Initial intracellular proteome profile of Aspergillus niger biofilms

Perfil inicial del proteoma intracelular de biopelículas de Aspergillus niger

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Abstract
An initial profiling of the intracellular proteome of Aspergillus niger ATCC 10864 biofilm cultures developed on polyester cloth was carried out by using 2D-PAGE and MS-TOF analysis and it was compared to the proteome of conventionally grown free-living submerged cultures. A number of 2D-PAGE protein spots from both types of cultures were subjected to MS-TOF analysis and data interrogation of the NCBI nr database available for this species. Proteomic maps showed different expression patterns in both culture systems with differentially expressed proteins in each case. In biofilm cultures, 19% and 32% of the selected protein spots were over-expressed and differentially expressed, respectively. On the contrary, in free-living cultures, 44% and 7% of the selected protein spots were over-expressed and differentially expressed, respectively. Although preliminary, results presented in this paper show that there are significant differences between the proteomes of A. niger biofilm and free-living mycelia. It seems that cell adhesion is the most important stimulus responsible for biofilm development which is the basis of Surface Adhesion Fermentation.

Keywords: Aspergillus niger, biofilms, proteome, MS-TOF, 2D-PAGE.

Resumen
Se realizó un perfil inicial del proteoma de biopelículas de Aspergillus niger ATCC 10864 desarrolladas sobre tela de poliéster mediante 2D-PAGE y análisis MS-TOF y comparado con el proteoma de cultivos sumergidos convencionales de micelio libre. De ambos tipos de cultivo se analizó un número de muestras proteicas de geles 2D-PAGE mediante MS-TOF y los resultados se compararon con la base de datos NCBI nr disponible para esta especie. Los mapas proteómicos mostraron patrones diferentes de expresión en cada caso. En cultivo de biopelículas, el 19% y el 32% de las muestras seleccionadas fueron sobre-expresadas y diferencialmente expresadas, respectivamente. Por el contrario, en cultivos sumergidos en micelio libre el 44% y el 7% de las muestras seleccionadas fueron sobre-expresadas y diferencialmente expresadas, respectivamente. Aunque preliminares, los resultados presentados en este trabajo muestran que existen diferencias significativas entre los proteomas de biopelículas y micelio libre de A. niger. Parece ser que la adhesión celular es el estímulo más importante para el desarrollo de biopelículas, las cuales son la base de la Fermentación por Adhesión a Superficies.

Palabras clave: Aspergillus niger, biopelículas, proteoma, MS-TOF, 2D-PAGE.

Introduction

The genus Aspergillus includes several species of outstanding biotechnological importance (Meyer 2008, Ward et al. 2006). Several Aspergillus species are used for the commercial production of enzymes and other biochemical products and their genes are being gradually investigated as an effort to understand their molecular activity in the fungal cell and to expand current industrial applications (Abe et al. 2006, Gouka et al. 1997, Gilseman et al. 2004, van den Hombergh et al. 1997, Kim et al. 2008). The genomes of A. nidulans, A. fumigatus, and A. oryzae were the first to be available (Galagan et al. 2005a, Galagan et al. 2005b, Machida et al. 2005, Gilseman et al. 2004, Nierman et al. 2005). Aspergillus niger genome has been recently sequenced (Pel et al. 2007, Semova et al. 2006).

The genome of Aspergillus comprises 8 chromosomes being those of A. niger and A. oryzae of the highest size with 33.9 and 37.0 Mb, respectively. A. niger genome contains ca. 14165 genes, with 1572 bp average length per gen and 0.42 genes density (genes/Kb) (Pel et al. 2007), and about 87% of its genes containing introns (Archer & Dyer 2004).

Contrary to the fast genome sequencing, filamentous fungal proteomic studies are moving slowly specially referred to secreted proteins (Medina et al. 2005). Although protein analysis is improved by advances in mass spectrometry (MS) and the continuous update of genomic data bases, sequences are not completely available. Generally, protein identification implies obtaining of MS patterns to be compared with all possible proteins coded by a genome available in a data bank (Reinders et al. 2004). When sequences are available, data obtained by MALDI-TOF MS (matrix assisted laser desorption/ionization - time of flight mass spectrometry) or MS-TOF (mass spectrometry - time of flight) peptide fingerprint may facilitate protein identification (Carberry & Doyle 2007, Eisenberg et al. 2000, Fenýo 2000).

