African Journal of Microbiology Research Vol. 7(10), pp. 905-908, 5 March, 2013
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.622
ISSN 1996-0808 ©2013 Academic Journals

Short Communication

Lack of *seqA* and *dam* genes induces the formation of cyclic fatty acids in *Salmonella typhimurium* at the entry of stationary phase

Chatti Abdelwaheb[1]*, Maalej Lobna[1], Ben Ghorbel Kloula Selma[1] and Landoulsi Ahmed[2]

[1]Laboratoire de Traitement des Eaux Usées, Centre des Recherches et des Technologies des Eaux (CERTE), Technopole Borj Cédria, BP 273- Soliman 8020.
[2]Unité de Biochimie des Lipides et interaction des macromolécules en Biologie, 03/UR/0902. Laboratoire de Biochimie et Biologie Moléculaire, Faculté des Sciences de Bizerte, Zarzouna. ; Université de Carthage.

Accepted 6 February, 2013

Membrane fatty acid composition of *dam* and/or *seqA* mutants of *Salmonella typhimurium* was investigated for exponential and stationary phase cells. Our results show that by reaching the stationary phase, the lack of *dam* and *seqA* genes enhanced the production of cyclic fatty acids (CFA), whereas the wild-type cells produced CFAs normally. Data from this study suggest that the membrane fatty acid composition of these mutants is phase-dependent.

Key words: Fatty acids, *Salmonella*, *seqA*, *dam,* growth phase.

INTRODUCTION

The DNA methylation affects several physiological parameters such as the regulation of chromosome replication, DNA segregation, mismatch repair, transposition and transcriptional regulation (Chatti and Landoulsi, 2008). Dam protein regulates gene expression and virulence of *Salmonella typhimurium* (Heithoff, 1999). Previous works confirmed that the lack of the *dam* gene attenuated its virulence in a mouse model (Heithoff et al., 1999). Moreover, *dam* deletion decreased both adhesion and invasion of cultured epithelial cells. Recently, it was demonstrated that Dam protein of *Salmonella enteritidis* affects LPS components and the expression of *std* fimbriae. Jakomin et al. (2008) proved that *std* operon is regulated by SeqA protein. Our previous results have demonstrated that lack of *seqA* attenuates virulence of *S. typhimurium* in the mouse model (Chatti et al., 2007). The *dam* mutation affects also the transcription of genes involved in environmental stress response (Oshima et al., 2002). Dam protein regulates the expression of various genes implicated in lipid (*accC, fabB*) and phosphatidic acid (*gpsA*) biosynthesis. Previous studies have shown that the *dam* and *seqA* alter the membrane fatty acid composition in both *Escherichia coli* (Daghfous et al., 2006) and *Salmonella typhimurium*.

The membrane is the first barrier between bacteria and all living cells and the environment. Several works have demonstrated that the membrane structure changes by growth conditions such as growth temperature (Diefenbach et al., 1992), pH (Russell et al., 1995) and carbon source. These factors can change membrane fluidity and lipid composition of bacteria. An effective way for this is to modify the ratio of saturated/unsaturated fatty acids of the phospholipids (Shinitzky, 1984). It was demonstrated that the value of the ratio SFAs/MUFAs of stationary phase was about two-fold higher as compared to an early log phase culture of *Corynebaterium* sp. and *Sphingomonas* sp. (Syakti et al., 2006).

Previous studies have indicated that cyclic fatty acids (CFA) synthase enhances C17 and C19 CFAs in the stationary phase (Kim et al., 2005). In *E. coli*, it has been suggested that the initiation of CFA synthesis at the entry

*Corresponding author. E-mail: chattiabdel@yahoo.fr. Tel: 216 79 325 199. Fax: 216 79 325 802.

