the electrophoresis unit on. Check water level in water-cooling machine and fill it up with water if it is low. Turn the machine on, press mode to check the designated temperature, which should be 10 °C.
8. Make 1 L of 2x running buffer and 1 L of 1x of running buffer and store them at 4 °C. Make agarose-sealing solution: weight 0.25 g of agarose and dissolve in 50 ml of 1x running buffer. Pulse for 15 sec each.
9. Setting up the Electrophoresis unit: Use tweezers to take out each strip and remove the excess buffer on both sides with clean paper towel. DO NOT touch the strips. Pour off the water on top of the gel and line the gel top with 1x running buffer. Use clean tweezers and place the strips on top of the long plate, with the gel side facing towards you. Add 1x buffer to the strips to lubricate it. Slide strips further down by using plastic strips. Remove all bubbles in between the strip and the gel.
10. Add agarose-sealing solution to the top of the strip to seal it. Repeat for the rest of the strips. Place the glass plates containing the gels into gel cradle and lower into the gel tank. Ensure the 1x buffer level in the outer chamber is on the designated bottom line before putting the upper chamber on.
11. Place the frame for upper buffer chamber on top of the glass plates and ensure it is all the way down by pressing the bottom down. Use a funnel and add the 2x running buffer into the upper chamber up to the middle line. Use a funnel and add 1x running buffer to the outer chamber until it reaches the top line. Place lid on top and turn on the Electrophoresis unit and the power pack.
12. Run overnight at 52 V, 96 mA, 5 W. Check if the current is going by looking for bubbles on the silver wire near the top. Run the gel until the leading line is 1 cm from the bottom.
13. Proteins can also be analyzed using equipment and reagents from other companies such BIORAD or Hoefer, according to manufacturer instructions.

6. Silver Staining of the Gels for Gel Visualization (Day 5-6)

1. Prepare a fix solution 1 (800 ml ethanol, 200 ml acetic acid, and 1,000 ml of Milli-Q water in fume hood into a plastic bucket). Solution is good for 6 gels. Turn all the machines off and take out the whole middle section of the Electrophoresis unit and pull it in sink. Also put the white stand in the sink. Pull out the top tray containing the 2x running buffer.
2. Disassemble the apparatus and remove the gels from the glass plates into fix solution 1. Gels can be identified by cutting off corners when they are removed from the glass plates, the number of corners cut corresponding to the gel order in the caster.
3. Place the container with the fix solution and the gels on a rocker for at least 1 hr to overnight at 4 °C.

4. Transfer the gels to fix Solution 2 (20 ml of 50% glutaraldehyde, 600 ml of ethanol, 5 g of potassium tetrathionate (302.46 g/mol), 136 g of sodium acetate (82.03 g/mol), and Milli-Q water to 2,000 ml) and place on a rocker for 1 hr.
5. Wash the gels 4x for 15 min each in Milli-Q water.
6. Stain the gels in Silver Nitrate Solution (4 g of silver nitrate (169.87 g/mol), 500 µl of formaldehyde, and Milli-Q water to 2,000 ml) for 30 min or up to 48 hr.
7. Wash gel for 1 min in Milli-Q water.
8. Transfer to developer Solution (60 g of sodium carbonate (105.99 g/mol), 300 µl of formaldehyde, 15 mg of sodium thiosulfate (158.11 g/mol), and Milli-Q water to 2,000 ml) for 5-30 min until the gel is stained.
9. Put the gels in Stop Solution (100 g of Tris-Base (121.14 g/mol), 40 ml of acetic acid, and Milli-Q water to 2,000 ml) for 10 min.
10. For storage, transfer the gels to Glycerol Solution (40 ml of glycerol or 400 ml if long term storage is desired, and 1,600 ml of Milli-Q water) for 10 min and then dry them using a gel dryer.
11. Gels can be visualized after staining using imaging systems such as a scanner/densitometer, photographed using light transilluminator or Gel imager. Image Analysis software such as PDQuest 2-D analysis from BIORAD can then be used to obtain quantitative and qualitative information from proteins in a sample.

