Monitoring virus-like particle and viral protein production by intact cell MALDI-TOF mass spectrometry

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A new application of intact cell MALDI-TOF MS (ICM-MS) methodology is described for monitoring the production of viral proteins and viral-like particles using the baculovirus/insect cells expression system. Various MALDI matrices, cell preparation methods, cell/matrix volume ratio and MALDI target application procedures were tested in order to obtain the highest intensity and reproducibility of intact insect cell spectra. The web interface, SPECLUST (http://bioinfo.thep.lu.se/speclust.html), was used to construct dendograms based on MALDI-TOF MS data for evaluation of fingerprint changes.

We demonstrate that insect cell mass spectrum fingerprints are characteristic of each viral protein/particle production. Their changes along the time for each production experiment correlate with the intracellular viral protein content determined by Western blot.

This work shows that this simple, fast and low cost assay, which requires low sample volume, is a powerful analytical tool that complements the most common analytical methods used for monitoring bioprocesses and has potential application in the biotechnological industry namely, in the production of recombinant proteins.

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1. Introduction

Since the late 80’s [1,2], matrix-assisted laser-desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) has been applied in many scientific research areas, mainly in the proteomics field [3]. More recently, the suitability of MALDI MS approaches for identification and characterization of clinically important microorganisms [4], including bacteria [2,3–10], fungi [11–13], viruses [14] and spores [15–18] has been probed. MALDI-TOF MS profiles have also been used to analyze single cells and tissue samples from animals namely, mollusk, insect neurons [19,20] and mammalian cells [21].

Besides detecting various metabolites or characteristic components of the cell envelopes [2,22,23] in the low mass range, intact cell MALDI (ICM-MS1) also provides unique insights into cell biology and chemistry based on the detection of chemicals or in the measurement of chemical changes in response to environmental, temporal or other external influences [8,24,25]. MALDI-TOF MS fingerprint comparisons in the mass range 2–20 kDa are currently considered as a rapid approach for bacterial identification, an application termed phyloproteomics [8,17,26–28]. This strategy is based on the computer-assisted comparison of spectral fingerprints obtained directly from intact cells [29–32] with internet-accessible protein sequence databases [17,33–36], for identification of specific biomarkers based on the molecular masses of proteins.

In the intact cell MALDI-TOF MS (ICM-MS) approach, cells are mixed with an energy-absorbing matrix and irradiated with UV-laser. The matrix promotes laser energy transfer to the cells that results in the ionization of cell-surface macromolecules. The quality of the spectra produced is highly dependent on the choice of matrix, composition of the matrix solvent and sample preparation procedure [37]. Matrix solvent influences the release of macromolecules from the cell and co-crystallization with the matrix compound affects the mass range of the molecules being ionized, allowing enhancing the differential ionization of specific compounds, such as proteins, lipopeptides or nucleotides [8,38].

In this study, a novel application for the ICM-MS method is reported. This methodology was used to monitor the production of viral proteins (VP) and virus-like particles (VLPs) by the insect cells Spodoptera frugiperda SF-9/baculovirus expression system. This is a widely used system for recombinant protein and VLP production.
as it allows high productivities, it does not contain contaminants from mammalian sources and the baculovirus expression vectors are flexible and easy to handle [39]. In this work, cells involved in real time production of Rotavirus VLP's, were used as a model to follow insect cell fingerprint changes by ICM-MS. These triple layered particles, contain, in the inner shell, 60 dimers of VP2 (102.7 kDa); in the middle shell, 260 trimers of VP6 (44.9 kDa) and in the outer shell, 780 monomers of the glycosylated VP7 (37.2 kDa) [40].

The ICM-MS experimental approach has many advantages, namely: (i) it is compatible with crude samples and sample clean up is minimal; (ii) there is no need for tagging or pre-select proteins or peptides of interest; (iii) uses simple instrumentation; (iv) has high sensitivity; (v) provides complementary information to immunochemical methods; (vi) is compatible with post-translational modifications; (vii) requires low amounts of sample and (viii) is low time consuming due to minimal sample preparation [41]. ICM-MS is therefore, a very interesting alternative to monitor the production of recombinant proteins avoiding high analytical costs, usually associated to immunochemical reagents, large sample volumes and time consuming protocols.