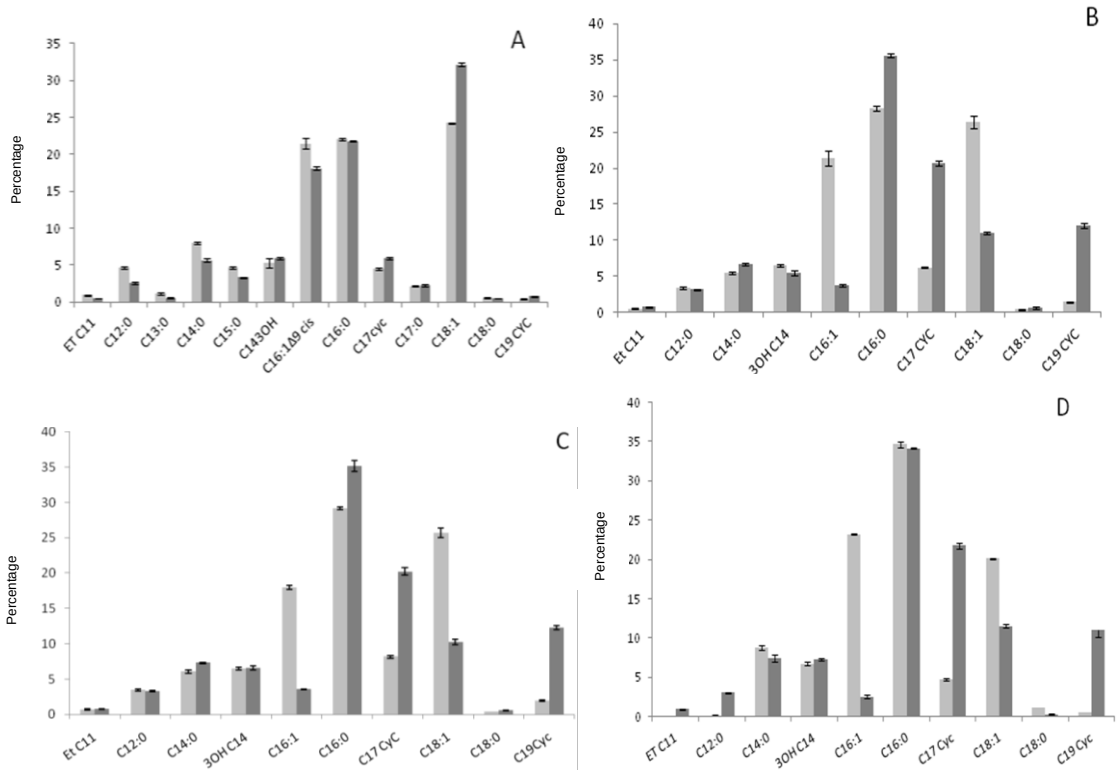

Figure 1. Analysis of the fatty acid composition of *S. typhimurium* (A), the *seqA* (B), *seqA/dam* (C) and *dam* (D) mutants at stationary and exponential growth phase.

to the stationary phase is due to the increased transcription of *cfa* (Eichel et al., 1999; Wang and Cronan, 1994). *RpoS* from *S. typhimurium* increases the CFA synthase level during the stationary phase (Kim et al., 2005).

In the present study, we investigated the fatty acid composition of both mid-log and stationary growth phase of *dam* and/or *seqA* mutants in *S. typhimurium*.

MATERIALS AND METHODS

Bacterial strains and culture conditions

S. typhimurium and its isogenic strains *dam*, *seqA* and *seqA/dam* were used in this study. Strains were kindly provided by Prof. Casadesus Josef. Bacteria were routinely cultured in nutrient broth at 37°C. Exponential and stationary phases corresponded to optical density (OD) of 0.5 and 1, respectively.

Bacterial irradiation

The bacterial suspension was centrifuged. The pellets were washed, then resuspended in 20 ml of NaCl (0.9%) and transferred to glass Petri dishes. Bacterial suspension was irradiated for the time desired. Measurements of incident intensity at the liquid surface were made with an Ultraviolet Products Vilbert-Lourmat digital radiometer. Dose expressed in $mW.s.cm^{-2}$ was calculated as the average incident intensity and was regulated by controlling the exposure time.

Fatty acid extraction

Cells were weighed and a direct methylation of the cellular fatty acids was performed using the MIDI System. 50 to 100 mg of biomass was transferred into a tube with a teflon-lined cap using 1 ml of MIDI Reagent 1 (NaOH 45 g, methanol 150 ml, deionized water 150 ml). After shacking, tubes were placed in a boiling water bath (98°C) for 5 min, shaken again and returned to the bath for 30 min. After cooling in a water bath, 4 ml of MIDI Reagent 2 (6 N hydrochloric acid 54.16 ml, methanol 45.83 ml) was added and the tube was sealed, vigorously shaken and heated at 80°C for 10 min. After cooling in a water bath, 1.5 ml of MIDI Reagent 3 (hexane 200 ml, methyl tert-butyl ether 200 ml) was added and the tubes vigorously shaken. Then, 2 ml of organic phase was transferred into another conical glass tube with Teflon cap and 6 ml of MIDI Reagent 4 (sodium hydroxide 10.8 g, deionized water 900 ml) was added and the tube vigorously shaken. 490 µl of the top phases were transferred to chromatographic vials and stored at -20°C until analysis.

