Impact of environmental conditions on surface proteins of *Clostridium difficile*

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Introduction

Clostridium difficile is the etiological agent of pseudomembranous colitis and of many cases of nosocomial diarrhoea. Its pathogenicity is mainly due to toxins A and B. The role of surface proteins in the first step of colonization could be of crucial importance in the pathogenicity. This colonization process involves various surface proteins, including the High Molecular Weight S-layer Protein (HMW-SLP) (1), the adhesins Cwp66 (Cell Wall Protein of 66 kDa) and Fbp68 (Fibronectin Binding Protein of 68 kDa), the flagellar proteins FliC and FliD and the protease Cwp84 (Cell Wall Protein of 84 kDa), which has been shown recently to cleave the S-Layer proteins precursor, and that may play a role in the dissemination of the bacteria (2,3).
The virulence genes expression could be influenced by environmental conditions such as temperature, osmolarity, pH, glucose and antibiotics. It has been shown that the expression of toxins A and B of *C. difficile* is negatively regulated by different environmental factors, particularly the glucose (4). Moreover, subinhibitory concentrations of some antibiotics increased the transcription of genes encoding some colonization factors (S-layer proteins, *cwp84* and *fbp68*)(5), as well as, an acidic pH increases the adherence of *C. difficile* to Vero cells (6).

The aim of this study was to analyze *in vitro* the effect of glucose and the accompanying decrease in pH resulting from glucose metabolism on the *C. difficile* surface protein expression.

Methods

➢ **Analysis of surface proteins expression:**
The strain 630 (TcdA+ TcdB+) was grown in tryptone yeast (TY) or TY supplemented with 0.5% glucose (TYG). Assays were repeated three times. For some assays, pH of media was maintained constant by addition of a buffering agent.

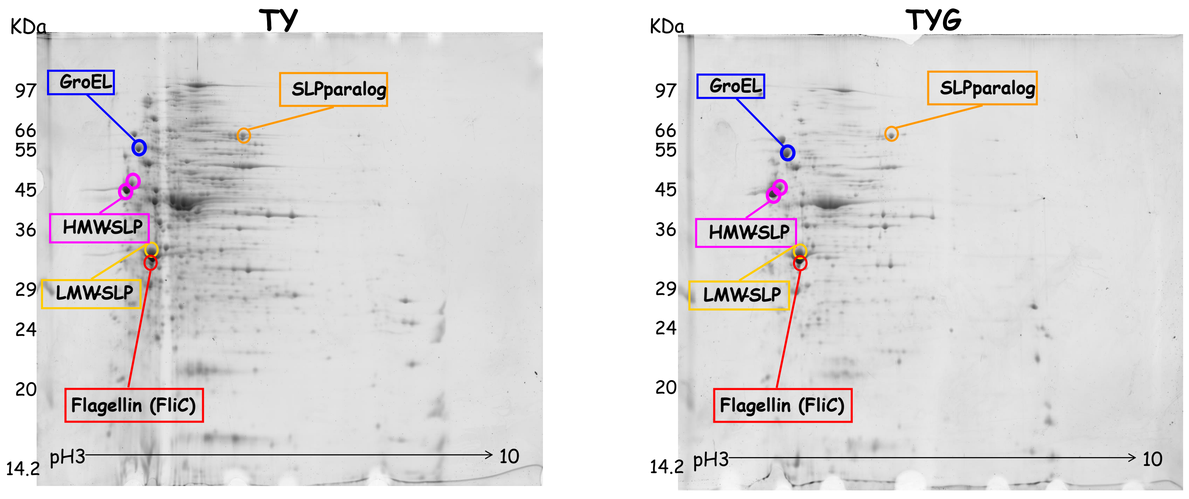
1.Whole cell-wall associated surface proteins analysis
The surface proteins were extracted by mutanolysine and identified by MALDI-TOF and MS/MS after 2D electrophoresis.

2. Quantitative analysis by Immunoblot
Immunoblot analysis were performed with specific antibodies. Quantitative differences of surface proteins expression were estimated by densitometry analysis with an imaging-system (ImageJ) and results were expressed as means ± standard deviations.

➢ **Analysis of surface proteins gene expression by real-time RT-PCR:**
-Total RNA was isolated using the FastRNA® Pro Blue Kit and the FastPrep® Instrument, according to the manufacturer's instructions;
- cDNA synthesis were performed using the SuperScript™ III RNase-H (Invitrogen);
- Real-time PCR were performed in the Light Cycler using the Fast Start DNA MasterPLUS SYBR Green I reagent (Roche Diagnostics).