2. Materials and methods

2.1. Cell line and baculovirus

Spodoptera frugiperda SF-9 and SF-21 cell lines were obtained from the American Type Culture Collection (ATCC, USA). SF-9 cells were used for production of baculovirus and VLPs, SF-21 cells were used for virus titration. Both cell types were cultured in serum free media SF900II (Gibco, Glasgow, UK) at 27 °C in 250 mL (working volume) Wheaton spinner flask stirred at 150 rpm or in 500 mL Erlenmeyers with 50 mL (working volume) at 90 rpm.

Autographa californica nucleopolyhedrovirus (AcNPV) recombinant baculovirus vectors, individually expressing bovine vp2 or vp6 or vp7 gene, under the control of polyhedron promoter, were constructed and kindly provided by Dr. Jean Cohen from Centre National de la Recherche Scientifique-Institut National Recherche Agronomique, Gif-sur-Yvette, France. The viruses were titrated using the assay described by Mena [42], based on cell viability. Infections were performed at a cell concentration at infection (CCI) of 1 × 10⁶ cell mL⁻¹. The recombinant baculovirus were amplified by infecting SF-9 cells at a multiplicity of infection (MOI) of 0.1 pfu cell⁻¹. Baculovirus were harvested with a cell viability of approximately 70%, corresponding to the 4–5 days post-infection [43], by centrifugation at 200 × g for 10 min. For proteins and VLP production a MOI of 5 pfu cell⁻¹ was used. The density of infected cells was determined by haemocytometer cell counts (Brandt, Werthein/Main, Germany) and the viability evaluated by trypan blue exclusion dye (Merck, Darmstadt, Germany) in 0.4% of phosphate-buffered saline (PBS).

2.2. Monitoring kinetics of VPs and VLP production

The following kinetic experiments were carried out in duplicate: (i) Control 1, non-infected insect cells; (ii) Control 2, insect cells infected with a wild type baculovirus; (iii) VP production, insect cells independently infected with monocistronic recombinant baculovirus for VP2, VP6 and VP7 production; and (iv) VLP production, insect cells co-infected simultaneously with VP2, VP6 and VP7 monocistronic recombinant baculovirus for VLP2/6/7 production.

Aiming at monitoring VPs production kinetics, samples were collected approximately every 12 h and centrifuged at 200 × g for 10 min. The cell pellet was washed three times with PBS and finally resuspended in 3 µL of 0.9% (w/v) NaCl for insect cell ICM-MS analysis. Total protein content was also assessed using the BCA Protein Assay Kit (Pierce, Rockford, USA).

2.3. VPs quantification

Intracellular viral protein quantification was done by Western blot, according to the protocols previously described [44]. The amount of each protein was normalized using a well characterized VLP standard, produced and purified using a method described elsewhere [45]. Immunochemical staining for recombinant protein detection was carried out with a polyclonal anti-Rotavirus serum for VP2 and VP7 detection and a monoclonal antibody produced against VP2, both kindly provided by Dr. Jean Cohen, Gif-sur-Yvette, France. Blots were developed after incubation with an alkaline phosphatase conjugated anti-rabbit IgG antibody (Sigma, St. Louis, USA) and an alkaline phosphatase conjugated anti-mouse IgG antibody (Sigma, St. Louis, USA) using 1-stepTM NBT/BCIP blotting detection reagent (Pierce, Rockford, USA). The product yield was estimated by densitometry analysis of the scanned images (ImageQuant® for Microsoft® WindowsNT®, Molecular Dynamics, Inc., USA, 1998).

2.4. Sample preparation for ICM-MS

The quality of Spodoptera frugiperda SF-9 cell suspension spectra was evaluated using three MALDI matrices. All matrix solutions, 2,5-dihydroxybenzoate (DHB), sinapinic acid (SA) and 2,6-dihydroxyacetophenone (DHAP), were prepared daily in 70% (v/v) acetonitrile 0.1% (v/v) trifluoroacetic acid with the concentration of 20 mg mL⁻¹.

Two sample MALDI target application procedures were tested: direct spotting of the insect cells on the MALDI target plate followed by the matrix solution which were air-dried prior to analysis; or by spotting the previously mixed cell suspension with the matrix solution. Different matrix:cell suspension volume ratios were tested for the direct spotting method 1:1 and 1:0.6. For the mixture spotting a 1:5 volume ratio has been used.

