JOURNAL OF PROTEOMICS 73 (2010) 1535–1541

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Genomic and proteomic evaluation of antibiotic resistance in *Salmonella* strains

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ARTICLE INFO

Article history:
Received 17 December 2009
Accepted 17 March 2010

Keywords:
Salmonella Typhimurium
Salmonella Enteritidis
Antibiotic resistance
Proteome
Biomarkers
Wild animals

ABSTRACT

Using *Salmonella* strains identical to those present in the gastrointestinal tract of different animals we aim to determine and compare the proteome of two serotypes, *Salmonella* Typhimurium and Enteritidis recovered from faecal samples of wild boars and wild rabbits, respectively. The presence of genes responsible for antibiotic resistance was detected by PCR. Proteomes of the two distinct serotypes were determined using 2-DE in order to identify proteins associated with antibiotic resistance or virulence. Through 2-DE we obtained a total of 229 spots from both strains. All were suitable for MALDI-TOF/TOF and, in correlation with bioinformatic databases, allowed accurate identification and characterization of proteins. S. Enteritidis recovered from wild rabbits was sensitive to all the antibiotics tested in contrast to S. Typhimurium isolated from wild boars which presented a resistance phenotype to ampicillin, streptomycin and chloramphenicol. Nevertheless, despite the different ratio of proteins observed in each proteome according to their biological function, no significant difference was observed in the involvement of these proteins in pathogenicity. Bearing in mind that serotypes are related to infectious processes in humans and animals, it is important to explore the proteome of new strains which might serve as protein biomarkers for biological activity.

1. Introduction

The increase of antibiotic resistance in different bacterial strains, including *Salmonella*, is a serious health problem bearing in mind the resulting infections in humans. Multiple antibiotic resistance (MAR) in *Salmonella* has been increasing and approximately 45% of the isolates of *Salmonella* Typhimurium reported to the Enter-net surveillance network in recent years showed this phenotype of multiple-resistance [1]. Different chromosomally mediated systems are present in

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doi:10.1016/j.jprot.2010.03.009

microorganisms such as *Salmonella,* and are probably implicated in low-level resistance to different antimicrobial agents through the expression of the *mar* locus that can contribute to the development of the MAR phenotype [2].

Proteomics is a major area of research developing in the post-genome era. Its application provides great opportunities to elucidate disease mechanisms. Significant progress has also been made on the characterization of bacterial pathogens through comparative proteomics correlated with mass spectrometry and bioinformatics [3]. The evaluation of protein profiles in response to various mechanisms of stress, such as the sensitivity to antibiotics or modifications related to antibiotic resistance could represent an integrating approach for the development of new therapeutic strategies and antimicrobial agents. Bacterial surface proteins are important for the host-pathogen interaction and they are frequently involved in disease pathogenesis [4]. Although the number of proteins detected in these studies represents only a small proportion of the predicted proteome, many genes may be induced and only expressed under certain conditions [5]. Detection and characterization of outer-membrane-proteins (OMP) is particularly desirable for discovery of biomarkers for the MAR phenotype since these include certain channel proteins, such as TolC, linked to efflux pump proteins, and porins which are intimately involved in molecular permeation and multiple antibiotic resistance [6,7]. Other potentially important proteins associated with the MAR phenotype were found in the inner membrane and periplasm, also termed the cell envelope; these include AcrB, an inner membrane drug anti-porter which is joined to AcrA, a periplasm fusion linker between the former and an OMP channel protein, such as TolC [8].

Escherichia coli is a common Gram negative bacterium that shares similar sequences and functions at the chromosomal level with *Salmonella enterica*. In fact, these two bacteria have a mutual ancient ancestor which enabled them to possess a layer of peptidoglycans and lipids at the cell wall permitting them to resist many of the known antibiotics more effectively [9]. *Salmonella* is responsible for a wide range of clinical manifestations in humans, including gastroenteritis. Although there are over 2000 serotypes of *Salmonella* described, only a small number of these are important pathogens in humans. On the other hand, it is important to distinguish two groups: *Salmonella* serotypes causing enteric fever and "non-typhoidal *Salmonella*" serotypes [10]. *Salmonella* is commonly related to food poisoning killing people worldwide, multiplying inside the host's macrophages. In fact, *Salmonella* serotypes are often associated with warm-blooded vertebrates and are normally transmitted via faecal contamination from an infected host and via food contamination [3].

