African Journal of Microbiology Research Vol. 4(16), pp. 1708-1712, 18 August, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2010 Academic Journals

Full Length Research Paper

The osmoprotective effect of L-carnitine factor on the bacterial growth of *Streptococcus agalactiae* and *Escherichia coli*

Maras Meltem[1]*, Akman Yildiz[2], Yula Erkan[3] and Gökmen Tülin[3]

[1]Department of Biology Education, Faculty of Education, Zonguldak Karaelmas University, 67300, Kdz. Eregli, Zonguldak, Turkey.
[2]Science and Art Center, Adana, Turkey.
[3]Department of Microbiology, Faculty of Medicine, Çukurova University, Adana, Turkey.

Accepted 23 June, 2010

In response to osmotic stress, gram positive bacteria *Streptococcus agalactiae* and enterobacteria *Escherichia coli* accumulates compatible solutes from the medium, stress response systems may enable bacteria to adapt cellular response and survival systems to a variety of stress conditions. *S. agalactiae* and *E. coli* strains have a transporter for carnitine that can respond to osmotic stress by regulating its own activity. Brain heart infusion (BHI) broth medium with 1 mM L-carnitine was the medium with the highest growth to be observed. The growth of the *E. coli* strain was faster than the growth of *S. agalactiae* strain. *S. agalactiae* and *E. coli* are known as bacteria being hard to grow at the laboratory tests for making clinical diagnosis. The medium with L-carnitine factor used in laboratory tests accelerates the bacterial growth.

Key words: Bacterial growth, carnitine, osmoprotectant, *Streptococcus agalactiae*, *Escherichia coli.*

INTRODUCTION

Carnitine has pleiotropic and beneficial effects in higher eukaryotic and beneficial effects in higher eucaryotic cells. Carnitine is available to both human and some animal species through de nove synthesis via a posttranslational modification of protein-bound lysine and is significant factor of exogenous food sources such as notable meat and dairy products (Woolard et al.,1999; Shirazi et al., 2007).

A role for carnitine as a stress protectant has been described in mammalian and bacterial systems. Studies in bacterial systems have revealed that carnitine shows a protective capacity as a compatible solute at osmotic stress conditions in procaryotes. (Rebouche and Seim, 1998; Ferreire et al., 2004; Franken et al., 2008). In this study, the possibility of similar functions in *Streptococcus agalactiae* and *Escherichia coli* was investigated. Adaptation mechanisms to physical environment of cells are not understood.

Osmolarity changes cannot be sensed via structure-specific ligant-receptor interactions that occur at specific resudies on receptor molecules (Racher et al., 2001). In general, bacterial survival under stress conditions requires rapid alterations in gene expression, controled by the association of different alternative sigma factors with RNA polymerase (Engeman et al., 2005; Okada et al., 2006). One of the seven types of σ subunits of RNA polymerase was resently proposed as a global regulator in the the osmoregulation network (Xizeng et al., 2010). The metabolism of L(-)- carnitine in *E. Coli* has been studied because of its implication in stress survival and anaerobic respiration, although its role is not totally understood. *E. coli* are able to metabolize carnitine but do not assimilate the carbon or nitrogen carnitine skeleton. In *E. coli*, the metabolization of L-carnitine is catalyzed by enzyme, L- carnitine dehydrotase. ATP-driven binding

*Corresponding author. E-mail: melmaras@karaelmas.edu.tr. Tel: +90 0372 319 19 50/109.

protein dependent transport systems are highly osmotically stimulated at the level of gene expression. It is involved in the transport of carnitine under high osmolarity (Bhuiyan and Seccombe, 1996; Canovas and Iborra, 2005; Bernal et al., 2007).

We examined the effect of the osmoprotective carnitine on *S. agalactiae* and *E. coli* under high-osmolarity conditions in a chemically defined medium.