In order to try improving MALDI-TOF spectra intensity [7,46], mechanical disruption of cells was done using glass beads under vigorous agitation for 1 min after the addition of matrix to the cell suspension (1:1). Obtained whole cell lysates were directly spotted on the MALDI plate for further analysis.

2.5. Data acquisition and processing

All mass spectra were obtained using a PerSeptive Biosystems MALDI-time-of-flight (TOF) MS Voyager-DE STR (Framingham, USA) equipped with delayed extraction, and a 337-nm N₂ laser. Mass spectra were acquired in linear delayed extraction mode using an acceleration of 20 kV and a low mass gate of 5000 Da. The laser power was set to just above the threshold of ionization. Spectra for individual samples were accumulated, averaging 150 shots taken across the width of the sample spot for m/z values between 5000 and 25 000.

The individual spectra were initially processed by Data Explorer 4.2 software (Applied Biosystems, Foster City, USA) used for baseline correction, noise removal and peak detection. For each spectra, m/z peaks relative intensities were computed with the most intense peak set to 100%. Peaks with 5% maximum peak area, relative intensity below 5%, and a mass resolution below 500 were ignored. From the peak lists obtained as previously described, m/z peaks were deleted if their relative intensity was below 20% for the mass range of 5000–7500 Da; below 10% for the mass range of 7500–10 000 Da; and below 5% for the mass range of 10 000–25 000 Da.

The web interface SPECLUST created by The Computational Biology & Biological Physics Group at Lund University
3. Results

3.1. Sample preparation and ICM-MS method optimization

Apart from the classical application of MALDI MS in proteomics (e.g., identification of isolated and purified proteins and peptides), it has also been used more recently with intact cells for identification of microbes and monitoring of recombinant protein productions in *E. coli* [25]. It is known that intact cell MALDI spectra profiles can be highly variable [46,47]. Therefore, it is important to investigate the effect of sample preparation procedure on spectra quality and data acquisition reproducibility. Cell treatment (cell suspension or whole cell lysate), appropriate MALDI matrices and several matrix:cell suspension ratios were evaluated. Two MALDI target plate sample application methods were also assessed. In each case, mass spectra were examined for number and relative abundance of *m/z* peaks detected. The best condition was direct spotting on the MALDI plate, using 2,6-dihydroxyacetophenone matrix in a matrix:cell suspension volume ratio of 1:1. The number of detectable peaks and their relative intensities were considerably higher when comparing with those detected using sinapinic acid matrix. No peaks were detected when using DHB matrix (Fig. 1).

Different methods for applying samples onto the MALDI target plate and insect cell treatment conditions were also evaluated. The *m/z* peaks that passed the acceptance criteria (described under Materials and methods) were listed for each spectrum and both common and new peaks were identified. The number of occurrences was determined for each *m/z* peak using Peaks In Common consensus analysis, a functionality of SPECLUST. Peaks were considered as common when appearing in more than 70% of the spectra and as new if fewer occurrences took place. Then, the percentage of common and new peaks was determined by dividing the number of each occurrence by the total number of peaks per spectra. The mean number of the overall detectable peaks was 23 for the direct application of insect cells onto the MALDI plate, 12 when previously mixing the cell suspension with matrix (1:1 ratio) and 14 for the cell lysate treatment. Fewer peaks were detected when diluting cell suspension in matrix. The higher number of detectable *m/z* peaks was obtained by direct spotting the cell suspension onto the MALDI plate (Fig. 2). In this condition, lower inter-spectra variability was also achieved, corresponding to an average of 6 new *m/z* peaks per spectra (26% of total detectable *m/z* peaks). To estimate the reproducibility associated with the selected method, the total number of detectable peaks and the number of peaks assigned as common was determined in 6 replicates. The outcome of this analysis revealed an average of 23 detectable peaks in which 15 and 17 of them are common to at least 90% and 70% of the analyzed spectra, respectively.

3.2. *S. frugiperda* cells fingerprint evaluation and the Dissimilarity Score

ICM-MS approach was already been used to study regulation of recombinant protein production in vivo by monitoring the *m/z* peak that corresponds to the molecular mass of the over expressed protein [25]. Similarly, we examined the presence of *m/z* peaks corresponding to the molecular masses of the expressed viral proteins in the MALDI-MS fingerprint of the intact insect cells, but no matching peaks were observed. This could be explained by the fact that the viral proteins VP6 and VP7 are secreted from the insect cells into the culture media, being the VP2 protein the only one that accumulates in the cellular cytoplasm. However, VP2 protein has a molecular mass of 102 kDa, making it more difficult to detect since MALDI ionization efficiency is reduced with protein size. Our strategy was then to evaluate if protein production could be indirectly monitored in vivo by studying the real-time changes in the MALDI-MS fingerprints of the intact insect cells during the bioreaction events.

