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Full Length Research Paper

Iron starvation induces expression of a putative xylanase gene in *Salmonella enterica* subsp. *enterica* serovar Enteritidis mini-transposon*5 lac*Z1 mutants

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In this study, *Salmonella enterica* subsp. *enterica* serovar Enteritidis mini-transposon*5 lac*Z1 mutant strains induced under iron starvation were analyzed. Inverse polymerase chain reaction (IPCR) was used to isolate iron starvation-induced DNA fragments upstream of mini-Tn*5* (containing a promoter-less *lac*Z gene) insertion in *S. enterica* subsp. *enterica* serovar Enteritidis mutants showing β-galactosidase activity during growth in Fe^{3+}-deprived media. Out of ten mutant strains analyzed, four (Ez188, Ez477, Ez1819 and Ez2508) were induced during growth in the presence of the iron chelator, 2,2'-dipyridyl. IPCR products of Ez188 and Ez2508 were reamplified by nested PCR. Sequence analysis of the Ez188 PCR product revealed that a putative xylanase gene was induced under iron starvation conditions.

Key words: *Salmonella* mutants, iron starvation, induced, genes, putative xylanase.

INTRODUCTION

Salmonella are gram-negative bacteria in the family *Enterobacteriaceae*. They are ubiquitous pathogens found in humans and their livestock, wild mammals, reptiles, birds and even insects (Falkow and Mekalanos, 1990). *Salmonella* species causes gastroenteritis, septicaemia and enteric fever (Falkow and Mekalanos, 1990). *Salmonella enterica* subsp. *enterica* serovar Enteritidis causes many episodes of bacterial food contamination all over the world. *Salmonella*, like most other microorganisms, require iron as an essential element in a variety of metabolic and informational cellular pathways (Miethke and Marahiel, 2007). Iron is needed for support of biological processes such as transport and storage of oxygen, reduction of ribonucleotides and dinitrogen, activation and decomposition of peroxides and electron transport (Bagg and Neilands, 1987). More than 100 enzymes acting in primary and secondary metabolism possess iron-containing cofactors such as iron-sulfur clusters or heme groups (Miethke and Marahiel, 2007). Although iron is the fourth most abundant element in the earth's crust, it is largely unavailable to microorganisms because under aerobic conditions at neutral pH, Fe (II) is oxidised to Fe (III) forming an insoluble hydroxide (Neilands, 1982).

The human body contains 3 - 5 g of iron but the element is bound intracellularly in haemoglobin, heme, ferritin and hemosiderin and extracellularly to transferrin and lactoferrin (Payne, 1998). The average amount of free iron in human serum is estimated at 10^{-24} M (Raymond et al., 2003); a concentration far less than the 0.06 µg/ml minimum required for survival of *Salmonella* species (Payne, 1988). When the intracellular iron concentration drops below the threshold of about 10^{-6} M (which is critical for microbial growth), siderophores are produced to scavenge for iron (Dertz and Raymond, 2003, 2004). Most pathogens possess an arsenal of iron acquisition systems which provide an advantage for microbial multiplication in different compartments with changing iron source composition and pH conditions (Rouault, 2006). The host uses several mechanisms to withhold iron including several iron-binding proteins such as ovalbumin, lactoferrin, ferritin and transferring (Rouault, 2006). The low iron concentration in the host makes *Salmonella* increase the expression of siderophores and virulence determinants (Litwin and Calderwood, 1993). Thus, the ability of *Salmonella* species to acquire iron in the host is

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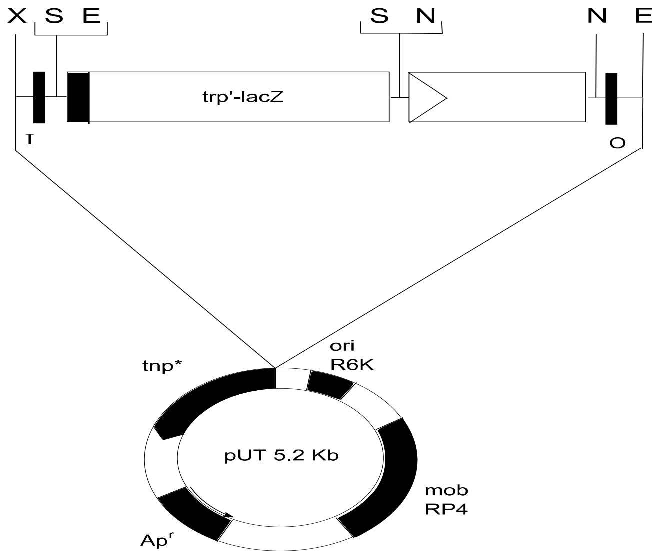

Figure 1. Organization of the promoter-probe minitransposon (mini-Tn*5lacZ*1) with its vector delivery plasmid (pUT/km). Details concerning the construction of pUT/km were described by Herrero et al. (1990). Adapted from de Lerenzo et al. (1990) and Herrero et al. (1990). Restriction sites: X, *Xba*I; S, *Sfi*I, E, *Eco*RI; N, *Not*I.

an essential adaptive component in pathogenesis (Litwin and Calderwood, 1993; Miethke and Marahiel, 2007).