MATERIALS AND METHODS

S. agalactiae strain and *E. coli* strain were used in the research. Brain heart infusion (BHI) broth medium were used for both cultures. The incubation of bacterial cells in BHI broth medium at 37°C was performed. *S. agalactiae* and *E. coli* bacteria, which grown in BHI broth medium, were washed for 3 times:

a) BHI broth saturated with 3% NaCl.
b) BHI broth suspensed with 2.5 cc (1 mM) L-carnitine.
c) BHI broth suspensed with 2.5 cc (1 mM) L-carnitine that contains 3% NaCl

From *S. agalactiae* and *E. coli* bacteria strains, 120 µl was added into test tubes and incubated at 37°C. The measurements of bacteria strains were performed in 600 nm. OD. The results of measurements were monitored in first 60 min, 6, 24 and 48 h.

Statistical differences between bacteria growth in BHI medium were assesed with one- samples *t* test at SPSS 13, with $p < 0.05$ considered statistically significant. The results were given as average ± standard deviation.

RESULTS

Recent studies on osmoregulated transport in some bacteria revealed an additional role of compatible solute transporters as a recovery system for carnitine, which leaks through the cytoplasmic membrane. *E. coli* cells are subjected to hyperosmotic shift with a membrane-impermanent osmolyte (Vermeulen and Kunte, 2004).

S. agalactiae and *E. coli* strains were incubated in BHI broth medium. These bacteria were washed in test tubes of BHI broth with 3% NaCl, BHI broth with 1mM L-carnitine, BHI broth suspensed with (1mM) L-carnitine that contained 3% NaCl. At the end of first 60 min, 6, 24 and 48 h, bacterial growths were measured in 600 nm.

Figure 1 shows the effect of osmoprotectants on the growth of the *E. coli* strain in (a) BHI broth (b) BHI broth with 3% NaCl (c) BHI broth with carnitine (d) BHI broth with carnitine and 3% NaCl.

In BHI broth without L- carnitine; no effect was observed towards the growth stimulation of *S. agalactiae* and *E. coli* bacteria (Figures 1 and 2a). In the BHI broth medium saturated with 3% NaCl without L- carnitine, NaCl was observed to be an inhibitory effect on the indicator types of *S. agalactiae* and *E. coli* strains (Figures 1 and 2b).

Figure 2 shows the effect of osmoprotectants on the growth of the *S. agalactiae* strain in (a) BHI broth (b) BHI broth with 3% NaCl (c) BHI broth with carnitine (d) BHI broth with carnitine and 3% NaCl.

The growth of *S. agalactiae* and *E. coli* were induced in the presence of L-carnitine. This induction was performed with 1 mM concentration of L-carnitine as the substrate in the growth medium. The bacterial growth in BHI broth with L-carnitine was determined to increase from 48 h to first 60 min (Figures 1 and 2c). At the end of 48 h, the growth of the *E. coli* strain was faster than the growth of *S. agalactiae* strain. BHI broth medium with 1 mM L-carnitine was the medium with the highest growth.

Table 1 shows the mean growth of *S. agalactiae* and *E. coli* strains in (a) BHI broth (b) BHI broth with 3% NaCl (c) BHI broth with (1 mM) L-carnitine (d) BHI broth with 1 mM L-carnitine and 3% NaCl.

An acceleration of the growth was seen in BHI broth medium suspensed with L-carnitine with 3% NaCl. The 3% NaCl in BHI broth showed inhibitory effect on the growth of bacteria (Figures 1 and 2d). However, the L-carnitine in the medium again was observed to increase the growth of *S. agalactiae* and *E. coli* bacteria (Table 1).