For this purpose, some factors such as cell growth phase on bioreactor or cell stress caused by baculovirus infection had to be evaluated, since they can imply changes on the insect cell’s fingerprint. To estimate the reproducibility of the acquired data pattern and reproducibility, we have undertaken assays with *S. frugiperda* Sf-9 cells collected from two different experiments. In Control 1, insect cells were simply left under optimal growing conditions in the bioreactor for 5 days. In Control 2, insect cells were infected with “empty” wild type baculovirus allowing evaluation of the infection impact on intact cell MALDI spectra fingerprint. For both experiments, ICM-MS spectra were acquired using the optimized conditions previously described for the various insect cell pellets, corresponding in total to 11 time checkpoints (approximately 12 h cycles) of the real bioreactor production.

After MS data acquisition and processing, 11 *m/z* peak lists were generated for each control group assay and submitted for consensus analysis. In SPECLUST, a new list is generated per control group containing the *m/z* values for the detectable peaks, where the mean *m/z* value is included in the common peaks. A simple mathematical expression has been developed to estimate the insect cell fingerprint differences between groups of submitted spectra. The Dissimilarity Score, *dS* (Eq. (1)), is the ratio between the total number of *m/z* peaks in the list generated by the consensus analysis, *Px*, and the mean number of *m/z* peaks per spectrum list in the analyzed group, which represents the mean value of the number of detectable peaks per spectra, *Px*; where *n* represents the number of spectra to be compared.

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dS = \frac{|Px|}{|P|} \quad 1 < dS < n
\]

Equal spectra have the same number of *m/z* peaks as the number of peaks in the list generated by the consensus analysis *P(x)*, and it will be equal to the mean number of detectable *m/z* peaks per spectra *Px*. So when comparing equal spectra, the Dissimilarity Score value is 1 (*dS* = 1).

Fig. 1. Effect of matrix on insect cell ICM-MS spectra: Insect cell suspension MALDI-TOF MS spectra using different matrices (DHB, SA and DHAP) and pictures of their co-crystallization are presented. All spectra were collected after direct spotting. A ratio of 1:1 of matrix:insect cell suspension was used. DHAP (20 mg mL\(^{-1}\)) in 70% acetonitrile 0.1% trifluoroacetic acid seems to be the optimal matrix since it originates spectra with more detectable peaks.
Fig. 2. Inter-spectra variability inherent to sample preparation method. Six MS spectra (R1–R6) were acquired after direct spotting of cell suspension: DHAP matrix solution (1:1) onto the MALDI plate. An average of 23 m/z peaks was detected per spectrum, 15 and 17 of them are common to at least 90% and 70% of the analyzed spectra, respectively. Vertical lines match common m/z peaks.

By opposition, if the compared spectra are completely different with no common peaks in the submitted spectra, the Dissimilarity Score will have the maximum value, equivalent to the number of peak lists that were submitted.

The Dissimilarity Score values are only comparable when submitting the same number of MS spectra per group. In the present work, we have analyzed 11 time checkpoints on cell samples collected from each bioreaction run, which allowed comparing dS between different kinetic experiments.

Higher dS values correspond to stronger differences among m/z peak lists of the analyzed time checkpoints. This indicates the occurrence of more extensive modifications on the insect cell fingerprint during the kinetic experiment. This becomes obvious when comparing the insect cells fingerprint variation along time in both experiments (Fig. 3). In Control 1 group, there are about 20 m/z peaks common to 70% of the spectra and 10 common to 90% of the spectra. This is within the estimated spectra variability (15 m/z peaks common to 90% of the spectra in a total of 23 detectable peaks), meaning there are no apparent changes in the fingerprint along the time. In contrast, in Control 2, the number of common m/z peaks decreases to 13 when considering 70% of the spectra and 5 when considering 90% (in a total of 34 detectable peaks). These values are considerably lower, and show that after infection, the insect cells fingerprint changes throughout time. This is also