7. Gel Silver Staining for Mass Spectrometry Analysis

1. Fix the gels in 50% methanol, and 5% acetic acid for 1 hr.
2. Wash in 50% methanol for at least 10 min to overnight.
3. Wash in distilled water 3x for 10 min.
4. Sensitize with 0.02% sodium thiosulfate (158.11 g/mol) 2x for 15 min.
5. Wash 3x in CHILLED distilled water 3x for 10 min.
6. Submerge gel in CHILLED 1% silver nitrate solution (169.87 g/mol) for at least 20 min to 1 hr at 4 °C
7. Wash 2x for 1 min with distilled water. Change tray/bucket.
8. Develop in 0.04% formaldehyde in 2% sodium carbonate anhydrous (105.99 g/mol) and discard when it turns yellow quite quick; have three batches and be ready to change every 5 min.
9. Wash in 5% acetic acid then store in 1% acetic acid.
10. Rinse 1.5 ml Eppendorf tubes (to hold get spot cut-outs) with USP grade ethanol and air-dry prior to use.
11. Clean forceps, razor blades and surfaces with 100% methanol.
12. Spray gloves and surfaces with ethanol.
13. Excise spots in a flow hood or clean air area. Spots can be also excised using an automated spot cutter.
14. Put them in Eppendorf tubes and ship on ice or keep in fridge.

Representative Results

Comparative analysis of the protein profiles extracted from the same bacterial culture on two different occasions showed similar patterns banding indicating successful protein extractions. Molecular weight proteins extracted ranged from 10-150 kDa. **Figure 1** shows representative Coomassie blue staining gel of the whole-cell protein extractions from *Burkholderia multivorans* (a member of the BCC) clinical isolates grown in LB or Yeast/Manitol (YEM) broth and harvested from stationary phase.

For 2-D gel electrophoresis analysis, 200 µg of total proteins from *Burkholderia pseudomallei* was used (**Figure 2**). Since this pathogen is recognized as a B-type biological warfare agent by the US Centers for Disease Control and Prevention[25], protein preparation procedures were carried out in Bio Safety Level 3 containment laboratory and accordingly with standard operation procedures. Proteins were resuspended in rehydration solution and applied to a 24 cm long immobilized pH 4-7 gradient and proteins were separated according to their isoelectric point (pI). Then strips were placed on a SDS-polyacrylamide gel and proteins were separated according to their molecular weight (MW). Subsequently, gel was silver stained for the protein spot visualization. Results showed the abundance of more than 500 protein spots that can be individually identified by mass spectrometry.

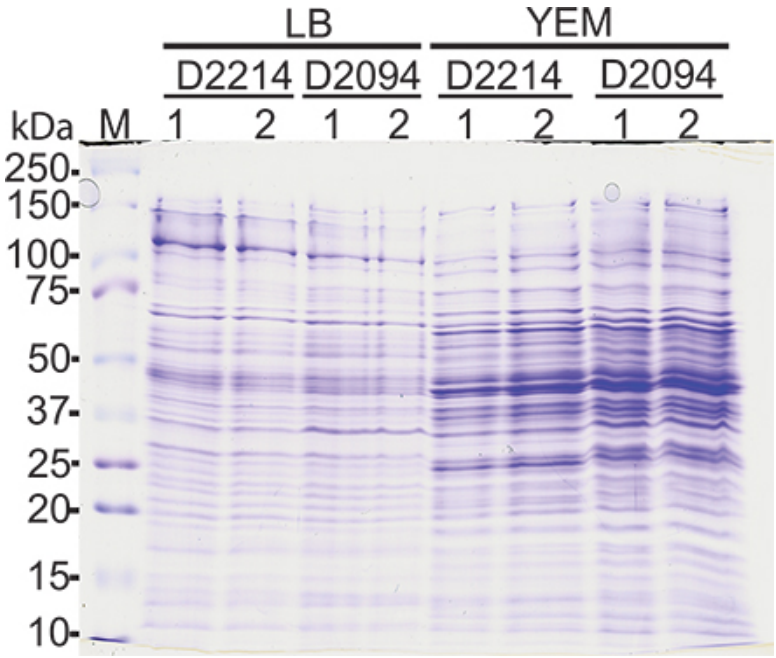

Figure 1. Total protein profiles of *Burkholderia multivorans* isolates. SDS-PAGE analysis of protein extracted at the stationary phase from D-2214 and D-2094 isolates grown in LB or Yeast/Manitol (YEM) broth in two different occasions (1 and 2). 4 µg of protein were loaded in each lane of a 12.5% gel and stained with Coomassie blue. Lane M, protein marker.

