Two-dimensional gel electrophoresis image analysis of two Pseudomonas aeruginosa clones

Análisis de imágenes bidimensionales de electroforesis en gel de dos clones de Pseudomonas aeruginosa

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Keywords

2D-GE; Image analysis; CellProfiler; P. aeruginosa C25; P. aeruginosa C50.

Abstract

A classical strategy to analyse the protein content of a biological sample is the two-dimensional gel electrophoresis (2D-GE). This technique separates proteins by both isoelectric point and molecular weight, and images are taken for subsequent analyses. However, analyses of 2D-GE images require standardized image analysis due to susceptibility of gels to get deformed, presence of overlapping spots and stripes, fuzzy and unstained spots, and others. This represent a difficulty for final users (researchers), which demand for free and user-friendly solutions. We have previously reported the standardization of a protocol to analyse 2D-GE images, and in the current study we applied it to two new bacterial isolates *Pseudomonas aeruginosa* C25 and C50. We first extracted periplasmic proteins after exposure to antibiotics, and we then run a 2D-GE analysis. Images were analysed using our standardized protocol, achieving the identification of protein spots using CellProfiler after pre-processing step. Comparison between strains was done using differential spot analysis, revealing a specific pattern in the protein expression between bacteria. These results will help to study the biological meaning of these strains using proteomic profiling under different conditions.

Palabras clave

2D-GE; análisis de imágenes; CellProfiler; P. aeruginosa C25; P. aeruginosa C50.

Resumen

Una estrategia clásica para analizar el contenido de proteínas de una muestra biológica es la electroforesis bidimensional en gel (2D-GE). Esta técnica separa las proteínas tanto por punto isoeléctrico como por peso molecular, y se toman imágenes para análisis posteriores. Sin embargo, los análisis de imágenes 2D-GE requieren un análisis de imagen estandarizado debido a la susceptibilidad de los geles a deformarse, la presencia de manchas y rayas superpuestas, manchas borrosas y sin teñir, y otros. Esto representa una dificultad para los usuarios finales (investigadores), que demandan soluciones gratuitas y fáciles de usar. Anteriormente informamos de la estandarización de un protocolo para analizar imágenes 2D-GE, y en el estudio actual lo aplicamos a dos nuevos aislados bacterianos Pseudomonas aeruginosa C25 y C50. Primero extrajimos proteínas periplásmicas después de la exposición a antibióticos y luego realizamos un análisis 2D-GE. Las imágenes se analizaron usando nuestro protocolo estandarizado, logrando la identificación de manchas de proteína usando CellProfiler después del paso de preprocesamiento. La comparación entre cepas se realizó mediante análisis de puntos diferenciales, que reveló un patrón específico en la expresión de proteínas entre bacterias. Estos resultados ayudarán a estudiar el significado biológico de estas cepas utilizando perfiles proteómicos en diferentes condiciones.

Introduction

The study of the protein content in biological systems is the main study subject of proteomics. This included not only to identify the particular proteins that are expressed that can explain a biological context, but also the comparison between conditions to recognize differential proteomic patterns [1].

A classical strategy to analyze the proteomic profile of a sample is the two-dimensional gel electrophoresis (2D-GE) [2]this technique is a powerful tool for the analysis and detection of proteins from complex biological sources. Proteins are separated according to isoelectric point by isoelectric focusing in the first dimension, and according to molecular weight by sodium dodecyl sulfate electrophoresis in the second dimension. Since these two parameters are unrelated, it is possible to obtain an almost uniform distribution of protein spots across a two-diminsional gel. This technique has resolved 1100 different components from Escherichia coli and should be capable of resolving a maximum of 5000 proteins. A protein containing as little as one disintegration per min of either 14C or 35S can be detected by autoradiography. A protein which constitutes 10 minus 4 to 10 minus 5% of the total protein can be detected and quantified by autoradiography. The reproducibility of the separation is sufficient to permit each spot on one separation to be matched with a spot on a different separation. This technique provides a method for estimation (at the described sensitivities. This technique separates proteins in a layer of polyacrylamide gel by both isoelectric point (pI, pH at which a molecule is electrically neutral) and molecular weight [3], creating spots that are then stained.

Analyses of 2D-GE images require standardized image analysis [3], due to susceptibility of gels to get deformed, presence of overlapping spots and stripes, fuzzy and unstained spots, and others. [1], [4]. However, the 2D-GE image analysis is not straightforward. This represent a difficulty for final users (such as microbiologist, biologist and researchers in general), which demand for user-friendly solutions. However, these user-friendly software are expensive commercial packages. Free options regularly requires command-line work, making it a drawback for researchers.