DISCUSSION

E. coli serves as a paradigm for studies of cellular osmoregulation (Romantsov et al., 2009). Delpech (2009) suggested that the stresses seem to induce instability in bacterial genome, which leads to gen duplication and mutation. In the stressful and variable environment, the increase in mutation rate might be considered advantageous, because population may occasionally give rise to be a mutant strain that is well adapted to the stressful forces. Van Derlinden et al. (2008) investigated the effect of high temperature for growth of *E. coli*. They observed that bacterial growth was good up to a temperature of 45°C in enriched BHI broth. High temperatures inhibit the methionine biosynthesis and therefore restrict growth. According to their research, the addition of methionine in the growth medium could not eliminate the growth disturbance.

These bacteria can tolerate salt concentration in the medium with L-Carnitine at 3% NaCl. Carnitine enhances growth of the wild type strains in the presence of NaCl. In *E. coli* and *S. agalactia,* the highest growth was observed in the 48 h and in BHI broth with carnitine. Growth of *E. coli* and *S. agalactia* in saline minimal medium is dependent on the presence of external carnitine. Siaterlis et al. (2009) assessed the effect on growth medium on cell survival of *Lactobacillus plantarum* during freeze drying. Sucrose, at a concentration between 5 and 10% offered better protection during freeze drying compare with sorbitol. They supported that the growth media affected the ability of the cells of survive during the stresses. A large amount of carnitine used in our study uptake via the membrane transporter in cytoplasm to resist osmotic pressure and to grow under conditions of

