



Contents lists available at ScienceDirect

## International Journal of Food Microbiology

journal homepage: [www.elsevier.com/locate/ijfoodmicro](http://www.elsevier.com/locate/ijfoodmicro)C-source metabolic profilings of foodborne Shiga-toxin producing *E. coli* match serogroup differentiations and highlight functional adaptationsStéphane Kerangart<sup>a</sup>, Benoit Cournoyer<sup>a,\*</sup>, Estelle Loukiadis<sup>a,b,1</sup><sup>a</sup> University of Lyon, Research group on "Bacterial Opportunistic Pathogens and Environment", UMR Ecologie Microbienne, CNRS 5557, INRA 1418, University Lyon 1, VetAgro Sup, 69280 Marcy L'Etoile, France<sup>b</sup> University of Lyon, VetAgro Sup, LMAP, National Reference Laboratory for *Escherichia coli* (including VTEC), Marcy L'Etoile, France

## ARTICLE INFO

## Keywords:

STEC  
Metabolic profilings  
Carbon sources  
INT-reduction assays  
O157  
O26  
O104:H4

## ABSTRACT

The tropism of pathogenic STEC for foodstuffs and cattle reservoir is related to functional specializations. An investigation of C-source utilization patterns among and between STEC serogroups was performed using omnilog phenotypic microarrays (OM). OM functional groupings were compared with STEC phylogroups, seropathotypes, EFSA's molecular risk assessment groups and serogroups. OM INT reduction activities of 37 STEC strains growing on 190 C-substrates were compared. Each strain had its own specific C-utilization profile but 23% of the substrates was used by all strains, 47% by none, and 30% was variably metabolized. Galactose, mannose, *N*-acetyl-glucosamine (GlcNAc), and *N*-acetyl neuraminic acid (Neu5Ac) found in the mucus layer of the bovine small intestine were metabolized by all strains. The 56 most informative substrates divided the C-utilization patterns (CP) into three clusters with: (A) harboring all O157 and O145 strains; (B) all O26 strains, and (C) strains of the other serogroups. Significant correlations between INT reduction values of pair of strains per CP group supported these differentiations. CP of group A and B strains were respectively defective in the use of galactonic acid- $\gamma$ -lactone and rhamnose. Most CP group C strains grew with *L*-lyxose. Adjusted Wallace coefficients analyses of the datasets indicated high probabilities for the prediction of the use of glycolic acid,  $\beta$ -hydroxybutyric acid, *L*-lyxose and *D*-galactonic acid- $\gamma$ -lactone and 5-keto-*D*-gluconic acid by a serogroup. The use of a C-substrate could be predicted from the classification of a strain into a phylogroup or seropathotype. Significantly lower numbers of C-substrates were used by seropathotype A strains like O157 ones. Improvements of STEC identification keys were proposed using the most discriminant C-substrates found in this study.

## 1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) strains are major worldwide foodborne pathogens, causing severe and sometimes lethal human infections, such as hemolytic uremic syndrome (HUS) cases, especially among young children (Croxen et al., 2013; Kaper et al., 2004). They are notably responsible of outbreaks and sporadic cases of acute illnesses (reaching each year 2,801,000 cases), and cause annually 3890 HUS cases and 230 deaths (Majowicz et al., 2016). The key virulence trait of STEC is the synthesis of Shiga toxins (Stx) but additional virulence factors boosting their pathogenicity have been described (Karmali, 2009). Most typical pathogenic strains harbor a LEE (locus of enterocyte effacement) involved in the intimate adherence to the host-cell membrane (Franzin et al., 2015). Recent outbreaks, such as the one involving STEC O104:H4 strains lacking the LEE, led to the observation of additional virulence properties (Beutin and Fach, 2014;

Navarro-Garcia, 2014). STEC virulence thus appears multi-factorial, and several factors remain to be identified (Karmali et al., 2010).