Since the competition for iron is an important adaptive response that influences the outcome of the host-*Salmonella* interaction and pathogenesis, characterisation of iron starvation-induced genes would help elucidate the molecular basis underlying the induction of *Salmonella* genes during host infection. Iron starvation-induced proteins in *Salmonella* are also strong immunogens.

During the acute-phase reaction occurring upon inflammation, iron storage is enhanced by an interleukin-1- and tumor necrosis factor-dependent increase of macrophage ferritin pool (Jurado, 1997). During infection, the concentration of lactoferrin receptors on macrophages is also increased (Jurado, 1997). Given that the hypoferremic response and the development of siderophore binding proteins are an intriguing example of pathogen-directed host defense system (Jurado, 1997), isolation of iron starvation-induced promoters or their genes can yield practical benefits in the form of novel vaccines and treatments.

Salmonellae survive in phagocytic cells including macrophages. Thus iron starvation-induced promoters or genes may lead to the development of recombinant vaccines that produce antigens during host infection only; this can prevent problems associated with production of vaccines in culture (Chinsembu, 1996a, 1996b). There-fore, the aim of this study was to identify some of the *S. enterica* subsp. *enterica* serovar Enteritidis (*S. enteritidis*) genes induced under iron starvation conditions.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions

S. enterica subsp. *enterica* serovar Enteritidis mini-transposon*5 lac*Z1 (mini-Tn5 containing the modified β-galactosidase reporter gene, *lac*Z1) mutants were used in this study. The mini-Tn5*lac*Z1 construct with its suicide vector delivery plasmid are shown in Figure 1. The mini-transposon contained a promoter-less *lacZ* gene. Construction of the vector was done according to Herrero et al. (1990) and that of *S. enterica* subsp. *enterica* serovar Enteritidis mini-Tn5*lac*Z1 mutants was done according to de Lorenzo et al. (1990). The eleven mutants were denoted as: Ez766, Ez033, Ez149, Ez188, Ez477, Ez1594, Ez1819, Ez1987, Ez2169, Ez2278 and Ez2508. The *lac*Z1 gene fusion in Ez766 was constitutively expressed; therefore Ez766 was used as a positive control. A *S. enterica* subsp. *enterica* serovar Enteritidis natural (wild type) isolate, 76 Sa88, was used as a negative control. Bacterial strains were streaked on Luria-Bertani (LB) medium (10 g/l bacto-tryptone, 5 g/l bacto-yeast extract, 10 g/l NaCl, pH adjusted to 7.4) containing 20 g/l bacto-agar. Overnight bacterial cultures were grown at 37°C from single colonies in LB broth.

Screening assays

The strategy used to isolate iron starvation-induced DNA fragments from *S. enterica* subsp. *enterica* serovar Enteritidis mutants is shown in Figure 2. *S. enterica* subsp. *enterica* serovar Enteritidis mini-Tn5*lac*Z1 mutants including the negative (wild type) and positive (Ez766) controls were screened for the expression of β-galactosidase during growth on iron-deprived medium. Iron (III) was removed from LB-agar medium by adding 2,2'-dipyridyl to 2 mM final concentration. 100 µl of 250 mg/ml 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (Xgal, 250 mg/ml in dimethyl formamide) were also added to the medium and mixed gently before pouring onto plates.

LB-gar and LB-agar/Xgal plates without 2,2'-dipyridyl were included as controls. Each mutant liquid culture (75 µl) was added to 75 µl of 80% glycerol in microtiter plate wells and transferred onto media using a sterilised replicator fork. Plates were allowed to dry for 5 min and incubated at 37°C overnight. Iron starvation-induced β-galactosidase expression was scored on the basis of colour: deep blue colonies were strongly induced (+++), blue colonies were moderately induced (++), pale blue colonies were weakly induced (+) and white colonies were not induced (-). Screening tests were carried out in triplicate and 4 independent assays were done. Only mutants that produced deep blue colonies were analysed further.