RESULTS AND DISCUSSION

The total FA content of the wild-type strain *Salmonella typhimurium* was compared in both the exponential and stationary growth phases. Our results showed an increase in the level of C18:1 and a slight decrease of C16:1 was observed at the stationary phase (Figure 1A). Similar results have been reported by Kim et al. (2005). The proportion of the total CFAs of logarithmic and

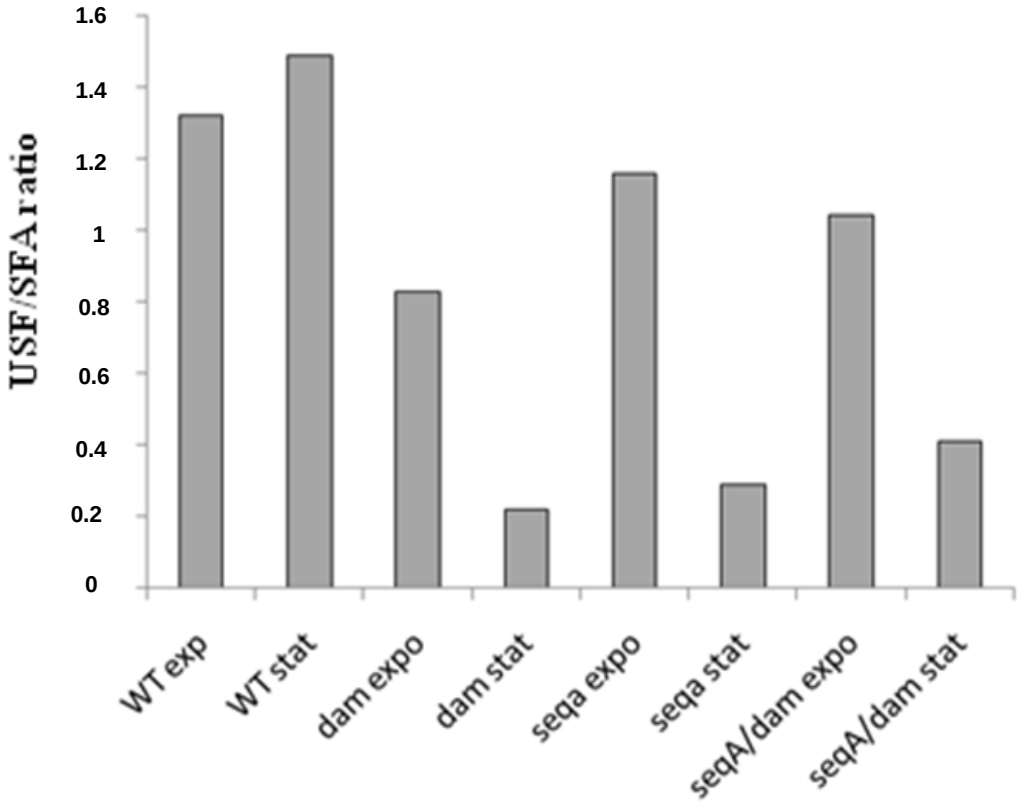

Figure 2. The USF/SFA ratios of *S. typhimurium* and its isogenic mutants *dam*, *seqA* and *dam/seqA* at exponential and stationary growth phase. WT: wild type; exp: exponential and stat: stationary.

stationary phase were about 6.91 and 8.79%, respectively. The analysis of the total CFAs showed a slight increase in both C17 and C19 by reaching the stationary phase. CFAs in *Salmonella* are growth phase-dependant in the membrane (Kim et al., 2005). The formation of CFA is a post-synthetic modification of the phospholipid bilayer occuring when the cells reach the stationary phase. The unsaturated fatty acids conversion to their cyclopropane derivatives protects bacteria from stress conditions (pH, temperature, etc.) (Brown et al., 1997).

In the exponential phase, the level of C17 and C19 CFAs was comparable between the wild-type and the *dam* mutant (Figure 1A and D). In the stationary phase, on the other hand, the *dam* mutant enhanced the production of both C19 and C17 CFAs, whereas the wild-type cells produced CFAs normally (Figure 1D). The increase in CFAs was accompanied by a decrease of C18:1 and C16:1. An increase in CFAs was also obtained for the *seqA* and *dam/seqA* mutants (Figure 1B and C). These results were accompanied by a decrease in both C16:1 and C18:1. These results indicate that *dam* and *seqA* mutations induced the formation of CFA at the entry of stationary phase (Figures 1B and D). C16 and C18 UFAs may be converted to the corresponding CFAs in *dam* and/or *seqA* mutants. Similar phenomenon was observed by Wang and Cronan (1994) in *E. coli* between WT and *rpoS* mutant. These authors have proved that this phenomenon is due to CFA synthase.