Proteomes of some Aspergillus species have been studied only in the last five years (Carberry & Doyle 2007, Kim et al. 2008). Thus, very few insights have been displayed on the proteomes of A. nidulans (Kim et al. 2008, Ström et al. 2005), A. oryzae (Oda et al. 2006, Zhu et al. 2004), A. flavus (Medina et al. 2004, Medina et al. 2005), A. fumigatus (Asif et al. 2006, Carberry et al. 2006), and A. niger (Wright et al. 2009).
Recently, a great deal of attention has been focused on the use of lignocellulosic biomass to produce bioethanol and other useful metabolites by means of its hydrolysis with lignocellulosic enzymes produced by various microorganisms (Bhat 2000). However, the cost of obtaining sugars from lignocellulosic biomass for fermentation is still high, mostly due to low enzyme yields of producing microorganisms (Gabel and Zacchi 2002). Submerged fermentation (SF) is the main process used for cellulase production but other fermentation techniques are being tested. Filamentous fungi are naturally adapted to growth on surfaces and in these conditions they show a particular physiological behavior due to differential gene expression which is different from that in SF; thus, they can be considered as biofilm forming organisms. Fungal biofilm fermentation (BF) depends on surface adhesion and a new fermentation category named surface adhesion fermentation (SAF) was proposed by Gutiérrez-Correa and Villena (2003).

We have recently showed that there is a differential gene expression in Aspergillus niger biofilms (Villena et al. 2009). From this point of view, the study of differential proteome expression of biofilms developed by filamentous fungi may be the starting line of an analysis of differential physiological behavior as compared to submerged cultures, which is needed to establish the role of cell adhesion and the growth on surfaces on the productivity of submerged industrial processes. The aim of the present work was to initiate the study of the intracellular proteome of Aspergillus niger during biofilm formation on polyester.

Material and methods

Microorganism

Aspergillus niger ATCC 10864 maintained on potato dextrose agar slants was used throughout the study. Spores were washed from 5-day agar-slant cultures with 10 mL of 0.1% Tween 80 solution, counted in a Neubauer chamber and diluted to give 1 x 10⁶ spores/mL. This suspension was used as inoculum at a proportion of 3% (v/v). Culture medium for both SF and BF was described elsewhere (Villena & Gutiérrez-Correa 2006).

Submerged and Biofilm Fermentation

For both types of fermentation systems 250 mL flasks containing 70 mL culture medium were used. For SF each flask was inoculated with 2.1 mL of the above spore suspension. For BF each flask containing a polyester 100/1 cloth square in 70 mL distilled water was also inoculated with 2.1 mL spore suspension, incubated for 15 min at 28 ºC in a shaker bath. After this contact period, the squares were washed twice with distilled water under agitation at 175 rpm for 15 min; then they were transferred to flasks containing 70 mL of the culture medium. All flasks were incubated at 28 ºC in a shaker bath at 175 rpm for 72 h.

Mycelial preparation

Fungal biomass was determined by measuring its dry cell weight. For SF samples, the entire content of a flask was filtered through pre-weighed filter paper (Whatman N.º 1) under suction; the filter paper was dried at 80 ºC for a constant weight. For BF samples, the liquid part was removed by decanting and then the same steps used for free suspension were followed. The biofilm was washed three times with distilled water and then dried as above. Samples were conserved at -70 ºC.

Protein preparation

For intracellular protein extraction 2 g 72 h-old mycelial samples were ground in a mortar with liquid nitrogen. Powdered biomass was gently suspended in 5 mL of extraction buffer (10mM Tris HCL pH8; 1mM EDTA, 2% (w/v) polivinilploipoíridolona PVPP) containing protease inhibitors (1μg/mL chymostatin, 1μg/mL aprotinin, 1μg/mL leupeptin and 2mM PMSF), and centrifuged at 8000 rpm for 30 min at 4 ºC; then, supernatant was collected.

Protein samples were precipitated with methanol-chloroform following the procedure of Wessel & Fluegge (1984). Protein samples were successively mixed with methanol (4 volumes), chloroform (1 volume), distilled water (3 volumes), and centrifuged at 12000 g for 1 min. The upper phase was carefully removed and 3 volumes of methanol were added followed by centrifugation at 9000 g for 2 min. The supernatant was discarded and the pellet was air-dried at room temperature. Protein concentration was estimated using either Bradford (1976) or Lowry et al. (1951) procedures.