*Taq*I digestion and Southern blotting

Total genomic DNA (5 µg) isolated from Ez188, Ez477, Ez1819, Ez2508 and *S. enterica* subsp. *enterica* serovar Enteritidis wild type (negative control) strains was digested with 20 units of *Taq*I restriction endonuclease at 65°C for 2.5 h. Digested DNA (4 µg) was run on 0.8% agarose gel until all smaller fragments were (less than 800 bp) migrated into the electrophoresis buffer. Separated DNA fragments were blotted onto 0.45 µm pore size Hybond-N membranes (Amersham Biosciences). Membranes were prehybridised with 1 mg/ml of Herring sperm DNA and hybridised at 65°C overnight with a probe of 25 ng of pUC18 DNA radio-labelled with [α^{32}P]dCTP (Amersham Biosciences). Hybridised filters were exposed to medical X-ray film (Fuji Photofilm, Japan) for 2 h at -70°C with an amplifying screen.

Plasmid pUC18 contained *lac*Z sequences hence it was used as

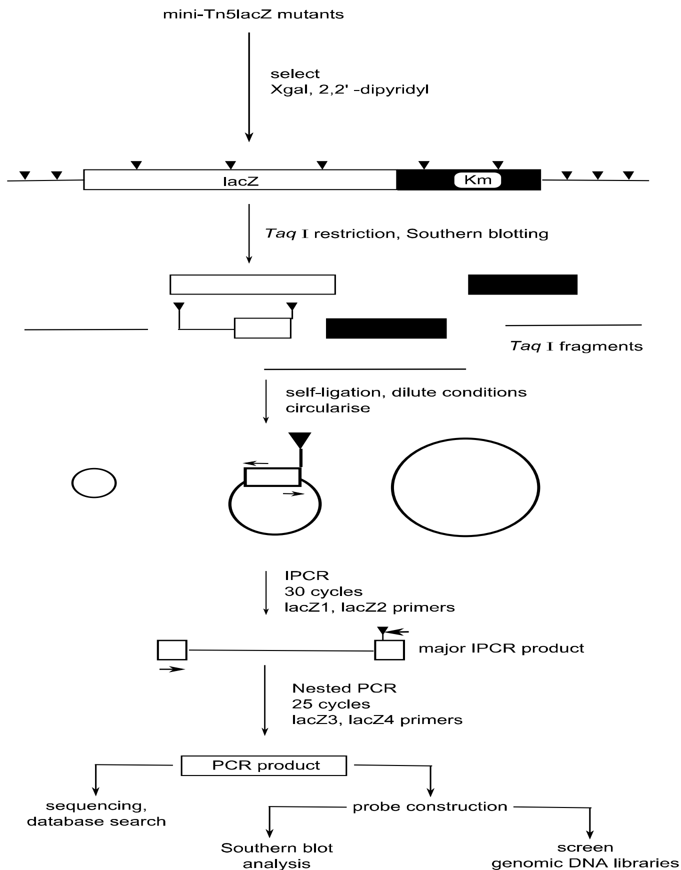

Figure 2. Flow chart showing the strategy used to analyse *S. enterica* subsp. *enterica* serovar Enteritidis mini-Tn*5lac*Z1 mutants. Mutant genomic DNA upstream of the lacZ region (arrowed box in circle) was amplified by IPCR and reamplified by nested PCR. The number and location of of TaqI restriction sites (black pointers) is hypothetical.

a probe to identify *Taq*I-digested fragments flanking the mini-transposon. *S. enterica* subsp. *enterica* serovar Enteritidis wild type did not contain sequences homologous to *lac*Z1 and *lac*Z2 primers and thereby allowed identification of *S. enterica* subsp. *enterica* serovar Enteritidis mini-Tn5 IPCR amplicons since such fragments were absent in the wild type control.

Self-ligation of *Taq*I-digested DNA

*Taq*I digested DNA (250 ng) of Ez188, EZ477, Ez1819, Ez2508, and *S. enterica* subsp. *enterica* serovar Enteritidis wild type (negative control) was self-ligated under dilute conditions in a volume of 50 µl (2 µl DNA [250 ng], 5 µl ligase buffer, 41 µl of sterile distilled water, and 5 µl [20 units] of T4 DNA ligase) at 12 - 14°C overnight.

Inverse polymerase chain reaction

Self-ligated DNA for Ez188, Ez477, Ez1819 and Ez2508 was amplified by inverse polymerase chain reaction (IPCR) using lacZ1 (5'-GGAATTCAAAGCGCCATTCGCCATTCAG-3') and lacZ2 (5'-GGAAGCTTATGGCAGGGTGAAAGCAGG-3') primers. The IPCR mixture was as follows: 5 µl of 10× PCR buffer, 8 µl of deoxynucleotide triphosphates (NTPs, 1.25 mM), 1.5 µl of lacZ1 (20 mM), 1.5 µl of lacZ2 (20 mM), 24 µl of sterile distilled water, 10 µl of DNA template (50 ng), and 0.5 units of DNA polymerase. Amplification was done in the Perkin Elmer Gene Amp PCR system 9600 thermocycler in two stages: 94°C (denaturation), 53°C (annealing), and 72°C (extension) for three cycles of one minute each and a further 35 cycles of 30 s each. IPCR products (20 µl) were run on 0.8% agarose gel. Correct IPCR products flanking mini-Tn5 sequences were excised from gel and purified using the Jetsorb kit (Genomed).