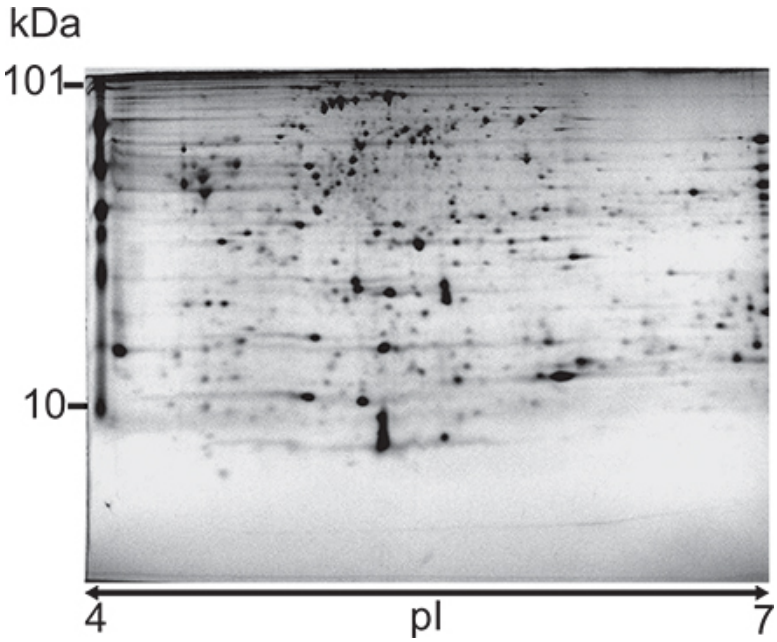

Figure 2. 2-D gel electrophoresis analysis of *Burkholderia pseudomallei* 1234B isolate. Representative silver-stained 2-D gel showing protein separation in the pI 4 to 7-range strip. Whole-cell protein extracts (200 µg) at the stationary phase were used and separated on a 15% SDS-PAGE gel.

Discussion

A method for proteins preparation has been described that can extract the majority of *Burkholderia* proteins with good reproducibility. This is demonstrated by obtaining the same protein profile from two independent preparations performed in different days using the same bacterial culture grown in LB or YEM broth as shown in **Figure 1**. Extraction was efficient for bacteria grown in liquid media; however we have not tested this method for bacteria grown on plates. This method was also used for further protein characterization using proteomic tools; our group has successfully studied proteome changes using 2-D gel electrophoresis and isotope tagging for relative and absolute quantitation (iTRAQ) proteomic techniques[15,23,24]. For the latter, proteins were extracted into a buffer containing 0.5 M EDTA in PBS to avoid any interference of PMSF with the iTRAQ experiments[15].

It is important to remark that during the protein extraction process and 2-D gel electrophoresis, all steps should be carried out at 4 °C or in cold conditions using ice buckets to minimize protein degradation, as well as to use plugged tips and wear nitrile gloves and cover all skin to prevent keratin contamination of any spots cut out for subsequent identification by mass spectrometry. When performing protein extractions, it is also important to consider that the PMSF has a short half-life time in aqueous solutions, according to the manufacturer, therefore the 0.1 mM stock solution in acetone should be made immediately before use. Alternatively, other serine inhibitors or protease inhibitors that are more soluble and stable, and less toxic can be used, although we have not tested them in our experiments.

The lysis buffer used in this procedure does not contain urea, which is important for the solubilization to extract both aqueous (cytosolic) proteins and less soluble proteins, including some membrane proteins[17]. But, during sample preparation for first-dimension isoelectric focusing (step 3 of this protocol), samples are resuspended in rehydration buffer that contains urea. Other proteomic techniques also involve similar treatment[26]. Before following with the rehydration step, it seems that the removal of interfering materials or low molecular weight impurities is crucial for high resolution, we recommend to use the 2-D Clean-up Kit in each protein samples as results improved. Previously loading the samples for first-dimension isoelectric focusing separation, make sure to have prepared strips of blotting paper the right size of the strip holders, as this is problematic to cut the right size in aseptic fashion on the spot (for step 3.4 of this protocol).