Two-dimensional electrophoresis.

Protein separation by 2D-PAGE was as follows: Precipitated proteins (600—1000 μg) were resuspended in 7 M Urea, 2 M thiourea, 4% (w/v) CHAPS, IPG buffer 0.5%, (v/v), 3 mg/mL DTT and loaded onto Immobiline Dry strips (IPG strip; Amersham) in the non linear pH range 3—10. Gels underwent active rehydration at 30 V for 10 h, followed by a further 9 h focusing with a total of 19200 V applied. Following IEF, gels were equilibrated with 2.5 mL of reducing buffer (50 mM Tris–HCl, 6M urea, 2% (w/v) SDS, 30% (v/v) glycerol, 0.002% (w/v) bromophenol blue) and 3 mg/mL DTT for 12 min followed by equilibration in alkylation buffer (50 mM Tris–HCl, 6M urea, 2% (w/v) SDS, 30% (v/v) glycerol, and 25 mg/mL iodoacetamide) for a further 5 min. The IPG strips (18 cm) were placed on homogenous 12.5% SDS–PAGE gels. Electrophoresis was performed at constant current of 152 mA and 5V/gel for 16 hours until bromophenol blue dye migrated to the end of the gel using Entan Daltsix Electrophoresis System (Amersham) with temperature maintained at 4 ºC using a recirculating water bath. Mass spectrometry compatible silver staining was performed according with Blum et al. (1987).

Mass spectrometry

Mass spectrometry was carried out using an MS-TOF Autoflex mass spectrometer (Brukers Daltonics, Yokohama, Japan). Briefly, protein spots were manually excised, destained with equal volume mixture of 30 mM K₃Fe(CN)₆ and 100 mM Na₂S₂O₃, and were in-gel digested with 25 mM de NH₄HCO₃ and 5 ng/μL trypsin at 37 ºC overnight. Digested protein peptides were extracted multiple times by sonication with 50% acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA), concentrated and desalted using Zip-Tip C18 reverse phase peptide separation matrix (Zip Tip® Millipore Corporation), and deposited (1 μL) with 1 μL α-cyano-4-hydroxycinnaminic acid (4-HCCA; 5 mg/200 μL of 50% (v/v) acetonitrile in 0.1% (v/v) aqueous trifluoroacetic acid) onto mass spectrometry slides, and allowed to dry prior to delayed extraction, reflecton ToF analysis at 20 kV. Protein identification was carried out by m/z data interrogation of the NCBI nr database available for Aspergillus using MATRIXSCIENCE Mascot Search (http://www.matrixscience.com/search_forms_elect.htm).


**Results and discussions**

Intracellular proteomes of *Aspergillus niger* biofilm and submerged cultures were compared by using 2D-PAGE and MS-TOF. Proteomic maps showed different expression patterns in both culture systems with differentially expressed proteins in each case.

Figure 1 shows the intracellular proteome map of *A. niger* biofilms. Although protein concentration was somewhat low probably due to the low protein solubility in the IEF solubilization buffer, we found an adequate protein resolution. Forty eight protein spots were chosen among those showing either differential or higher expression levels. From the spots marked in the map 19% and 32% were over-expressed and differentially expressed proteins in biofilm cultures, respectively (Fig. 1). Highly stained spots were chosen for further MS-TOF analysis (56% of the 48 initial proteins), identifying 55% and 56% over-expressed proteins in biofilm cultures, respectively (E= 0 in both cases).