In this scenario, we have previously reported the standardization of a protocol to analyze 2D-GE images using the Costa Rican bacteria Pseudomonas aeruginosa AG1 as model [5]. Now, in this work we applied our protocol to two new isolates, P. aeruginosa C25 and C50, which are two clones obtained from the former strain when exposed to high ciprofloxacin (antibiotic) concentrations. P. aeruginosa is an opportunistic bacteria able to infect immunocompromised hosts, which is frequently associated with antibiotic multi-resistance [6]. The three Costa Rican isolates have a multi-resistance profile. They are categorized as a high risk clones because are coming from a strain causing infections in hospitals. Thus, the goal of this study was to implement and assess an image analysis protocol using our previously reported protocol to identify protein spots in 2D-GE gels images from two P. aeruginosa strains C25 and C50.

To achieve this, we first extracted periplasmic proteins of P. aeruginosa C25 and C50 after exposure to antibiotics, and we then run a 2D-GE analysis. Images were analyzed using our standardized protocol, by identifying spots using CellProfiler. Then, comparison between conditions was done using differential spot analysis.

Methods

For the extraction of periplasmic proteins of P. aeruginosa C25 and C50, we followed the protocol by [5], [7]. Briefly, cells were cultured until the exponential phase in LB medium. The 2D-GE was performed using strips for separation by isoelectric point (GE HealthCare Immobiline Dry Strip GelsTM), and a SDS-GE gradient was done for the molecular weight separations. Images were taken using ChemiDoc™ photo viewer (BioRad®).

The processing step included an image alignment using bUnwarpJ package in the ImageJ program [8]and speci\u001ccally in biomedical applications that require inter or intra modality image alignment. We have developed an ImageJ plugin called bUnwarpJ, which elastically registers pairs of images. This simple and easy-to-use plugin can be used by researchers and clinicians to create anatomical atlases, segment images using atlases, align pairs of images

distorted by both physical and acquisition related distortions, etc. Registering two images consists on \u001cnding the image transformation that maps corresponding pairs of pixels between the original and \"distorted\" images. We use here the term distorted in a wide sense, to account not only for \"sensu strictu\" image distorsions, but also for anatomical variations between or within individuals. We use an algorithm that simultaneously calculates the direct and inverse transformations and minimizes the similarity error between the target and source images after imposing a consistency constraint. This approach provides bidirectional registration \ u0015from image A to B or from B to A\u0015 in a single computation. We use B-splines to represent both images and deformations and make use of a powerful optimizer to converge fast to the best image alignment. Our plugin allows guiding the registration process using the image similarity, the consistency of the deformations, vector-spline regularization and/or a set of optional landmarks, which can be calculated and fed from other ImageJ plugins such as the automatic extractors Scale-Invariant Feature Transform (SIFT. In this program, five spots were used as reference for the deformation of images and to achieve the alignment. Identification of spots was done using our previously reported protocol [5]. Briefly, CellProfiler (https://CellProfiler.org/) was used to analyze images following the next steps: images inversion, primary object recognition and segmentation, manual editing, intensity measuring and visualization of objects.

To compare 2D-GE images, a differential spot analysis was implemented. Pairs of images were compared to identify shared spots using an analysis of primary objects (segmentation) of overlapping spots, identification of exclusive spots in each image using the no-overlapping regions, and the subsequent representation spot borders separating shared (red circles) or exclusive dots (green or blue circles).

