Anaerobe 17 (2011) 4–14

Contents lists available at ScienceDirect

Anaerobe

journal homepage: www.elsevier.com/locate/anaerobe

Clinical Microbiology

Anaerobe/aerobe environmental flux determines protein expression profiles of *Bacteroides fragilis*, a redox pathogen

Eliane de Oliveira Ferreira [a], Edwin Yates [b,*], Michel Jourlin [c], Jian Liu [d], Regina M.C. Pilotto Domingues [a], Morris Goldner [d,e,f,**]

[a] *Laboratorio de Biologia de Anaerobios, Departamento de Microbiologia Medica, IMPPG UFRJ, 2194-902 Rio de Janeiro, RJ, Brazil*
[b] *School of Biological Sciences, University of Liverpool, Liverpool L69 7ZB, UK*
[c] *Laboratoire Traitement du Signal et Instrumentation, UMR CNRS SS26, Universite Jean Monnet, Saint Etienne 42023, France*
[d] *Faculty of Applied Health Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1*
[e] *Departement de Biologie Medicale, Universite Laval, Quebec, Canada G1K 7P4*
[f] *School of Occupational and Public Health, Ryerson University, 350 Victoria Street, Toronto, Canada M5B 2K3*

ARTICLE INFO

Article history:
Received 17 September 2010
Accepted 22 September 2010
Available online 15 October 2010

Keywords:
Redox level
Bacteroides fragilis
Pathogenic potential
Outer-membrane protein (OMP) profiles
OMPs suggestive of unique participation

ABSTRACT

The oxidation-reduction (redox) of the environment characterizes the *Bacteroides fragilis* pathogenic potential. Previously, using 3D confocal laser scanning microscopy, the bacteria prepared from cultures grown under oxidizing conditions (Eh_7 *ca.* + 100 mV) were able to penetrate into Hela cell monolayers. In contrast, when grown under reducing conditions (Eh_7 *ca.* − 60 mV), there were no bacteria evident within Hela cells. The influence of the anaerobe/aerobe environmental flux during the process of the anaerobe infection could be significant. In *B. fragilis* peritonitis, this may depend on the occurrence of aerobiosis as opposed to anaerobiosis. To this end, three clinical *B. fragilis* strains, two infectious and one non-infectious, were grown under oxidizing and reducing conditions; then, the outer membrane protein expressions derived from these strains were assessed, following sarcosyl extraction and SDS-PAGE. The differences between the protein profiles from these strains when cultured under oxidizing and reducing conditions were found to be statistically significant for the two infectious strains, but not for the non-infectious strain. OMP profiles under aerobic conditions compared to anaerobic conditions exhibited products with a range of apparent molecular weights suggestive of unique participation in the interaction with the host cell.

1. Introduction

Abrupt displacement of *Bacteroides fragilis* from an intestinal reducing to a peritoneal oxidizing environment can result in peritonitis. This clinical observation suggested that *B. fragilis* would respond to changes in oxidation-reduction (redox) conditions. Previous attempts to clarify this observation based on redox levels, designated by Eh in millivolts (mV) adjusted to pH 7 (Eh_7), have revealed a potential pathogenic characteristic. Three-dimensional (3D) confocal laser scanning microscopy (CLSM) indicated that *B. fragilis* penetrated into Hela cell monolayers [1]. This redox effect occurred for the bacteria poised for growth under oxidizing but not reducing conditions. When *B. fragilis* bacteria were cultured under oxidizing conditions, examination by scanning electron microscopy had shown that the bacteria were widely dispersed, but under reducing conditions they were densely aggregated [2]. Using appropriate software, an adapted algorithm was applied that separated the Hela cell image into regions of nucleus and cytoplasm [3]. CLSM examination allowed the necessary subtle information to be extracted thus shaping and limiting the cytoplasmic area for treatment of consecutive laser cuts which enabled this image to be reassembled in 3D [4]. Then, it could be demonstrated that *B. fragilis* bacteria grown under oxidizing conditions were located *inside* the Hela cell.

In earlier CLSM studies, the *B. fragilis* bacteria in preparation for interaction with Hela cells were poised for growth at either frankly reducing, mildly oxidizing or relatively oxidizing conditions by varying the concentration of cysteine as the redox reagent [5]. Coupling metabolic energy to membrane potential results in the fine tuning of metabolism to environmental changes. These are

* Corresponding author.
** Corresponding author. Faculty of Applied Health Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1.
E-mail addresses: eayates@liv.ac.uk (E. Yates), morris.goldner@ryerson.ca (M. Goldner).

1075-9964/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.anaerobe.2010.09.004

perceived to control gene expression when presented with changes in redox environment [6]. Hence, *B. fragilis* cells will undergo significant alterations in their physiology while growing in aerobic versus anaerobic environments. Both input and internal signals explain how signaling molecules, which are based on communication modules that send and receive protein phosphorylation signals, enable bacteria to transduce adaptive responses [6,7]. When grown under frankly reducing conditions (Eh_7 *ca.* - 60 mV), no bacteria were evident within Hela cells; in contrast under mildly oxidizing conditions (Eh_7 *ca.* + 20 mV) bacterial cells were observed to project from the Hela cell surface, and by comparison under relatively oxidizing conditions (Eh_7 *ca.* + 100 mV), *B. fragilis* bacteria penetrated *into* the Hela cells. Thus, when responding to particular redox conditions [1,3], this extracellular microorganism of the intestinal flora produces an unexpected biological response: distinct bacterial penetration (Diagram 1a).