The ratios of UFA/SFA were calculated (Figure 2). This ratio showed an important decrease by reaching the stationary phase of *dam* and/or *seqA* mutants. However, this ratio was without change for the wild type strain. The increase of UFA/SFA ratio induces the fluidity of bacterial membranes (Casadei et al., 2002) and regulates their lipid composition (Teixeira et al., 2002).

REFERENCES

Bligh EG, Dyer WJ (1959). A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

Brown JL, Ross T, McMeekin TA, Nichols PD (1997). Acid habituation of *Escherichia coli* and the potential role of cyclopropane fatty acids in low pH tolerance. Int . J. Food Microbiol. 37:163-173.

Casadei MA, Manas P, Niven G, Needs E, Mackey BM (2002). Role of membrane fluidity in pressure resistance of *Escherichia coli* NCTC 8164. Appl. Environ. Microbiol. 68:5965-5972.

Chatti A, Daghfous D, and Landoulsi A (2007). Effect of *seqA* mutation on *Salmonella Typhimurium* virulence. J. Infect. 54:241-245.

Chatti A, Landoulsi A (2008). The DNA-methylation state regulates virulence and stress response of *Salmonella*. C. R. Biol. 331:648-654.

Daghfous D, Chatti A, Marzouk B, Landoulsi A (2006). Phospholipid changes in *seqA* and *dam* mutants of *Escherichia coli*. C. R. Biol. 329:271-276.

Diefenbach R, Heipieper HJ, Keweloh H (1992). The conversion of cis into trans-unsaturated fatty acids in Pseudomonas putida P8: evidence for a role in the regulation of membrane fluidity. Appl. Microbiol. Biotechnol. 38:382-387.

Eichel J, Chang Y-Y, Riesenberg D, Cronan JE Jr (1999). Effect of ppGpp on Escherichia coli cyclopropane fatty acid synthesis is mediated through the RpoS sigma factor. J. Bacteriol.181:572-576.

Heithoff DM, Sinsheimer RL, Low DA, Mahan MJ (1999). An essential role for DNA adenine methylation in bacterial virulence. Science 284:967-970.

Jakomin M, Chessa D, Baumler AJ, Casadesus J (2008). Regulation of the *Salmonella enterica std* Fimbrial Operon by DNA Adenine Methylation, SeqA, and HdfR. J. Bacteriol. 190:7406-7413.

Kim BH, Kim S, Kim HG, Lee J, Lee IS, Park YK (2005). The formation of cyclopropane fatty acids in Salmonella enterica serovar Typhimurium. Microbiol. 151:209-218.

Oshima T, Wada C, Kawagoe Y, Ara T, Maeda M, Masuda Y, Hiraga S, Mori H (2002). Genome-wide analysis of deoxyadenosine methyltransferase-mediated control of gene expression in *Escherichia coli*. Mol. Microbiol. 45:673-695.

Russell NJ, Evans RI, terSteeg PF, Hellemons J, Verheul A, Abee T (1995). Membranes as a target stress adaptation. Int . J. Food Microbiol. 28:255-261.

Seshasayee ASN (2007). An Assessment of the Role of DNA Adenine Methyltransferase on Gene Expression Regulation in E. coli. PLoS ONE 3:e273

Shinitzky M (1984). Membrane fluidity and cellular functions. In Physiology of Membrane Fluidity, pp.1-51[M Shinitzky, editor]. Boca Raton, FL:CRC Press, Inc.

Syakti AD, Mazzella N, Torre F, Acquaviva M, Gilewicz M, Guiliano M, Bertrand J-C, Doumenq P (2006). Influence of growth phase on the phospholipidic fatty acid composition of two marine bacterial strains in pure and mixed cultures. Res. Microbiol. 157:479-486.

Teixeira H, Gonçalves MG, Rozès N, Ramos A, San Romao MV (2002). Lactobacillic acid accumulation in the plasma membrane of Oenococcus oeni: a response to ethanol stress? Microb. Ecol. 43:146-153.

Wang AY, Cronan JE Jr (1994). The growth phase-dependent synthesis of cyclopropane fatty acids in *Escherichia coli* is the result of an RpoS (KatF)-dependent promoter plus enzyme instability. Mol. Microbiol. 11:1009-1017.