Reamplification of IPCR products, sequencing, and database analysis

IPCR products (Ez188 and Ez2508) purified from gel were reamplified by nested PCR using lacZ3 (5'-CTAGACGTTTCCCAGTCACGAC-3') and lacZ4 (5'-GCGGATCCTTTCGGCGGTGAAATTATCG-3') primers. The PCR reaction mixture consisted of 2 µl of template DNA, 5 µl of 10 × PCR buffer, 8 µl of dNTPs (1.25 mM), 1.5 µl of lacZ3 (20 mM), 1.5 µl of lacZ4 (20 mM), 32 µl of sterile distilled water, and 0.5 units of DNA polymerase. Twenty five cycles (denaturation, 94°C / 20 s; annealing, 53°C / 20 s; extension, 72°C / 1 min) were performed using programmes 64 - 65 in the thermocycler described above. Amplicons were purified from gel, stored at -20°C and only Ez188 was later sequenced. Database search was done by using the BLASTN 2.2.23+ network service (Altschul et al., 1990). The search parameters were: program (blastn); word size (28); expect value (10); hitlist size (100); match/mismatch scores (1- 2); gapcosts (0, 0); low complexity filter (yes); filter string (L;m;), and genetic code (1).

RESULTS

Table 1 shows the induction of β-galactosidase activity in *S. enterica* subsp. *enterica* serovar Enteritidis mini-Tn*5lac*Z1 mutants cultured in iron-deprived medium. The results indicate that β-galactosidase expression in four *S. enterica* subsp. *enterica* serovar Enteritidis mutants (Ez188, Ez477, Ez1819 and Ez2508) was induced during growth in iron-deprived medium as demonstrated by the deep blue colonies. Ez033, Ez149 and Ez2278 produced pale blue colonies in LB-broth-Xgal. This showed that these mutants were weakly induced even in the presence of iron (III). Mutants that were weakly induced and non-induced in LB broth-dipyridyl-Xgal were not analysed further.

In order to identify *S. enterica* subsp. *enterica* serovar Enteritidis genomic DNA fragments containing the miniTn*5lac*Z1 construct, radio-labelled pUC18 DNA was hybridised to *Taq*I digested DNA in Southern analysis. Based on Southern blot results (Table 2), the 1.1 kb (Ez188), 1.0 kb (Ez2508), and 0.9 kb (Ez1819) fragments were selected as the correct IPCR products upstream of mini-Tn*5lac*Z1 (Figure 3). Ez477 DNA was difficult to amplify by IPCR. The 1.1 kb Ez188 and 1.0 kb Ez2508 IPCR amplicons were isolated from gel and reamplified by nested PCR (Figure 4). BLAST nucleotide results showed

Table 1. Iron starvation induced β-galactosidase expression in *S. enterica* subsp. *enterica* serovar Enteritidis mutants.

Bacterial strain	Iron starvation-induced β-galactosidase activity score		
	LB-dipyridyl-Xgal	LB-Xgal	LB
S. enterica subsp. *enterica* serovar Enteritidis wild type	-	-	-
Ez766	+++	+++	-
Ez033	+	+	-
Ez149	+	+	-
Ez188	+++	-	-
Ez477	++	-	-
Ez1594	+	-	-
Ez1819	+++	-	-
Ez1987	+	-	-
Ez2169	+	-	-
Ez2278	+	-	-
Ez2508	+++	-	-

+++ deep blue colonies; ++ moderately blue coloies; + pale blue colonies; - white colonies. Scores were taken after overnight incubation. Ez766 and *S. enterica* subsp. *enterica* serovar Enteritidis wild type were included as positive and negative controls, respectively.

Table 2. Sizes of expected IPCR fragments versus observed sizes in Southern and IPCR analyses.

Mutant strains	Molecular sizes of fragments in base pairs		
	Southern blot	Observed IPCR	Expected IPCR
Ez188	1,800	1,100	1,210
Ez477	2,500	*	1,910
Ez1819	1,600	900	1,010
Ez2508	1,700	1,000	1,110

Based on the length of fragments obtained in Southern analysis, sizes of expected IPCR products were calculated by subtracting 590 bp (unamplified 'core' region between lacZ1 and lacZ2 sequences) (Herrero et al., 1990; de Lorenzo et al., 1990) from corresponding fragments identified in Southern hybridisation. Observed and expected sizes of IPCR products were not exactly the same due to problems with resolution of fragments on gel. *Not amplified.

that Ez188 sequences had 96% similarity to a putative xylanase coding region of *S. enterica* subsp. *enterica* serovar Typhimurium strain LT2 (Figure 5).