All selected over-expressed proteins (spots 3B, 4B, 9B, 10B, 25B, and 26B) were hypothetic, i.e., proteins that their sequences do not match those of known sequence and function, yet they show shared domains with known proteins (Table 1). Thus, proteins 3B and 9B match an *A. nidulans* hypothetic protein that shares domains with an outer mitocondrial membrane protein porin of *N. fischeri* (E value= 5e-155). Protein 4B is strongly related to ubiquinol-cytochrome C reductase of *A. fumigatus* (E= 0) and with hypothetical protein of *A. niger* (E value= 0) and *A. oryzae* (E value= 0). Protein 10B is related with both an *A. flavus* and *A. clavatus* cytrochorme b5 reductase (E= 6e-174 and 4e-171, respectively). Protein 25B shares domains with a hypothetical protein of *A. nidulans* (E= 3e-26) and with a putative β-xylosidase of *P. marneffei* (E= 1e-121). Finally, protein 26B is nearly related to a hypothetical protein of *A. niger* (E value= 0) and *A. oryzae* (E value= 0) and with AMP deaminases of both *A. fumigatus* and *A. terreus* (E= 0 in both cases).

Table 1. Over-expressed intracellular proteins in *Aspergillus niger* biofilms identified by MS-TOF analysis.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Mr (Da)</th>
<th>pI</th>
<th>accession</th>
<th>NCBI Protein description</th>
<th>E value</th>
<th>Related proteins (E value)</th>
<th>Score</th>
<th>Searched/ matched peptides</th>
<th>Sequence coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3B</td>
<td>28891</td>
<td>9</td>
<td>gi</td>
<td>40741129</td>
<td>Hypothetical protein AN4402.2 [Aspergillus nidulans FGSC A4], 284 aa</td>
<td>92</td>
<td>Outer mitochondrial membrane protein porin [Neosartorya fischeri NRRL 181] (5e-155)</td>
<td>19/8</td>
<td>20%</td>
</tr>
<tr>
<td>4B</td>
<td>47605</td>
<td>9</td>
<td>gi</td>
<td>40739821</td>
<td>Hypothetical protein AN8273.2 [Aspergillus nidulans FGSC A4], 458 aa</td>
<td>38</td>
<td>Hypothetical protein An09g06650 [Aspergillus niger] (0), Hypothetical protein [Aspergillus oryzae RIB40] (0)</td>
<td>18/5</td>
<td>11%</td>
</tr>
<tr>
<td>9B</td>
<td>28891</td>
<td>9</td>
<td>gi</td>
<td>40741129</td>
<td>Hypothetical protein AN4402.2 [Aspergillus nidulans FGSC A4], 284 aa</td>
<td>57</td>
<td>Outer mitochondrial membrane protein porin [Neosartorya Fischer NRRL 181] (5e-155)</td>
<td>12/5</td>
<td>17%</td>
</tr>
<tr>
<td>10B</td>
<td>51220</td>
<td>8.79</td>
<td>gi</td>
<td>40739927</td>
<td>Hypothetical protein AN3862.2 [Aspergillus nidulans FGSC A4], 468 aa</td>
<td>36</td>
<td>Cytochrome b5 reductase, putative [Aspergillus flavus NRRL3357] (6e-174)</td>
<td>9/4</td>
<td>11%</td>
</tr>
<tr>
<td>25B</td>
<td>12378</td>
<td>7.85</td>
<td>gi</td>
<td>40743794</td>
<td>Hypothetical protein AN2633.2 [Aspergillus nidulans FGSC A4], 110 aa</td>
<td>44</td>
<td>Beta-xylosidase, putative [Penicillium marneffei ATCC 18224] (1e-21)</td>
<td>8/3</td>
<td>26%</td>
</tr>
<tr>
<td>26B</td>
<td>99961</td>
<td>5.95</td>
<td>gi</td>
<td>40744930</td>
<td>Hypothetical protein AN8872.2 [Aspergillus nidulans FGSC A4], 878 aa</td>
<td>52</td>
<td>Hypothetical protein An03g06970 [Aspergillus niger] (0), AMP deaminase Amd1 [Aspergillus fumigatus A293] (0)</td>
<td>5/5</td>
<td>6%</td>
</tr>
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</table>
Differentially expressed proteins in biofilm cultures are presented in Table 2. As above, most of the spots were hypothetic proteins. Protein 16B matches putative calcium P-type ATPase similar to that of *A. fumigatus* and *A. flavus* (E= 0 in both cases), while protein 21B matches α-sarcin – a type of fungal RNase (E= 9e-101). Other spots were of hypothetic proteins (see Table 2 for details).