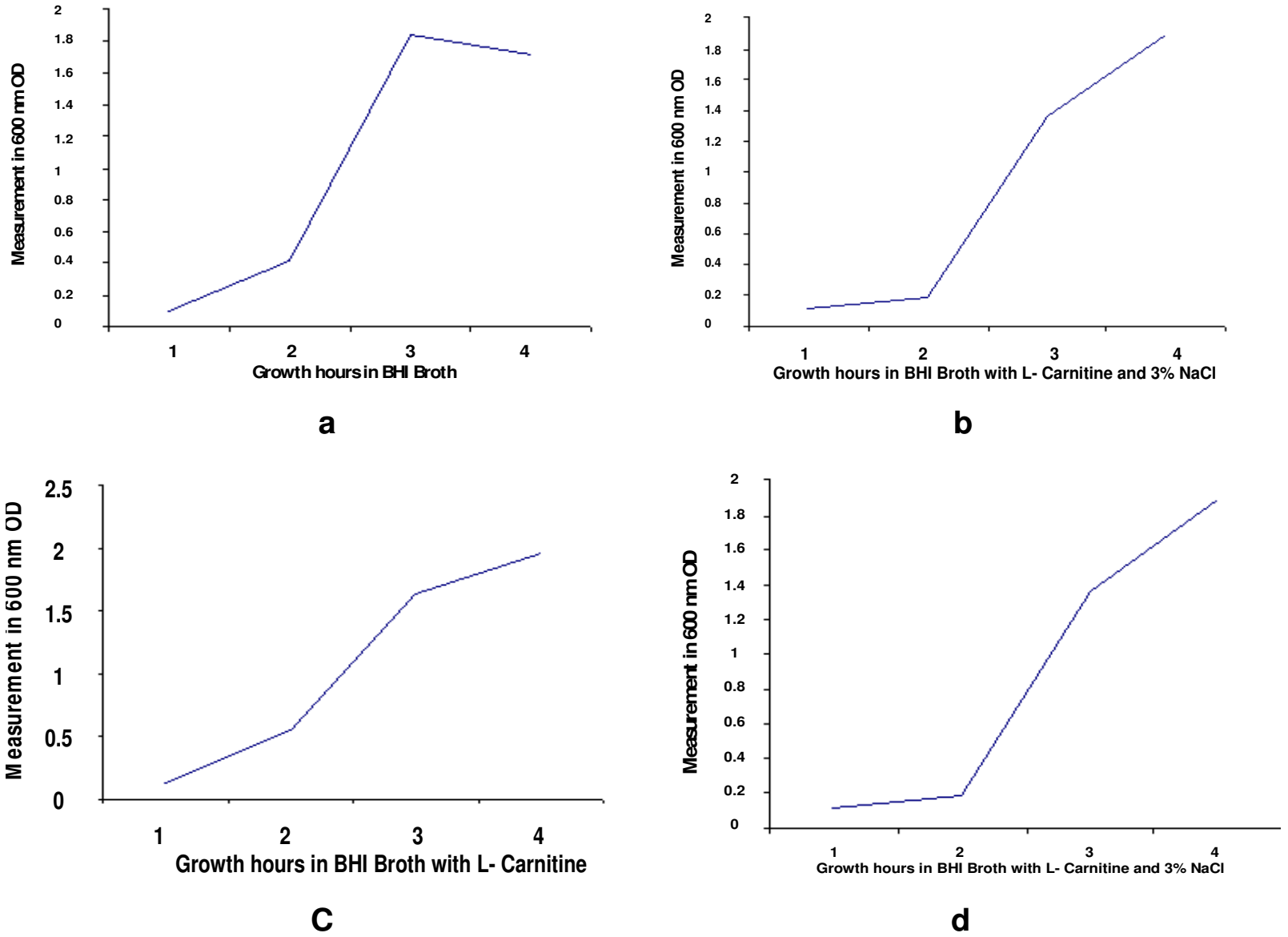

Figure 1. The effect of osmoprotectants on the growth of the *Escherichia coli* strain in (a) BHI broth; (b) BHI broth with 3% NaCl; (c) BHI broth with carnitine; (d) BHI broth with carnitine and 3% NaCl.