DISCUSSION

The transposon Tn*5* is a composite element in which inverted repeats of the mobile insertion element IS*50* bracket a segment containing genes for resistance to antibiotics (De Bruijm and Lupski, 1984). It has been greatly utilised in the insertion mutagenesis of many gram-negative bacteria because of its advantages (Sasakawa and Yoshikawa, 1987; Simon et al., 1989). In order to simplify the generation of insertion mutants, a series of Tn*5*-derived mini-transposons were constructed by de Lorenzo et al. (1990). The mini-Tn*5lac*Z1 construct used for the generation of *S. enterica* subsp. *enterica* serovar Enteritidis mutants contained a promoterless β-galactosidase reporter gene and formed operon fusions (Hererro et al., 1990). The enzyme β-galactosidase converted Xgal into a deep blue precipitate that was highly visible for scoring. Therefore, expression of β-galactosidase during growth in media containing the Fe^{3+} chelator (2,2'-dipyridyl) and Xgal suggested the existence of Fe^{3+} starvation-inducible promoter(s) upstream of the mini-transposon.

The strains Ez033, Ez149, Ez1819 and Ez2278 produced pale blue colonies on LB-Xgal without 2,2'-dipyridyl. These pale blue colonies were probably due to basal β-galactosidase induction. Ez188, Ez1819 and Ez2508 showed more β-galactosidase activity than Ez477. Increased activity in some mutants may have been due to increased transcription attributable to a stronger promoter. However, the same effect may mean that the intervening DNA region between the promoter and the *lac*Z gene may be shorter.

Since the 1990s, mutant fusions of *lac*Z and 2,2-dipyridyl have been widely employed in the analysis of iron regulated genes (Hassan and Sun, 1992; Postle,

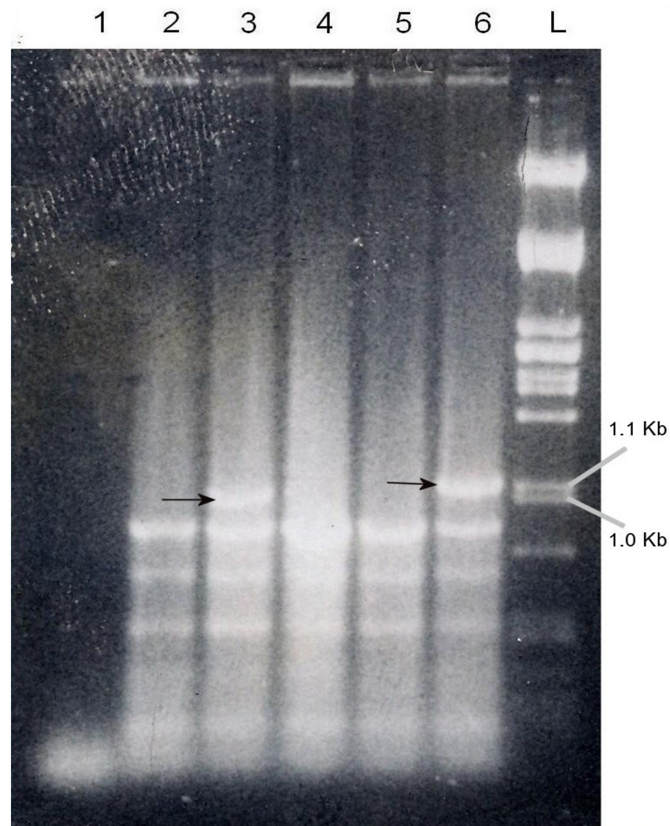

Figure 3. Agarose gel electrophoresis of IPCR products. Lane 1, IPCR no DNA control; lane 2, wild type DNA (negative control); lane 3, Ez 2508; lane 4, Ez1819; lane 5, Ez477; lane 6, Ez188; lane 7, 1 µg of λ/*Pst*I DNA ladder (14.3 to 0.072 kb size range).