Intracellular proteomic map of *A. niger* conventionally grown as free-living mycelium in SF is presented in Fig. 2. From the spots marked in the map, 44% were over-expressed and only 7% were differentially expressed proteins. Unfortunately, none of the differentially expressed protein spots could be analyzed due to the low concentration of them. However, 42% of the over-expressed proteins were MS-TOF analyzed. Most of the over-expressed proteins were identified and only 3 of them were hypothetic (see Table 3 for details). Spot 2FM is a hypothetical protein sharing domains with ribosomal proteins and it is closely related to both *A. fumigatus* L19e 60S ribosomal protein (E= 0) and an unnamed protein product of *A. niger* (E= 0). Spot 11FM did not share domains with other proteins and it showed similarity to a conserved hypothetical protein of *A. fumigatus* (E= 2e-48).

Since protein expression levels depend on regulatory systems, proteomes are highly dynamic but this allows comparative studies under different conditions (Carberry & Doyle 2007). As it has been described above, intracellular proteome analysis of *A. niger* grown under biofilm and submerged fermentation conditions demonstrated that there are different protein expression patterns in both fermentation systems. Although most of the proteins found in BF are hypothetic, they showed shared domains with known proteins which, in turn, may help to assign their functions. Protein identification through MS patterns mainly depends on the quality of the annotated gene sequences available in the database banks. In this sense, as significant fungal expressed sequence tags (EST) data is lacking, particularly for *A. niger* and other *Aspergillus* species, gene prediction strongly depends on de novo prediction (Galagan et al. 2005a). Semova et al. (2006) could sequence and identify only 650 out of 4856 new genes from 12820 ESTs obtained from 15052 transcripts of *Aspergillus niger* N402, FGSC#4732. In this work, we have used the NCBI database with *A. niger* sequences released by April 2005 and *A. nidulans* orthologues, since this species has 64% protein homology with it whereas 20% of the genes do not have homology (Semova et al. 2006). However, only 7.5% ORFs of *A. nidulans* have an assigned function (Mogensen et al. 2006) from which a complete identification is less probable. This also explains some low aligning scores obtained in our MS TOF analysis. Although all over- or differentially expressed proteins could not be completely identified there are interesting differences between both fermentation systems. Thus, *A. niger* biofilms differentially expressed a putative calcium P-type ATPase which is important both in the homeostatic maintenance of calcium concentration in the endoplasmic reticulum and in cation-dependant functions of Golgi apparatus (Vashit et al. 2002); this protein is probably involved in cAMP-mediated signaling (Bencina et al. 2005). Also, another differentially expressed protein found in biofilms is an *A. giganteus* α-sarcin – a cytosolic basic protein with ribonucleolytic activity – with biotechnological potential as anti-tumor agent (Moreno et al. 2006, Olmo et al. 2001).