The same procedures were followed for bacterial cultures grown under oxidizing and reducing conditions. The most recent

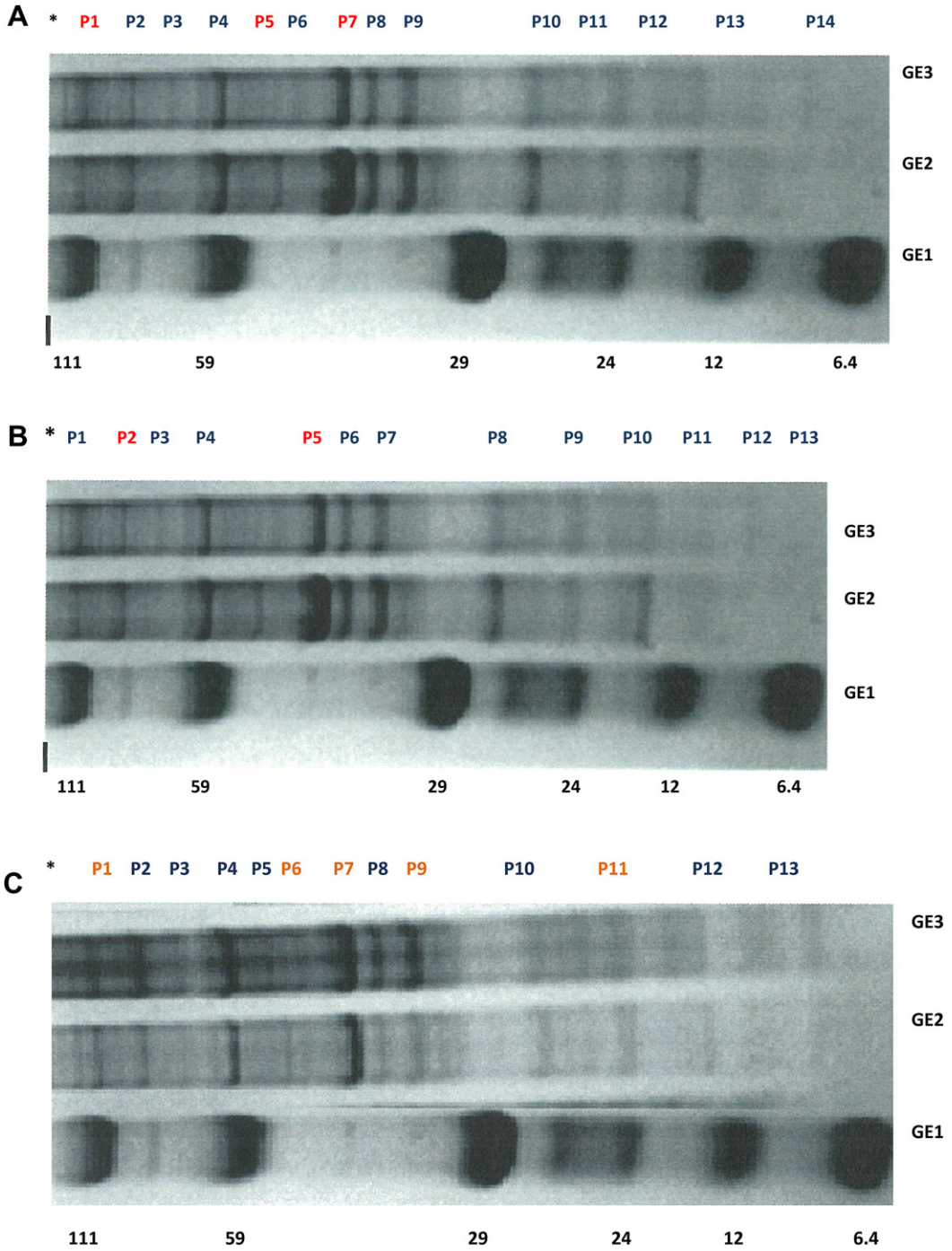

Fig. 1. A, B and C. PAGE profiles for the OMP expression of the three strains grown under reducing and oxidizing conditions. (A) 1A – SDS-PAGE Analysis of OMPs of *B. fragilis* Strain 1081.(B) 1B - SDS-PAGE Analysis of OMPs of *B. fragilis* Strain MC2.(C) 1C – SDS-PAGE Analysis of OMPs of *B. fragilis* Strain RBG-A. Under Oxidizing and Reducing Conditions. Lane GE1 – Molecular Weight Standard (KDa); Lanes GE2 and GE3 – OMPs of Strain 1081, MC2, and RBG-A Obtained Under Oxidizing and Reducing Conditions, respectively *Numerical Designation of Peaks (P).