Fig. 3. SF-9 intact cell fingerprints. Intact cell fingerprints along time for non-infected (Control 1) and infected (Control 2) are presented in the left and right side, respectively. A strong variation of the fingerprint along the kinetic experiment was observed for the insect cells infected with wild type baculovirus, in accordance with the calculated dS values. Vertical lines match common m/z peaks.
confirmed by the values of the Dissimilarity Score. For both groups the score limits are 1 for ideally equal spectra and 11 for spectra without common peaks. For Control 1 $dS = 3.75$ is lower than Control 2 $dS = 4.5$, which confirms that insect cells fingerprint in Control 2 group suffers more changes along time than in Control 1. Apparently, optimal bioreactor working conditions without other external stimuli do not cause changes in cells fingerprint. However, significant changes could be detected when infection is induced.

3.3. Fingerprinting the bioproduction of VP2, VP6, VP7 and VLP 267 in insect cells by ICM-MS

So far, it has been proved that the infection process itself induces fingerprint changes on insect cells, but there is still a question left to answer: if fingerprint evolution is affected by the infection mode and assembly of VLP's. Two possibilities were evaluated: infection with baculovirus expressing one recombinant protein and co-infection with three baculovirus for co-expression of three recombinant proteins. To address this question, the groups of fingerprints for each viral protein production were analyzed using two different approaches: (i) the Dissimilarity Scores were calculated for each group and (ii) a cluster analysis was performed for each kinetic experiment using SPECLUST.

By comparison of the Dissimilarity Score values for the kinetic experiments, it is possible to detect changes in the MS profiles of the insect cells along the production time when the viral proteins are produced separately (VP2 or VP6 or VP7) or when VLP's are produced by co-expression of the three genes (VP2, VP6 and VP7). All the determined values are above the $dS$ for Control 1 (3.75) where no relevant fingerprint changes occurred. For insect cells producing VP2, VP6, VP7 and VLP 2/6/7, the $dS$ are 4.7, 5.5, 5.6 and 4.4, respectively. These values are in accordance with a visual comparison of the spectra, showing fingerprint profile changes for each bioproduction opposed to more stable fingerprints in Control 1 (Fig. 4).

In order to analyze these changes in further detail, we performed a hierarchical clustering of the mass spectra for each group. The main goal was to compare and correlate results from a Western blot traditional assay with the obtained ICM-MS spectra from insect cells in the various stages of the protein production.

In the SPECLUST cluster analysis, each $m/z$ peak list is initially assigned to its own cluster. Distances are calculated between each pair of $m/z$ peak lists. The closest pair is found and merged to a new cluster. Distances between the new cluster and each of the old
clusters are calculated. This procedure is repeated until there is only one single cluster. Then, a score for matching m/z peaks is created, where the similarity between two m/z peak lists is calculated. This is then translated into a distance measure, which varies between 1 for completely different set of m/z peaks, and zero for a perfect match. This is the starting point in clustering the m/z peak lists and building a dendogram.

To interpret the cluster analysis of the insect cells fingerprints, it was verified that, for distances smaller than 0.6 in the dendograms scale, the grouped spectra were similar [50]. Considering this scale limit, several interesting spectra grouping occurred in each protein/particle production. In detail, for insect cells producing VP2 there are two main spectra groups formed in the dendogram (Fig. 4), one representing times 0–46.5 h and another spectra group that includes the three last time points, collected from 96.5 to 119.0 h.

When comparing with methods routinely used to monitor protein production during bioreaction (e.g. Western blot), in the first group of similar spectra, the turning point in fingerprint change is at 46.5 h, which is consistent with an increase of intracellular protein concentration value related to the initiation of VP2 production (Fig. 5A). The second group of similar spectra (96.5 to 119.0 h) is distinct from the first one, corresponding to the terminus of the bioproduction process. It has been previously reported that VP2 accumulates intracellularly when VP6 is not co-expressed [48]. There are still two time points remaining, 56.0 and 71.2 h, where the spectra are distinct from one and other and from the two formed groups. This is possibly related to a temporary decrease in cell viability (data not shown).

In cells producing VP6, there is also fingerprint grouping which can be related to biological events. Two main groups of similar spectra (Fig. 4) were obtained: the first group includes the time points 0.0 and 14.0 h, which is, in fact, the starting of the protein production (Fig. 5B). In the second group, spectra are similar from the 38.0 to 72.5 h time points, which is consistent with a stable intracellular concentration of VP6 (Fig. 5B). In the last phase of protein production the data do not correlate with each other, which is in accordance with random variation in the intracellular protein concentration, possibly associated with protein excretion events.