Although we could not identify any of the differentially expressed proteins in *A. niger* SF due to their very low concentrations, some of the over-expressed proteins were related to stress conditions. Thus, cyclophilin-like peptidyl prolly cis transisomerase is related to endoplasmic reticulum stress (Damveld et al. 2005) and 6 beta-hydroxyhyoscyamine epoxidase is related to secondary metabolism. A peroxosomal like protein of unknown function was also over-expressed (Aign & Hoheisel 2003). Finally, a subunit of pyruvate dehydrogenase E1 (Table 3, spot 14FM) was over-expressed as it has been recently found in *A. nidulans* under hypoxic conditions (Shimizu et al. 2009).
<table>
<thead>
<tr>
<th>Spot</th>
<th>Mr (Da)</th>
<th>pI</th>
<th>Accession</th>
<th>NCBI Protein description</th>
<th>Score</th>
<th>Related proteins</th>
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<tr>
<td>11B</td>
<td>24160</td>
<td>4.73</td>
<td>gi</td>
<td>49525279</td>
<td>Unnamed protein product [Candida glabrata]</td>
<td>27</td>
<td>Mitochondrial Ribosomal protein MRP8 (2–70)</td>
<td>30/4 16%</td>
</tr>
<tr>
<td>12B</td>
<td>50549</td>
<td>8.93</td>
<td>gi</td>
<td>40728089</td>
<td>Hypothetical protein AN6630.2 [Aspergillus nidulans FGSC A4]. 460 aa.</td>
<td>38</td>
<td>Hypothetical protein [Aspergillus oryzae RIB40] (0) Citrate synthase Citi1, putative [Aspergillus flavus NRRL3357] (0) 2-methylcitrate synthase, mitochondrial precursor [Aspergillus terreus NH2624] (0)</td>
<td>10/4 8%</td>
</tr>
<tr>
<td>13B</td>
<td>43391</td>
<td>6.91</td>
<td>gi</td>
<td>40743549</td>
<td>Hypothetical protein AN5646.2 [Aspergillus nidulans FGSC A4]. 417 aa.</td>
<td>59</td>
<td>Hypothetical protein [Aspergillus oryzae RIB40] (0) 3-ketoacyl-coA thiolase peroxisomal A precursor [Aspergillus flavus NRRL3357] (0)</td>
<td>19/7 16%</td>
</tr>
<tr>
<td>14B</td>
<td>43391</td>
<td>6.91</td>
<td>gi</td>
<td>40743549</td>
<td>Hypothetical protein AN5646.2 [Aspergillus nidulans FGSC A4]. 417 aa.</td>
<td>59</td>
<td>Hypothetical protein [Aspergillus oryzae RIB40] (0) 3-ketoacyl-coA thiolase peroxisosomal A precursor [Aspergillus flavus NRRL3357] (0)</td>
<td>19/7 21%</td>
</tr>
<tr>
<td>15B</td>
<td>38975</td>
<td>8.63</td>
<td>gi</td>
<td>40743825</td>
<td>Hypothetical protein AN2713.2 [Aspergillus nidulans FGSC A4]. 345 aa.</td>
<td>44</td>
<td>Hypothetical protein AN3471.2 <a href="0">Aspergillus nidulans FGSC A4</a> Hypothetical protein AN6966.2 <a href="0">Aspergillus nidulans FGSC A4</a></td>
<td>24/6 16%</td>
</tr>
<tr>
<td>16B</td>
<td>126249</td>
<td>6.65</td>
<td>gi</td>
<td>6688831</td>
<td>Hypothetical protein NCU05154 [Neurospora crassa OR74A]. 1152 aa.</td>
<td>51</td>
<td>P-type calcium ATPase <a href="0">Aspergillus fumigatus AF293</a> P-type calcium ATPase, putative <a href="0">Aspergillus flavus NRRL3357</a></td>
<td>19/9 8%</td>
</tr>
<tr>
<td>17B</td>
<td>60416</td>
<td>8.13</td>
<td>gi</td>
<td>40745893</td>
<td>Hypothetical protein AN1884.2 [Aspergillus nidulans FGSC A4]. 544 aa.</td>
<td>42</td>
<td>Cytochrome P450 monoxygenase, putative <a href="0">Aspergillus fumigatus A1163</a> Conserved hypothetical protein <a href="0">Aspergillus terreus NH2624</a> Hypothetical protein An11g02990 <a href="0">Aspergillus niger</a></td>
<td>12/5 7%</td>
</tr>
<tr>
<td>20B</td>
<td>78048</td>
<td>8.83</td>
<td>gi</td>
<td>40793880</td>
<td>Hypothetical protein AN6752.2 [Aspergillus nidulans FGSC A4]. 696 aa.</td>
<td>37</td>
<td>Hypothetical protein <a href="0">Aspergillus oryzae RIB40</a> Fatty-acyl coenzyme A oxidase (Pox1), putative <a href="0">Aspergillus fumigatus A1163</a> Conserved hypothetical protein <a href="0">Aspergillus terreus NH2624</a></td>
<td>14/6 9%</td>
</tr>
<tr>
<td>21B</td>
<td>19712</td>
<td>9.26</td>
<td>gi</td>
<td>2311</td>
<td>Ribonuclease alpha-sarcin.177 aa.</td>
<td>42</td>
<td>a-sarcin precursor <a href="9e%E2%80%93101">Penicillium daleae</a> Gigantin [Aspergillus giganteus] (4e–96)</td>
<td>33/6 30%</td>
</tr>
</tbody>
</table>

