Identification of plasmids in a Salmonella Typhimurium septicemic isolate without the classical 95 kb virulence plasmid

Bergeron, N.[1]*
Daigle, F.[2], Letellier, A.[1], Quessy, S.[1]

[1]Research Chair in Meat Safety, CRIP-GREMIP, Faculty of Veterinary Medicine, Université de Montréal, Québec, Canada.
[2]Department of Microbiology and Immunology, Faculty of Medicine, Université de Montréal, Québec, Canada.

* 3200 Sicotte, C.P. 5000, St-Hyacinthe, Québec, Canada, J2S 7C6
e-mail: nadia.bergeron@umontreal.ca; fax: 450-778-8157

Abstract

In this study, we report the characterization of plasmids from a Salmonella Typhimurium strain isolated from a septicemic pig. This isolate did not possess the classical 95 kb plasmid associated with virulence, but contained several low molecular weight plasmids. This isolate was as well one of the most invasive in intestinal epithelial cell lines (58.34 ± 7.32%) and showed no acquire resistance to tested antimicrobial agents. We therefore sequenced these plasmids. The size of the first plasmid, pST36-4-b5, was 3.6 kb and the size of pST36-1-b6, the second one, was 4.9 kb. They contained some open reading frames (ORF) that carry some genetic information for replication and mobilization. These plasmids contained also information for enzymatic functions and some hypothetical proteins also found in various bacterial species. Finally, a third plasmid has been partially characterized. The size of this plasmid is higher than the two other plasmids. These plasmids contained several genes of unknown function that will need to be further studied for their putative role in virulence.

Introduction

S. Typhimurium is an important zoonotic agent and a pathogen in swine. Infections caused by septicemic strains of S. Typhimurium are associated with significant mortalities in mature pigs and therefore with economic losses for the porcine industry. However, in most cases, the majority of affected pigs will become asymptomatic carriers and can be the source of meat contamination during evisceration process at slaughter. It is thus important to better characterize these isolates in order to understand pathogenesis of infection and develop appropriate control measures. The aim of this study was to characterized plasmids present in one isolate of S. Typhimurium associated with septicemia in swine.

Material and Methods

Bacterial strains. A collection of isolates that have been characterized in previous studies was used in the present study (Bergeron et al., 2007; Bergeron et al., 2009; Bergeron et al., 2010). One isolate from septicemic pig was chosen for plasmids characterization. Based on previous findings, this highly virulent isolate did not possess the classical 95 kb plasmid associated with virulence but contained many low molecular weight plasmids. We assumed that this isolate would likely contain new and unknown virulence factors. This isolate was one of the most invasive in intestinal epithelial cell lines (58.34 ± 7.32%) (Bergeron et al., 2009) and showed no acquire resistance to tested antimicrobial agents (Bergeron et al., 2010).

Characterization of plasmids from this isolate. The donor E. coli SM10λpir (Δasd) strain (Miller and Mekalanos, 1988), containing pLOF/Km, a Tn10-based transposon plasmid (Herrero et al., 1990), was conjugated with the recipient S. Typhimurium isolate. The donor strain was grown in LB broth with DAP (2,6-diaminopimelic acid) (50 µg/ml), kanamycin (50 µg/ml), and ampicillin (100 µg/ml) and the isolate was grown in a LB medium only at 37 °C overnight without agitation. The cultures were centrifugated at low speed and resuspended in LB broth with DAP. The conjugation was made on LB agar with DAP and IPTG (isopropyl-beta-D-1-thiogalactopyranoside) (20 mg/ml) at 37°C for 6 hours and was kept at room temperature overnight. The conjugants were plated on selective media.
The conjugants were pooled and grown overnight for plasmid extraction using the QIAGEN® Plasmid Midi kit (QIAGEN, Mississauga, Ontario, Canada) in accordance with the manufacturer's instructions. Plasmids DNA were visualized on 0.7% (w/v) agarose/EtBr gel. A Supercoiled DNA Ladder (Invitrogen Canada Inc., Burlington, Ontario, Canada) for low molecular weight plasmids was used as marker.

The plasmids were transformed into chemically competent E. coli DH5ć cells. The cells were plated on LB agar supplemented with kanamycin. Plasmids from different clones were extracted using the QIAprep® Miniprep Kit (QIAGEN), in accordance with the manufacturer's instructions, and these were visualized on 0.7% (w/v) agarose/EtBr gel.

Sequencing and analysis of plasmids. Plasmids were sequenced using primers specific to the transposon at Université de Montréal's Institute for Research in Immunology and Cancer (IRIC), Montréal, Québec, Canada. The sequences were submitted at the National Center for Biotechnology Information (NCBI) data banks for homology searches.