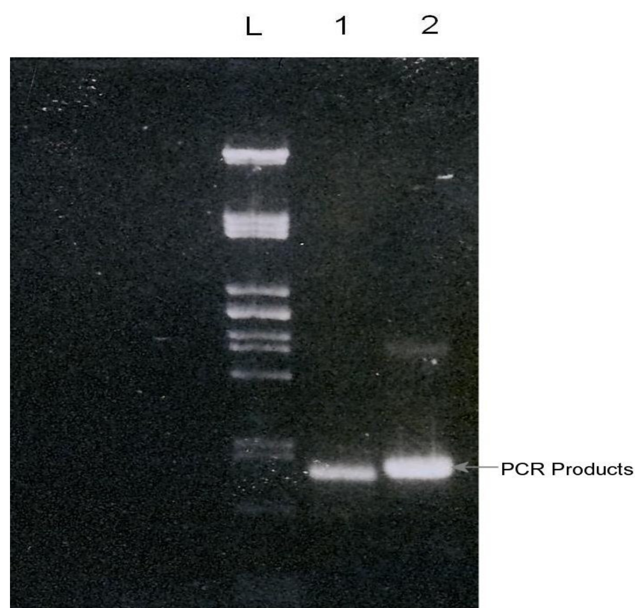

Figure 4. Agarose gel electrophoresis of PCR products. Lane 1, 1 µg of λ/*Pst*I DNA ladder (14.3 to 0.072 kb size range); lane 2, Ez2508; lane 3, Ez188.

1990). Further, IPCR is the method of choice for amplification of unknown DNA sequences flanking a core region of known sequence (Triglia et al., 1988). The most important step in preparing the DNA for IPCR is the ligation reaction. DNA fragments are ligated in such a way that they form monomeric circles (Figure 2). The formation of circular templates is formed under dilute conditions whereby the total DNA fragment concentration is less than the local concentration of the one terminus in the neighbourhood of another on the same molecule (Dugaiczyk et al., 1975).

Iron starvation-inducible genes are under the regulation of the 17 kDa ferric uptake regulation (Fur) repressor. In *Salmonella*, Fur exerts control over a series of genes involved in the synthesis, excretion and recovery of the siderophore enterochelin (Hall and Foster, 1996).

An active Fur repressor (containing Fe^{2+}) also prevents RNA polymerase from binding to promoters of iron starvation-induced genes (Litwin and Calderwood, 1993). Thus, in order to allow transcription of iron regulated genes, 2,2'-dipyridyl inactivates the Fur-Fe^{2+} complex (Hassan and Sun, 1992; Hall and Foster, 1996). The chemical 2,2'-dipyridyl was also shown to induce anaerobic expression of a gene encoding an isozymic form of superoxide dismutase (Hassan and Sun, 1992; Dubrac and Touati, 2000). This suggests that regulation of iron starvation-induced genes by fur could be essential for biological defense against O_2 radicals and toxicity in phagocytes. In contrast, some bacterial genes such as the one encoding iron superoxide dismutase are positively regulated by Fur (Dubrac and Touati, 2000). Fur has pleitropic effects on *Salmonella* gene expression and cellular physiology (Miethke and Marahiel, 2007), but an alternative sigma factor, σ^{54}, was also shown to be responsible for recruiting core RNA polymerase during growth under low iron conditions (Cullen et al., 1994).

Our results showed that a putative xylanase gene (McClelland et al., 2001) in *S. enterica* subsp. *enterica* serovar Enteritidis mutants was induced during iron starvation conditions. Xylanases (endo-1,4-β-xylan xylanohydrolase) are *O*-glycoside hydrolases that catalyze the random hydrolysis of internal β-1,4-D-xylosidic linkages of xylan, a major component of hemicellulose found in wood, thus making these enzymes very biotechnologically significant in the paper industry (Collins et al., 2002). Xylanases are found in several fungal and bacterial species including *Salmonella*, *Escherichia coli* and *Pseudomonas* (Collins et al., 2002).

Xylanase genes were reported to be under a pH regulatory system (Eisendle et al., 2004). Iron starvation-induced genes such as those involved in siderophore biosynthesis and uptake are also governed by both iron and pH control (Eisendle et al., 2004). It was suggested that because uptake of siderophores required contransport with protons (Winkelmann, 2001); there was cross-talk between siderophore metabolism and pH sensing (Eisendle et al., 2004). Our results also suggest that xylanase genes

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Query  7        GTCCCGGGATAGTCTCCCTTTCTGATGGACAATATGCATTAACGAGCGGGCAAACAGGTA  66
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  3292067  GTCCCGGGATAGTCTCCCTTTCTGATGGACAATATGCATTAACGAGCGGGCAAACAGGTA  3292126

Query  67       ACTTCTGCCCGGGTAATCCGTTACCCGACTTGATATTAACATTGTCATGAGGTGATATCA  126
                |||||||||||||||||||||||||||||| |||||||||||||||||||||||||||||
Sbjct  3292127  ACTTCTGCCCGGGTAATCCGTTACCCGACT-GATATTAACATTGTCATGAGGTGATATCA  3292185