Table 2. Differentially expressed intracellular proteins in Aspergillus niger biofilms identified by MS-TOF analysis.
Table 3. Over-expressed intracellular proteins in Aspergillus niger free-mycelium (mycelia pellets) identified by MS-TOF analysis.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Mr (Da)</th>
<th>pI</th>
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<td>1FM</td>
<td>18861</td>
<td>8.87</td>
<td>gi</td>
<td>4322946</td>
<td>Cyclophilin-like peptidyl prolyl cis-trans isomerase [Aspergillus flavus NRRL3357] (5e-81)</td>
<td>170</td>
<td>Peptidyl-prolyl cis-trans isomerase [Aspergillus clavatus NRRL 1] (7e-81)</td>
<td>10/9 51%</td>
</tr>
<tr>
<td>2FM</td>
<td>305669</td>
<td>6.58</td>
<td>gi</td>
<td>40739159</td>
<td>Hypothetical protein ANS840.2 [Aspergillus niger FGSC A4], 2788 aa.</td>
<td>39</td>
<td>60S ribosomal protein L19 [Aspergillus terreus NIH2624] (0)</td>
<td>11/8 3%</td>
</tr>
<tr>
<td>5FM</td>
<td>54818</td>
<td>5.17</td>
<td>gi</td>
<td>40745270</td>
<td>ATPB_NEUCR ATP synthase beta chain, mitochondrial precursor [Aspergillus niger FGSC A4], 513 aa.</td>
<td>131</td>
<td>ATP synthase F1, beta subunit [Aspergillus fumigatus AF293] (0)</td>
<td>18/12 31%</td>
</tr>
<tr>
<td>6FM</td>
<td>34370</td>
<td>8.64</td>
<td>gi</td>
<td>140746425</td>
<td>RL5_NEUCR 60S ribosomal protein L5 (CPR4) [Aspergillus niger FGSC A4], 301 aa.</td>
<td>16</td>
<td>60S ribosomal protein L5, putative [Neosartorya fischeri NRRL 1811] (1e-172)</td>
<td>10/2 6%</td>
</tr>
<tr>
<td>7FM</td>
<td>18441</td>
<td>5.36</td>
<td>gi</td>
<td>2769700</td>
<td>Allergen Asp F3 [Neosartorya fischeri NRRL 181] (1e-92)</td>
<td>48</td>
<td>Allergen Asp F3 [Aspergillus clavatus NRRL 1] (1e-89)</td>
<td>13/4 31%</td>
</tr>
<tr>
<td>8FM</td>
<td>22840</td>
<td>7.21</td>
<td>gi</td>
<td>3869086</td>
<td>CAP59 [Cryptococcus bacillisporus], 199 aa.</td>
<td>58</td>
<td>Hypothetical protein An04g04630 [Aspergillus niger] (1e-11)</td>
<td>7/4 16%</td>
</tr>
<tr>
<td>11FM</td>
<td>16626</td>
<td>5.72</td>
<td>gi</td>
<td>40741469</td>
<td>Hypothetical protein AN8625.2 [Aspergillus niger FGSC A4], 150 aa.</td>
<td>35</td>
<td>Conserved hypothetical protein [Aspergillus fumigatus AF293] (5e-48)</td>
<td>12/3 13%</td>
</tr>
<tr>
<td>13FM</td>
<td>47377</td>
<td>5.46</td>
<td>gi</td>
<td>2118302</td>
<td>6 beta-hydroxyhyoscynamine epoxidase [Aspergillus oryzae RIB40], 438 aa.</td>
<td>80</td>
<td>Hypothetical protein [Aspergillus oryzae RIB40] (0)</td>
<td>14/8 28%</td>
</tr>
</tbody>
</table>

Continúa ...
Conclusions

It seems that cell adhesion is the most important stimulus responsible for biofilm development and its particular morpho-genetic and physiological responses derived from this biological process in accordance to our former hypothesis, which is the basis of Surface Adhesion Fermentation. Although preliminary, results presented in this paper show that there are significant differences between the proteomes of A. niger biofilm and free-living mycelia. This is in agreement with our previous results on transcriptomics analysis in the same culture conditions (Villena et al. 2009). New insights will be obtained with the new available genomes of A. niger CBS 513.88 (Pel et al. 2007) and ATCC1015 (draft version in: http://genome.jgi-psf.org/Aspni5/Aspni5.home.html), and the recent attempt to use proteomic data for A. niger genome annotation (Wright et al. 2009). We are conducting a global transcriptomic and proteomic analysis of A. niger biofilms to clarify the process of cell adhesion as related to biofilm fermentation.

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Literature cited


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