Proteomics is a new molecular approach to studying bacteria. It has proven especially invaluable for, among other things, extracting information on different processes, including antibiotic resistance, and searching for new targets during the development of new antimicrobial agents. In this study a proteomic survey of *Salmonella* isolates with different serotypes and antibiotic resistance phenotypes, recovered from faecal samples of wild rabbits and wild boars from the North of Portugal, was carried out in order to gain information about the proteins they express.

2. Materials and methods

2.1. *Isolation of Salmonella and serotypification*

All the samples were individually packed and transported to the laboratory in refrigerated conditions. All the samples were analysed by standard culture methods according to ISO Norm 6579:2002 applied to *Salmonella* detection in animal faeces. Briefly, the diluted samples were incubated at 37 °C for 18 ± 2 h, afterwards 0.1 ml was inoculated in agar that was incubated at 41.5 ± 10 °C for 24 h ± 3 h. After this, one loop of each agar positive plate was streaked onto the surface of two selective solid media: Hektoen (Oxoid® — CM419) and agar (Oxoid® — CM469). Colonies of presumptive *Salmonella* were confirmed by biochemical tests (Oxidase reaction, Triple Sugar Iron Agar (Oxoid®— CM277), Urea broth (Merk® — 1.08483), L-lysine descarboxylation medium (Oxoid® — CM308S) and serological agglutination with Poli A–I & Vi antiserum (Difco® — 222641). All strains used in this study are listed in Table 1. *Salmonella* isolates were serotyped from each positive sample according to the Kauffmann–White scheme in the National Reference Laboratory for *Salmonella*.

2.2. *Extraction of DNA and characterization of antibiotic resistance genes*

The presence of genes encoding TEM, SHV, OXA, CTX-M, and CMY β-lactamases was studied by polymerase-chain-reaction (PCR) in all ampicillin-resistant isolates using primers and conditions previously reported [11]. In addition, the presence of *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(E) genes was studied by PCR for the tetracycline-resistant isolates. The following genes were also studied by PCR: *aad*A1 and *aad*A2 (in streptomycin-resistant isolates), *aac*(3)-I, *aac*(3)-II and *aac*(3)-IV (in

Table 1 – Resistance profiles of the different samples of *Salmonella* serotypes displayed in the 1-D gels.

Number	Sample	Serotype	Resistance profile
1	J27(1)	S. Typhimurium	AMP; TET; STR; CHL
2	J32(2)	S. Typhimurium	AMP; TET; STR; CHL
3	C71(1)	S. Typhimurium	AMP; TET; STR; CHL
4	P40(a)	S.Typhimurium	AMP; TET; STR; CHL
5	P16(1)	S. Typhimurium	AMP; TET; STR; CHL
6	P20(2)	S. Enteritidis	AMP; TET; STR; NAL; CHL
7	P29(2)	S. Rissen	AMP; TET; STR; NAL; SXT
8	J45(1)	S. Rissen	AMP; TET; SXT
9	P1(1)	S. Enteritidis	AMP; TET; NAL; SXT
10	AVT14(1)	ND	TET; SXT
11	C12(1)	S. Rissen	AMC; AMP; TET; STR; SXT
12	C16(1)	S. Typhimurium	AMP; TET; AK; STR; CHL
13	C40(2)	S. Typhimurium	AMP; STR; CHL
14	P57(c)	S. Enteritidis	TOB; STR
15	C37(1)	S. Enteritidis	–
16	J15(2)	S. Typhimurium	AMP; STR; CHL

J—wild boars; C—wild rabbit; P—swine; AVT— ostrich; AK—amikacin; AMC—amoxicillin–clavulanic acid; AMP—ampicillin; CHL—chloramphenicol; NAL—nalidixic acid; STR—streptomycin; SXT—sulfamethoxazole–trimethoprim; TET—tetracycline; TOB—tobramycin; ND—not determined.