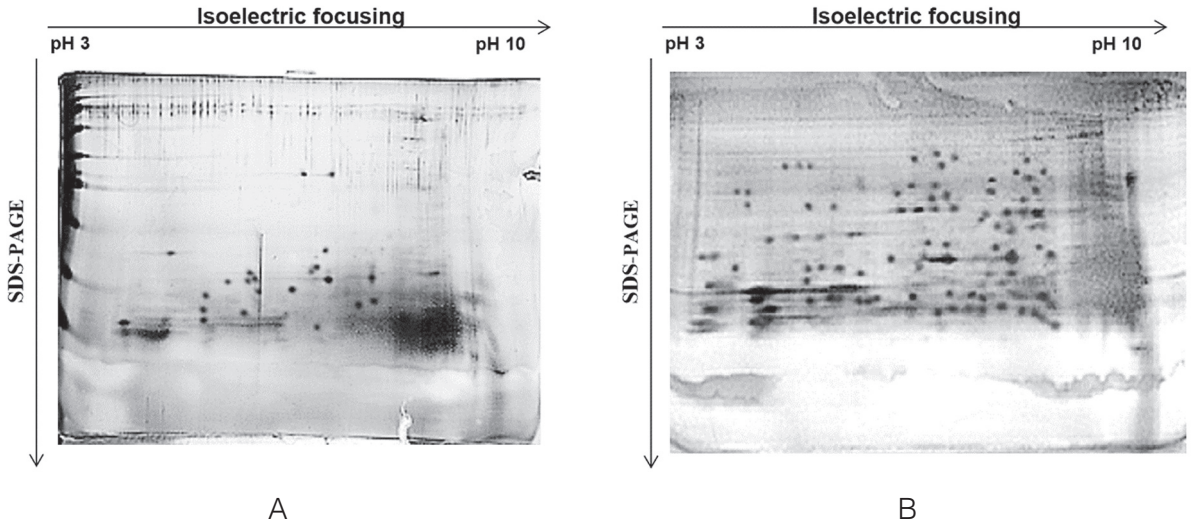

Figure 1. Example of two-dimensional gel electrophoresis (2D-GE) of P. aeruginosa C25 (A) and C50 (B) after growing in LB medium. Assays was performed after cells were growth in LB medium.

Results and discussion

Proteomics is considered an essential field for the systematic analysis of biological systems, an assessment of changes in the abundance of proteins that occur in living organisms and that can be studied at various levels [4].

The two-dimensional gel electrophoresis 2D-GE is a classical technique used to analyze the protein content in biological samples [1]. Here we first performed a 2D-GE assay for the bacterial clones P. aeruginosa C25 and C50, as shown in Fig. 1-A-B.

However, 2D-GE image analysis requires specific protocols due to image complexity [3]. In this way, we previously established a standardized protocol to identify protein spots using CellProfiler and other image analysis tools [5].

For the pre-processing step, bUnwarpJ package in the ImageJ program was used to align images. According to this pipelines, five points between the target image (to be modified) and a reference image are selected as common denominator to make the alignment, creating a deformation field and grid (Fig. 2-A-B).