Before running a 2-D SDS-polyacrylamide gel electrophoresis, it is necessary to ensure that the gel percentage fits the expected sizes of interest (a 12.5% percentage gel of can resolve protein in the range of 14-100 kDa). After second dimension gel electrophoresis is completed, visualization of proteins can be achieved by Coomassie blue or silver staining; the latter staining is more sensitive with detection limit is as low as 0.1 ng/protein/spot[20]. Protein spots visualized with silver staining are suitable for further analysis by mass spectrometry. However, it is important to mention that silver stain solution should not contain glutaraldehyde since this agent crosslinks with proteins; therefore it is not compatible with further mass spectrometry analysis[20]. Alternatively, to detect and quantify differentially expressed proteins, another gel-based approach such as Two Dimensional-Difference In Gel Electrophoresis (2D-DIGE) together with automated software can be used. This approach employs distinct fluorescent tags (*e.g.* Cy 3, 5 and 2) that are used to label samples and a universal internal standard prior to second dimension electrophoresis overcoming, to some degree, the disadvantages of variation and reproducibility of 2-D electrophoresis[27]. Additionally, gels can be stained after the second dimension with SyproRuby, which is an organometallic ruthenium chelate stain designed for proteomic applications. It can detect protein spots with similar sensitivity to that silver staining, but with greater sensitivity than Coomassie blue. After staining gels can be photographed with laser scanner or transilluminator[28].

In conclusion, this video describes a whole-cell bacterial protein extraction procedure and 2-D gel electrophoresis method that can allow the study of global changes in the proteome in *Burkholderia* species.

Disclosures

The authors declare that they have no competing financial interests.

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References

1. Drevinek, P. & Mahenthiralingam, E. *Burkholderia cenocepacia* in cystic fibrosis: epidemiology and molecular mechanisms of virulence. *Clin. Microbiol. Infect.* **16**, 821-830, doi:10.1111/j.1469-0691.2010.03237.x (2010).
2. Suarez-Moreno, Z.R., *et al.* Common Features of Environmental and Potentially Beneficial Plant-Associated *Burkholderia*. *Microb. Ecol*. doi:10.1007/s00248-011-9929-1 (2011).
3. Wiersinga, W.J., van der Poll, T., White, N.J., Day, N.P., & Peacock, S.J. Melioidosis: insights into the pathogenicity of *Burkholderia pseudomallei*. *Nat. Rev. Microbiol.* **4**, 272-282 (2006).
4. White, N.J. Melioidosis. *Lancet.* **361**, 1715-1722 (2003).
5. Mahenthiralingam, E., Urban, T.A., & Goldberg, J.B. The multifarious, multireplicon *Burkholderia cepacia* complex. *Nat. Rev. Microbiol.* **3**, 144-156 (2005).
6. Speert, D.P., Henry, D., Vandamme, P., Corey, M., & Mahenthiralingam, E. Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. *Emerg. Infect. Dis.* **8**, 181-187 (2002).
7. Dance, D.A., Wuthiekanun, V., Chaowagul, W., & White, N.J. The antimicrobial susceptibility of *Pseudomonas pseudomallei*. Emergence of resistance *in vitro* and during treatment. *J. Antimicrob. Chemother.* **24**, 295-309 (1989).
8. Thibault, F.M., Hernandez, E., Vidal, D.R., Girardet, M., & Cavallo, J.D. Antibiotic susceptibility of 65 isolates of *Burkholderia pseudomallei* and *Burkholderia mallei* to 35 antimicrobial agents. *J. Antimicrob. Chemother.* **54**, 1134-1138 (2004).
9. Govan, J.R.W., *et al.* Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet.* **342**, 15-19 (1993).
10. Thongboonkerd, V., *et al.* Altered proteome in *Burkholderia pseudomallei* rpoE operon knockout mutant: insights into mechanisms of rpoE operon in stress tolerance, survival, and virulence. *J. Proteome. Res.* **6**, 1334-1341 (2007).
11. Park, K.H., Lipuma, J.J., & Lubman, D.M. Comparative proteomic analysis of *B. cenocepacia* using two-dimensional liquid separations coupled with mass spectrometry. *Anal Chim. Acta.* **592**, 91-100 (2007).
12. Wongtrakoongate, P., Mongkoldhumrongkul, N., Chaijan, S., Kamchonwongpaisan, S., & Tungpradabkul, S. Comparative proteomic profiles and the potential markers between *Burkholderia pseudomallei* and *Burkholderia thailandensis*. *Mol. Cell Probes.* **21**, 81-91 (2007).
13. Harding, S.V., *et al.* The identification of surface proteins of *Burkholderia pseudomallei*. *Vaccine.* **25**, 2664-2672 (2007).