Query  127      TTTG---GGCCAG--ATAATCATCCGGAACCGGCGC  157
                ||||   ||||||  |||||||||||||||||||||
Sbjct  3292186  TTTGCCCGGCCAGCCATAATCATCCGGAACCGGCGC  3292221
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Figure 5. BLAST alignment results of query nucleotide sequences (Ez188 in red) and database sequences. Ez188 sequences reveal 96% similarity to a putative xylanase gene.

may be part of the molecular cross-talk network between iron starvation-induced genes and pH regulated genes. Previous observations that the role of fur in cellular physiology extends beyond that of regulatory iron utilization (Hall and Foster, 1996) also lend credence to suggestions of a cross-talk between siderophore metabolism and pH sensing. Fur was also found to regulate the acid tolerance response (ATR) in *Salmonella* (Hall and Foster, 1996).

Xylanase genes were also linked to the uptake of iron-siderophores in the phytopathogenic bacterium *Xylella fastidiosa* (Blanvillain et al., 2007). Bacterial uptake of iron-siderophore complexes depends on ABC-type transporters and in the case of gram negative bacteria, outer membrane receptors are the first gate of iron-siderophore recognition (Miethke and Marahiel, 2007). Energy for transport of iron-siderophore complexes is supplied by the TonB complex which acts as a transducer of a proton motive force (Miethke and Marahiel, 2007). TonB-dependent receptors (TBDRs) located in the outer membrane of gram negative bacteria are mainly known to transport iron-siderophore complexes into the periplasm (Blanvillain et al., 2007).

Interestingly, a carbohydrate utilization locus containing a TBDR ("CUT locus") is also involved in expression of xylanases (Blanvillain et al., 2007). The expression of genes encoding TBDRs involved in iron transport is regulated under iron depletion conditions and repressed under iron repletion conditions via the fur repressor (Blanvillain et al., 2007). In *X. fastidiosa*, two genes encoding enzymes putatively involved in xylan degradation were induced during heat stress (Koide et al., 2006). Many of the heat shock proteins were also induced under various environmental stress conditions such as nutrient starvation and changes in osmolarity or pH of the medium (Koide et al., 2006). Heat shock causes low oxygen pressure that leads to ferrous ion transport. Taken together, the induction of xylanases during iron starvation conditions is therefore well supported by previous studies. Xylan is present in agar from seaweeds (Collins et al., 2002). Therefore, it is plausible that in times of iron starvation, *Salmonella* may turn to xylan foraging as a source of carbon.

Conclusion

This study identified mini-Tn5lacZ1 *S. enterica* subsp. *enterica* serovar Enteritidis mutant fusions induced under iron deprivation conditions. IPCR enabled the amplification of genomic DNA upstream of the mini-Tn5 insertion in some mutants. Sequencing of nested PCR products revealed that iron starvation conditions induced a putative xylanase gene in *S. enterica* subsp. *enterica* serovar Enteritidis miniTn*5lac*Z1 mutants. Future studies will focus on the characterization of the xylanase gene and protein and how this xylanase gene possibly cross-talks with other genes that regulate responses to stresses such as pH and heat shock. Proteomic studies will also help to elucidate how whole biological systems with interconnected pathways respond to myriad environmental stimuli.

REFERENCES

Altschul SF, Gish W, Miller W, Meyers EW, Lipman DJ (1990). Basic local alignment search tool. J. Mol. Biol., 215: 403-410.

Bagg A, Neilands JB (1987). Ferric uptake regulation protein acts as a repressor, employing iron (II) as a cofactor to bind the operator of an iron transport operon in Escherichia coli. Biochemistry, 26: 5471-5477.

Blanvillain S, Meyer D, Boulanger A, Lautier M, Guynet C, Denance N, Vasse J, Lauber E, Arlat M (2007). Plant carbohydrate scavenging through TonB-dependent receptors: a feature shared by phytopathogenic and aquatic bacteria. PLos One 2: e224. Doi:10.1371/journal.pone.0000224.

Chinsembu KC (1996a). Molecular mechanisms of *Salmonella* pathogenicity: Review of recent advances. UNZA J. Sci. Technol., 1: 57-67.

Chinsembu KC (1996b). Iron and *Salmonella*: towards a new breed of vaccines. Zambian J. Med. Health Sci., 1: 2-4.