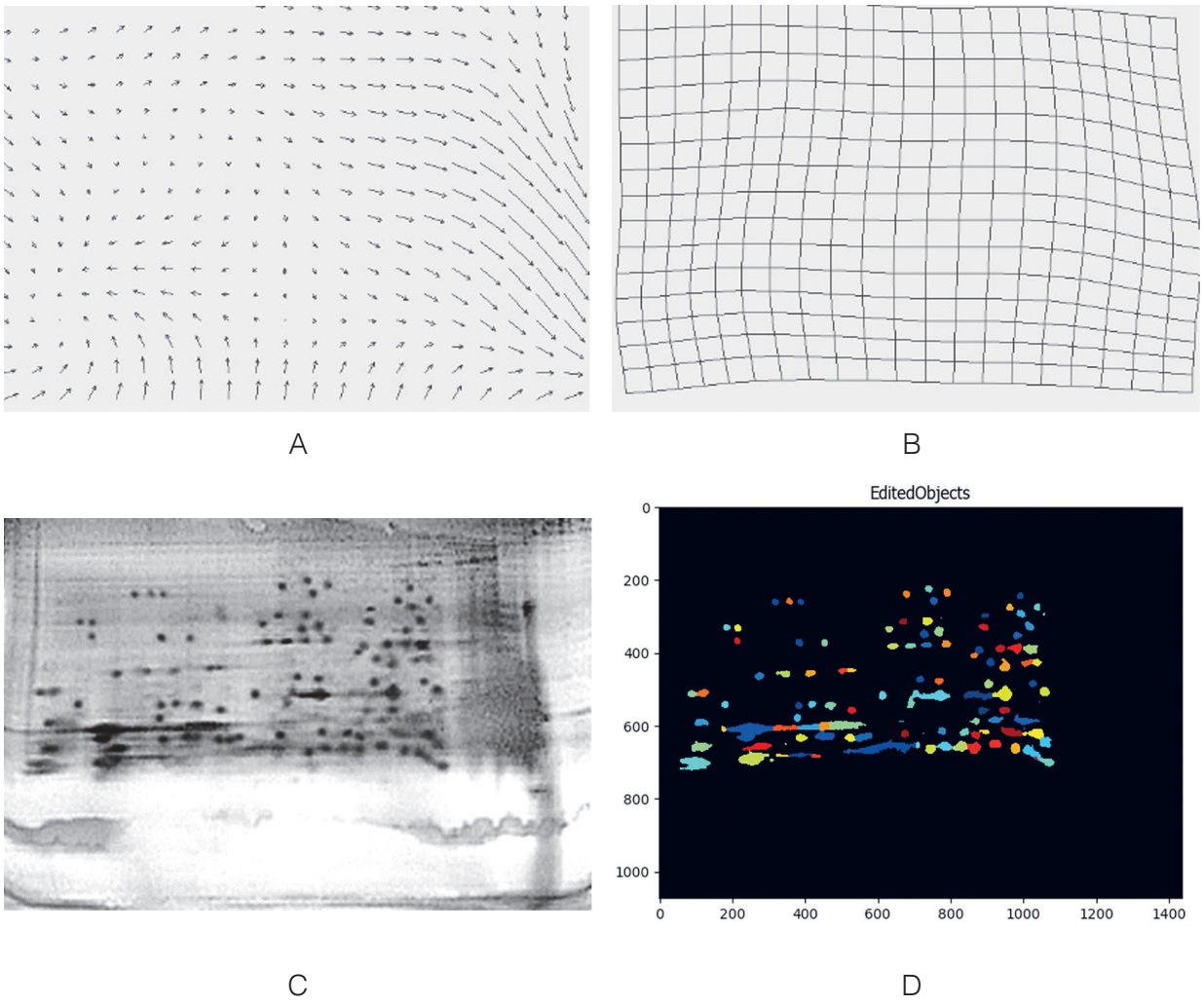

Figure 2. Analysis of 2D-GE images. Examples of deformation field (A) and deformation grid (B) to align images against a reference in the pre-processing step. (C) Example of a raw image used for the identification of spots using CellProfiler pipeline, as resulted in (D).

As shown in Fig. 2-C-D, identification of spots was achieved using CellProfiler software. Different metrics were used to optimize the segmentation algorithm, as previously described [5]. Although automatic spot recognition is sensitive to complex regions, manual edition helped to solve these drawbacks. Commercial solutions have similar tools to deal with this particular features that are common un 2D-GE image analyses [3].

With a modified protocol, the pipeline was also able to recognize common and shared spots when comparison of proteomic profiles of the two strains was done.

For this, a new consensus image was built using image operations (pixel operations), making possible the identification of common spots, which were identified in the same way as before but using the new image. After subtraction of shared dots, exclusive spots were marked and a final visualization was done in the initial images, as shown in Fig. 3.

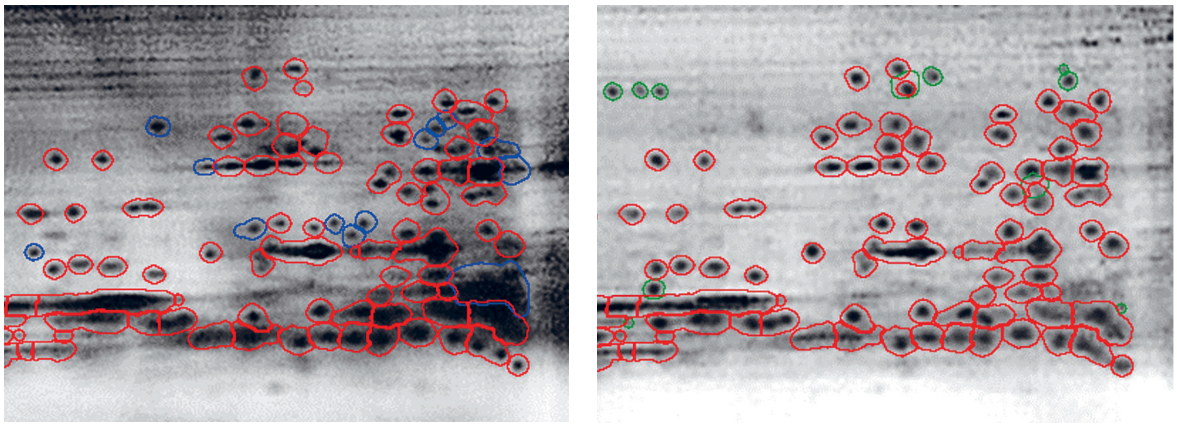

Figure 3. Example of the differential spot identification with 2D-GE images from two P. aeruginosa strains C25 (A) and C50 (B). Shared spots were identified using red circles, and exclusive spots were marked as blue or green spots.

Regarding the CellProfiler program, this is a known tool used for cell imaging, for example for microscopy images. However, as we have demonstrated before [5] and here, it is possible to use the algorithms to recognize spots in 2D-GE images. See our previous work for details of the implementations, more details of the pipeline and comparison of samples [5].

In summary, in this work we presented a new analysis of 2D-GE images using a standardized protocol to identify spots and compare conditions by proteomic profile. This was done using two P. aeruginosa clones, in which was possible to identify both shared and exclusive dots. Although this work is focused on the image analysis, these results will help us to apply this protocol to study P. aeruginosa strains under different experimental conditions, including antibiotics or other stressors and their effect on the proteomic profile of the bacteria.

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