VP7 is a glycosylated secreted protein, an interesting correlation with the results obtained in the Western blot analysis is observed. There seems to be two groups of similar spectra: time points 0.0 and 10.3 h, and the rest of the time points. For the first group intracellular VP7 concentration is almost zero, however, after this time period its concentration increases and is maintained constant till the end of the bioreaction (Fig. 5C). This behavior is eventually related to the fact that VP7 is secreted to the extracellular medium early in the bioreaction process, thus, it does not accumulate intracellularly, and it does not trigger major fingerprint changes.

For the VLP 2/6/7 particle production, insect cells undergo even more complex biological processes: infection and replication of three monocistronic baculovirus, the co-expression of three recombinant proteins, and their assembly into VLPs, among other important events. There is a curious phenomenon of spectra grouping, due to similarity between distant time point’s fingerprints (Fig. 4). This is observed up to 47.8 h post-infection, where the points 0.0 and 33.0 h group together as so do points 9.0–24.0 h and 47.8 h. These variations in intracellular total protein concentration are eventually related to the several steps involved in the baculovirus replication process, in which baculovirus proteins are expressed (Fig. 5D).

3.4. Infection stress versus viral protein production: distinct specific fingerprints?

Firstly, since it was determined that the infection process itself induces fingerprint changes on the insect cells, it was decided to evaluate if the fingerprint changes of the insect cells producing viral particles and proteins were different and could be correlated with a particular kinetics of viral protein production. Secondly, we tried to understand if the fingerprint changes were specific of each particular recombinant protein being produced with this expression system. To address these questions dendograms were constructed using both the fingerprints of the different protein kinetics and the fingerprints of Control 2 (Fig. 6). Taking into account the cluster analysis results, the overall conclusion is that the fingerprint of the first time points of the infection are similar either for infection with wild type baculovirus and for cells producing the different viral proteins. This reveals that in these first time points, fingerprints show only the contribution of the infection processes and infection stress related events. As infection proceeds in time, fingerprints start to differentiate from the initial time point pattern, and no longer are similar with the fingerprint of cells infected with wild type
baculovirus. This indicates that the production of each viral protein or VLP has an impact on insect cell fingerprint. To determine if each fingerprint change, for the production of a particular viral protein or VLP is specific and unique, cluster analysis was also performed comparing pairs of two of all protein production (data not shown) (VP2 vs. VP6; VP2 vs. VP7; VP2 vs. VLP 2/6/7; VP6 vs. VP7; VP6 vs. VLP 2/6/7; VP7 vs. VLP 2/6/7). This cluster analysis shows that each fingerprint profile depends on the specific protein(s) that is (are) being produced, showing the same cluster behavior as described for the comparison of each protein(s) production(s) with Control 2, where only the fingerprints of the first time points are similar.

4. Discussion

The usefulness of mass spectrometry approaches in bioanalysis of recombinant protein from biotechnological production has already been described when using liquid chromatography coupled with a mass spectrometer [49] as being potentially useful as it may offer rapid development and improved assay specificity. In this paper we present a new application for the described mass spectrometry method intact cell MALDI-TOF MS (ICM-MS). This MALDI-MS approach has application in the biotechnological industry allowing monitoring the production of viral proteins and virus-like particles. This technique is presented here as a complementary analytical method to monitor recombinant protein production during bioreaction and using the established pattern for the expression system/recombinant protein produced. Herein this method has been optimized for insect cells/baculovirus expression system and the production of Rotavirus proteins and virus-like particles. The experimental results obtained show that this is a highly reproducible method for each recombinant protein that allows checking if production is in fact occurring, by monitoring fingerprint changes observed when insect cells are producing the viral proteins. Moreover, the proposed method indicates the bioproduction phase allowing monitoring the lag periods. These fingerprint changes are independent of cell stress due to infection and are characteristic of each protein or VLP production.

This very simple, fast (10 min sample preparation) and low cost assay requires low sample volumes and can be used as a complement or even as a substitute of common analytical methods for bioproduction monitoring. However, careful method optimization is still required when applied to other biological samples, since sample preparation procedure can be substantially different.

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References