More than 380 STEC serotypes have been reported in humans so far but only ten of them have been consistently associated with severe cases and foodborne outbreaks (Caprioli et al., 2005; Mathusa et al., 2010). To improve the assessment of clinical and public health risks associated with STEC, Karmali et al. (2003) proposed a seropathotype classification (using a gradient from seropathotype A – high risk- to seropathotypes D and E – minimal risk). This classification considers the prevalence of STEC serotypes and their association with human outbreaks, including the severity of the clinical outcomes (Karmali et al., 2003). Serotypes O157:H7 and O157:NM have been classified in the seropathotype A, those of serotypes O26:H11; O103:H2; O111:NM; O121:H19 and O145:NM into seropathotype B, and those of O91:H21 and O113:H21 into seropathotype C. Recently, a new scheme for STEC categorization according to potential risk for human health was

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**Table 1**Serotype, seropathotype, molecular risk assessment and phylogenetic groups, and origin of the 37 O157 and non-O157 *E. coli* strains used in this study.

| Serogroup | Strain         | Serotype | Pathotype      | STEC seropathotype <sup>a</sup> | STEC molecular risk assessment group <sup>b</sup> | Phylogenetic group <sup>c</sup> | Origin  | References                     |
|-----------|----------------|----------|----------------|---------------------------------|---|---------------------------------|---|--------------------------------|
| O157      | 620            | O157:H7  | STEC           | A                               | I   | D                               | Host independent origin (Food-bovine meat)            | King et al., 2014              |
|           | RD9            | O157:H7  | STEC           | A                               | I   | D                               | Host independent origin (Food-bovine meat)            | King et al., 2009              |
|           | 540            | O157:H7  | STEC           | A                               | I   | D                               | Host independent origin (Food-bovine meat)            | Kerangart et al., 2016         |
|           | FCH6           | O157:H7  | STEC           | A                               | I   | D                               | Host independent origin (Food-raw goat's milk cheese) | Espié et al., 2006             |
|           | EDL 933        | O157:H7  | STEC           | A                               | I   | D                               | Host gut (Human)                                      | Perna et al., 2001             |
|           | Sakai          | O157:H7  | STEC           | A                               | I   | D                               | Host gut (Human)                                      | Hayashi, 2001                  |
|           | m622           | O157:H7  | STEC           | A                               | I   | D                               | Host independent origin (Food-raw milk cheese)        | Franz et al., 2011             |
| O26       | m638           | O157:H7  | STEC           | A                               | I   | D                               | Host gut (Human)                                      | Franz et al., 2011             |
|           | 21765          | O26:H11  | STEC           | B                               | I   | B1                              | Host independent origin (Food-raw cow milk cheese)    | Espié et al., 2006             |
|           | 2245/98        | O26:H11  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Zimmerhackl et al., 2010       |
|           | 64/36          | O26:H11  | AEEC           | ND                              | ND  | B1                              | Host independent origin (Food-raw ewe milk)           | Neto et al., 2012              |
|           | 3073/00        | O26:H11  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Zimmerhackl et al., 2010       |
|           | 37.40          | O26:H11  | STEC           | B                               | I   | B1                              | Host independent origin (Food-bovine meat)            | Neto et al., 2012              |
|           | 279/8          | O26:H11  | STEC           | B                               | I   | B1                              | Host independent origin (Food-raw cow milk cheese)    | Kerangart et al., 2016         |
|           | 11368          | O26:H11  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Ogura et al., 2009             |
|           | MB 2659 (PH10) | O26:H11  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Possé et al., 2007             |
|           | PMK5           | O103:H2  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Mariani-Kurkdjian et al., 1993 |
| O103      | 262.03.18.1    | O103:H-  | <i>E. coli</i> | ND                              | ND  | B1                              | Host independent origin (Food-bovine meat)            | This study                     |
|           | MB 2652 (PH3)  | O103:H2  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Possé et al., 2007             |
|           | MB 2653 (PH4)  | O103:H2  | STEC           | B                               | I   | B1                              | Host gut (Human)                                      | Possé et al., 2007             |
|           |                |          |                |                                 |   |                                 |   |                                |
| O111      | CL37           | O111:H8  | STEC           | B                               | I   | ND                              | Host gut (Human)                                      | Zhang et al., 2002             |
|           | J43            | O111:H8  | STEC           | B                               | I   | ND                              | Host gut (Bovine)                                     | Bibbal et al., 2015            |
|           | 622-4          | O111:H8  | STEC           | B                               | I   | ND                              | Host independent origin (Food-raw cow milk cheese)    | Kerangart et al., 2016         |
| O145      | 2513-21        | O145:H28 | STEC           | B                               | I   | D                               | Host independent origin (Food-raw cow milk cheese)    | Kerangart et al., 2016         |
|           | 991            | O145:H28 | STEC           | B                               | I   | D                               | Host independent origin (Food-bovine meat)            | Kerangart et al., 2016         |
|           | MB 2817 (PH88) | O145:H28 | STEC           | B                               | I   | D                               | Host gut (Human)                                      | Possé et al., 2007             |

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