14. Madeira, A., Santos, P.M., Coutinho, C.P., Pinto-de-Oliveira, A., & Sa-Correia, I. Quantitative proteomics (2-D DIGE) reveals molecular strategies employed by *Burkholderia cenocepacia* to adapt to the airways of cystic fibrosis patients under antimicrobial therapy. *Proteomics.* **11**, 1313-1328 (2011).
15. Zlosnik, J.E.A. & Speert, D.P. The Role of Mucoidy in Virulence of Bacteria from the *Burkholderia cepacia* Complex: A Systematic Proteomic and Transcriptomic Analysis. *J. Infect. Dis.* **202**, 770-781, doi:10.1086/655663 (2010).
16. Riedel, K., Carranza, P., Gehrig, P., Potthast, F., & Eberl, L. Towards the proteome of *Burkholderia cenocepacia* H111: setting up a 2-DE reference map. *Proteomics.* **6**, 207-216 (2006).
17. Weiss, W. & Gorg, A. Sample solublization buffers for two-dimensional electrophoresis. *Methods Mol. Biol.* **424**, 35-42 (2008).
18. von der Haar, T. Optimized protein extraction for quantitative proteomics of yeasts. *PLoS One.* **2**, e1078 (2007).
19. O'Farrell, P.H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* **250**, 4007-4021 (1975).
20. Gorg, A., Weiss, W., & Dunn, M.J. Current two-dimensional electrophoresis technology for proteomics. *Proteomics.* **4**, 3665-3685 (2004).
21. Gygi, S.P., Rist, B., Griffin, T.J., Eng, J., & Aebersold, R. Proteome analysis of low-abundance proteins using multidimensional chromatography and isotope-coded affinity tags. *J. Proteome Res.* **1**, 47-54 (2002).
22. Ross, P.L., *et al.* Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol. Cell Proteomics.* **3**, 1154-1169 (2004).
23. Velapatiño, B., Limmathurotsakul, D., Peacock, S.J., & Speert, D.P. Identification of differentially expressed proteins from *Burkholderia pseudomallei* isolated during primary and relapsing melioidosis. *Microbes. Infect.* **14**, 335-340 (2012).
24. Chung, J.W. & Speert, D.P. Proteomic identification and characterization of bacterial factors associated with *Burkholderia cenocepacia* survival in a murine host. *Microbiology.* **153**, 206-214 (2007).
25. Rotz, L.D., Khan, A.S., Lillibridge, S.R., Ostroff, S.M., & Hughes, J.M. Public health assessment of potential biological terrorism agents. *Emerg. Infect. Dis.* **8**, 225-230 (2002).
26. Thon, J.N., *et al.* Comprehensive proteomic analysis of protein changes during platelet storage requires complementary proteomic approaches. *Transfusion.* **48**, 425-435 (2008).
27. Wu, T. In New and Emerging Proteomic Techniques. *Methods Mol. Biol.* **328**,71-95 (2006).
28. Lopez, M.F., *et al.* A comparison of silver stain and SYPRO Ruby Protein Gel Stain with respect to protein detection in two-dimensional gels and identification by peptide mass profiling. *Electrophoresis.* **21**, 3673-3683 (2000).