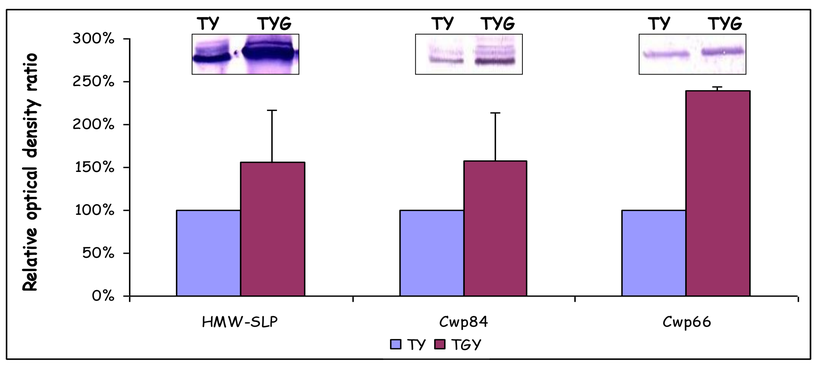
Results

Effect of glucose on surface protein expression



Resolution of surface proteins by 2-D PAGE. Proteins were extracted from a 15h growth of *C. difficile* 630 strain in TY or TYG.

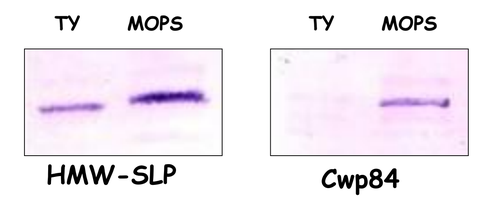
The cultures exhibited comparable growth in TY or TYG. The identification of the most important spots by MS/MS showed no striking qualitatives differences among the surface proteins. The flagellin, HMW-SLP, LMW-SLP, GroEL and one SLPA paralog were identified.



Effect of glucose on the surface expression of HMW-SLP, Cwp84 and Cwp66. Surface proteins were extracted at 8h.

The addition of glucose to the growth medium of *C. difficile* culture results in an increased in the expression of several surface proteins at 8h (HMW-SLP, Cwp84 and Cwp66) and 15h (results no shown). The pHs for the glucose containing cultures decreased from 7.4 to 6.0 that normally results from glucose metabolism

Effect of glucose independently of pH

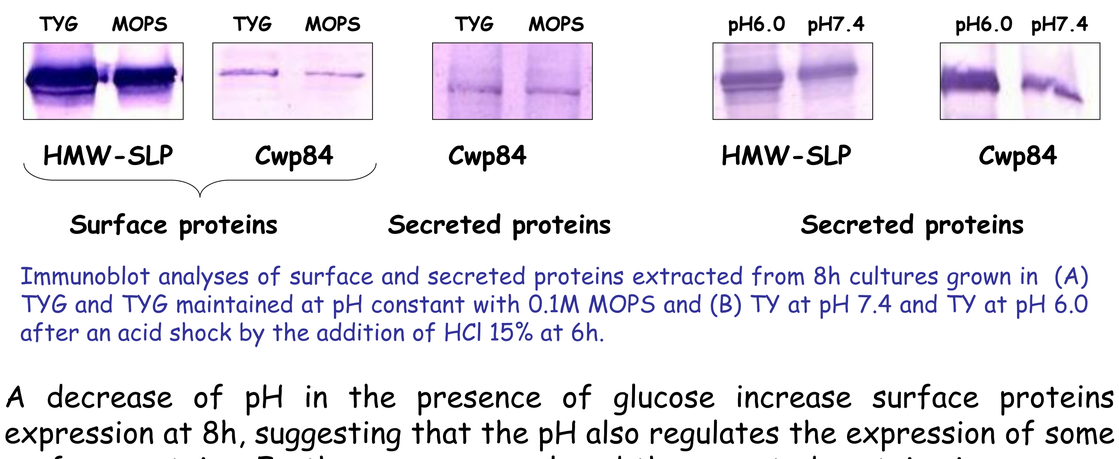


Immunoblot analyses of surface proteins extracted from cultures grown in TY and TYG maintained at pH constant with 0.1M MOPS (extraction from 8h cultures).

We observed an increase of surface proteins expression when the pH of TYG was maintained constant at 7.4. Therefore, the glucose effect on surface proteins expression occurs independently of the decrease in pH. This suggests that the glucose regulates positively the colonization factors expression.

This observation on surface protein expression was correlated with the RNA synthesis. Transcription of *cwp84* and *slpA* was increased 4- and 3-fold respectively, as compared with control culture without glucose.

Effect of pH



Immunoblot analyses of surface and secreted proteins extracted from 8h cultures grown in (A) TYG and TYG maintained at pH constant with 0.1M MOPS and (B) TY at pH 7.4 and TY at pH 6.0 after an acid shock by the addition of HCl 15% at 6h.

A decrease of pH in the presence of glucose increase surface proteins expression at 8h, suggesting that the pH also regulates the expression of some surface proteins. Furthermore, we analyzed the secreted proteins in presence or absence of glucose : HMW-SLP and Cwp84 were released into the medium at an acidic pH.

Conclusion

This study demonstrates that glucose and pH could regulate the expression of some surface proteins potentially involved in the colonization process. Therefore, *C. difficile* could increase the expression of these surface proteins in response to glucose and the localization of secreted and surface proteins in response to pH.

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