Results

Plasmids characterization. Two plasmids from the isolate were sequenced (pST36-4-b5 and pST36-1-b6) and a third plasmid is currently being characterized. This strain was chosen for plasmids characterization based on highly invasive criteria and absence of virulence plasmid (approximately 95 kb) and presence of several low molecular weight plasmids. The size of pST36-4-b5 and pST36-1-b6 is 3.6 kb and 4.9 kb respectively. The characterisation of the third plasmid is not ended yet, but it has more than 6.5 kb in size. The smaller plasmid contained 8 open reading frames (ORF) ranging in size from 120 bp to 726 bp. The second one contained 12 ORFs ranging in size from 102 bp to 1554 bp. Plasmids pST36-4-b5 and pST36-1-b6 possess similar functions: plasmid mobilization, plasmid replication, enzymatic functions, but the majority of ORFs are genes with unknown function. Tables I and II show ORFs identified with potential functions in plasmids pST36-4-b5 and pST36-1-b6 in a S. Typhimurium septicemic isolate.

Discussion

In this study, plasmids present in a S. Typhimurium isolate associated with septicemia in swine were characterized in order to analyze the genetic basis of this isolate and found new or unknown virulence factor.

The characterization of pST36-4-b5 and pST36-1-b6 indicates that these plasmids carry some genetic information for replication and mobilization, although it does not seem to possess all the information for a functional plasmid mobilization system. Beta-galactosidase-α protein, a hydrolyse enzyme was found in each plasmid. This enzyme is involved in aerobic glycolyse for digestion of lactose in glucose and galactose. The plasmid pST36-4-b5 possesses the LabA (low-amplitude and bright)-like proteins. These proteins belong to a well conserved group of bacterial proteins with no known function in Enterobacteriaceae. In cyanobacteria, the gene labA modulates the circadian gene expression by the negative feedback regulation of KaiC (Taniguchi et al., 2007). The same plasmid possesses a winged helix-turn-helix (WHTH) DNA-binding domain of the GntR family of transcriptional regulators. The GntR family has many members distributed among almost all bacterial species for the regulation of various biological processes. The plasmid pST36-1-b6 possesses an ORF that code for a histidyl-tRNA synthetase. It is responsible for the attachment of histidine to the 3' OH group of ribose of the appropriate tRNA. This domain is primarily responsible for ATP-dependent formation of the enzyme bound aminoacyl-adenylate. Finally, other ORFs showed similarities with hypothetical proteins found in Enterobacteriacea, Vibrio cholerae, and Citrobacter were found to both plasmids. Despite some technical difficulties, it will be important to continue the characterization of the third plasmid. This plasmid being larger, it can be more likely associated with virulence associated genes.

Conclusion

The S. Typhimurium isolate from septicemic pig characterized in this study possesses plasmids containing several genes of unknown function that should be further studied for their putative role in virulence. Although genes that possess similarities with existing proteins appeared to be involved in housekeeping functions, we cannot exclude participation of these proteins in virulence since some metabolic enzymes have already been shown to participate in both virulence and bacterial metabolism.

References

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Table I. Open reading frames (ORF) identified in plasmid pST36-4-b5 (3.6 kb) in a *S.* Typhimurium septicemic isolate.

ORF	Length (bp)	Frame from...to (bp)	e-value	Putative product	Source and reference number
1	348	683...1030	$5e^{-41}$	Plasmid mobilization	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491YP_002221413
2	213	1187...1399	$7e^{-19}$	Plasmid mobilization	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491YP_002221413
3	195	1290...1484	$1e^{-27}$	RNAI modulator protein	*Salmonella enterica* subsp. *enterica* serovar Typhimurium strain G8430 YP_198405
4	120	1795...1914	$8e^{-11}$	Beta-galactosidase alpha protein	Broad host range expression vector pRK415iq AB032164
5	726	2290...3015	$1e^{-66}$	LabA_like proteins	*Escherichia coli* strain B088 ZP_06662273
6	228	3366...3562	$2e^{-18}$	WHTH_GntR	*Escherichia coli* strain B088 ZP_06662274

Table II. Open reading frames (ORF) identified in plasmid pST36-1-b-65 (4.9 kb) in a *S.* Typhimurium septicemic isolate.

ORF	Length (bp)	Frame from...to (bp)	e-value	Putative product	Source and reference number
A	300	218...517	0.13	Histidyl-tRNA synthetase	*Desulfatibacillum alkentivorans* strain AK-01 YP_002431917
B	1554	1605...3158	0.0	Mobilization protein MobA	*Salmonella enterica* strain 79500 NP_203138
C	357	3148...3504	$3e^{-59}$	Mobilisation protein MbeC	*Escherichia coli* strain ETEC H10407 CBJ04269
D	225	3503...3727	$2e^{-36}$	Regulatory protein Rop	*Salmonella enterica* subsp. *enterica* serovar Virchow strain SL491 YP_002221408
E	120	4209...4328	$8e^{-11}$	Beta-galactosidase alpha protein	Broad host range expression vector pRK415iq AB032164