Collins T, Meuwis MA, Stals I, Claeyssens M, Feller G, Gerday C

(2002). A novel family 8 xylanase, functional and physicochemical characterization. J. Biol. Chem., 277: 35133-35139.
Cullen PJ, Foster-Hartnett D, Gabbert KK, Kranz RG (1994). Structure and expression of the alternative sigma factor, RpoN, in *Rhodobacter capsulatus*; physiological relevance of an autoactivated *nifU2-rpoN* super-operon. Mol. Microbiol., 11: 51-65.
De Bruijm FJ, Lupski JR (1984). The use of transposon *Tn*5 mutagenesis in the rapid generation of correlated physical and genetic maps of DNA segments cloned into multicopy plasmids-review. Gene, 27: 131-149.
de Lorenzo V, Herrero M, Jakubzik U, Timmis NK (1990). Mini-*Tn*5 transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in gram negative Eubacteria. J. Bacteriol., 172: 6568-6572.
Dertz EA, Raymond KN (2003). Siderophore and transferrins, pp. 141-168. In L Que, Jr, Tolman WE (eds) Comprehensive coordination chemistry II, vol.8. Elsevier Ltd, Philadelphia, PA.
Dertz EA, Raymond KN (2004). Biochemical and physical properties of siderophores, pp. 3-17. In JH Crosa, AR Mey, SM Payne (eds) Iron transport in bacteria. ASM Press, Washington, DC.
Dubrac S, Touati D (2000). Fur positive regulation of iron superoxide dismutase in Escherichia coli: functional analysis of the *sodB* promoter. J. Bacteriol., 182: 3802-3808.
Dugaiczyk A, Boyer HW, Goodman H (1995). Ligation of EcoRI endonuclease-generated DNA fragments into linear and circular structures. J. Mol. Biol., 96: 171-184.
Eisendle M, Oberegger H, Buttinger R, Illmer P, Haas H (2004). Biosynthesis and uptake of siderophores is controlled by the PacC-mediated ambient-pH regulatory system in *Aspergillus nidulans*. Eukaryotic Cell, 3(2): 561-563.
Falkow S, Mekalanos J (1990). The enteric Bacilli and Vibrios. In: Microbiology, BD Davis, R Dulbecco, HN Eisen and HS Ginsberg (eds), Lippincott Co., Philadelphia, pp. 561-587.
Hall HK, Foster JW (1996). The role of fur in the acid tolerance response of Salmonella typhimurium is biologically and genetically separable from its role in iron acquisition. J, Bacteriol., 178: 5683-5691.
Hassan H, Sun HC (1992). Regulatory roles of Fnr, Fur and Arc in expression of manganese-containing superoxide dismutase in Escherichia coli. PNAS USA 89: 3217-3221.
Herrero M, de Lorenzo V, Timmis NK (1990). Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram negative bacteria. J. Bacteriol., 172: 6557- 6567.
Jurado RL (1997). Iron, infections, and anaemia of inflammation. Clin. Infect. Dis., 25: 888-895.
Koide T, Vencio RZN, Gomes SL (2006). Global gene expression analysis of the heat shock response in the phytopathogen *Xylella fastidiosa*. J, Bacteriol., 188: 5821-5830.
Litwin CM, Calderwood SB (1993). Role of iron in regulation of virulence genes. Clin. Microbiol. Rev., 6: 137-149.
McClelland M, Sanderson KE, Speith J, Clifton SW, Latreille P, Courtney L, Porwollik S, Ali J (2001). Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature, 413: 852-856.
Miethke M, Marahiel MA (2007). Siderophore-based iron acquisition and pathogen control. Microbiol. Mole. Biol. Rev., 71(3): 413-451.
Neilands JB (1982). Microbial envelope proteins related to iron. Ann. Rev. Microbiol., 36: 285-309.
Payne SM (1988). Iron and virulence in the family Enterobacteriaceae. Crit. Rev. Microbiol., 16: 81-111.
Postle K (1990). Aerobic regulation of the *Escherichia coli* tonB gene by changes in iron availability and the fur locus. J. Bacteriol., 172: 2287-2293.
Raymond KN, Dertz EA, Kim SS (2003). Enterobactin: an archetype for microbial iron transport. PNAS USA 100: 3584-3588.
Rouault TA (2006). The role of iron regulatory proteins in mammalian iron homeostasis and disease. Nat. Chem. Biol., 2: 406-414.
Sasakawa C, Yoshikawa M (1987). A series of Tn5 variant drug-resistant markers and suicide vectors for transposon mutagenesis. Gene, 56: 283-288.
Simon R, Quandt J, Klipp W (1989). New derivatives of transposon Tn5 suitable for immobilisation of replicons, generation of operon fusions and induction of genes in gram negative bacteria. Gene, 80: 161-169.
Triglia T, Peterson MG, Kemp DT (1988). A procedure for in vitro amplification of DNA segments that lie outside the boundaries of known sequences. Nucl. Acids Res., 16: 8186.
Winkelmann G (2001). Siderophore transport in fungi, In G Winkelmann (ed) Microbial transport systems. Wiley-VCH, Weinheim, Germany. pp. 463-479.