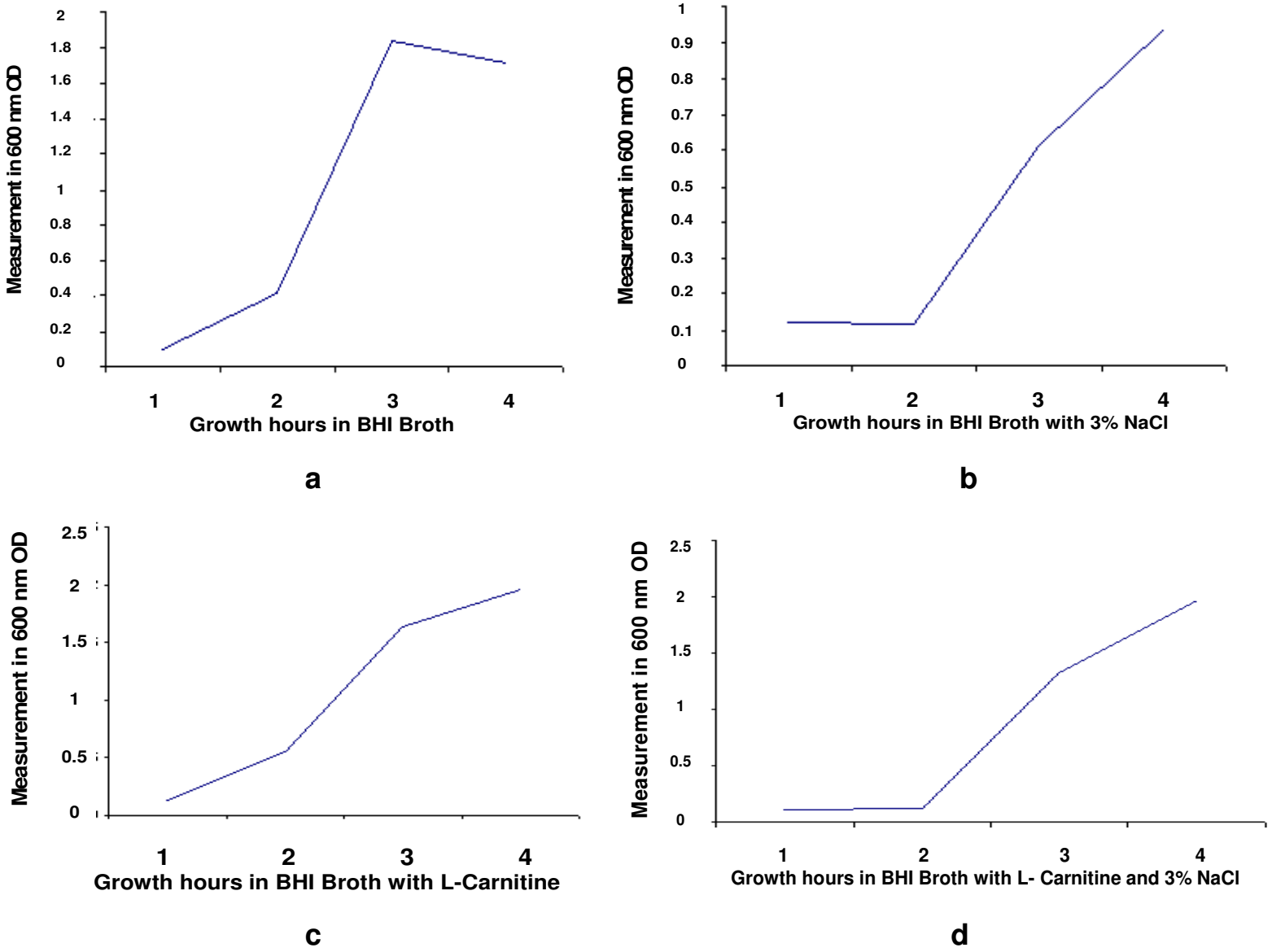

Figure 2. The effect of osmoprotectants on the growth of the *Streptococcus agalactiae* strain in (a) BHI broth; (b) BHI broth with 3% NaCl; (c) BHI broth with carnitine; (d) BHI broth with carnitine and 3% NaCl.

Table 1. The mean growth L-carnitine and 3% NaCl.

The means of bacterial growth in different BHI broth medium	The mean of growth for *E. coli*	The mean of growth for *S. agalactiae*
BHI Broth medium	1.245 ± 1.01	1.020 ± 0.88
BHI broth saturated with 3% NaCl	0.845 ± 0.85	0.447 ± 0.40
BHI broth suspensed with (1mM) L-carnitine	1.375 ± 0.94	1.067 ± 0.86
BHI broth suspensed with 2,5 cc (1mM) L-carnitine that contains 3% NaCl	0.887 ± 0.88	0.882 ± 0.92

high osmolarity. *S. agalactiae* and *E. coli* strains is tought to have a transporter for carnitine that can respond to osmotic stress by regulating its own activity. Lowering the carnitine concentration in the medium causes a decrease in growth rate. Carnitine is effective as an osmoprotectant, and is a key factor of osmotic adaptation by bacteria but much remains to be learned about its function of osmoregulatory systems. (Vermeulen and Kunte, 2004; Cánovas et al., 2006; Romantsov et al., 2009).

Today; *S. agalactiae* is widely observed as an invasive infection factor in the adult age groups not being pregnant. *S. agalactiae* meningitis is rare among adults and it can be seen in patients with predisposing factor. This species is hard to identify in laboratory tests for making a clinical diagnosis.

S. agalactiae and *E. coli* are known as bacteria being hard to grow at the laboratory tests for making clinical diagnosis. In this study, the mediums with L-carnitine factor used at laboratory diagnosis was determined to accelerate the bacterial growth. Adding L-carnitine factor into medium at laboratory tests will be get easier, the diagnosis of bacteria like *S. agalactiae* and *E. coli*.

REFERENCES

Bernal V, Sevilla A, Canovas M, Iborra JL (2007). Production of L-carnitine by secondary bmetabolism of bacteria. Microbion cell Factories, 6(31): 1-17.

Bhuiyan J, Seccombe DW (1996). The effects of 3-Hydroxy-3-Methtlglutaryl-CoA reductase inhibition on tissue levels of carnitine and carnitine acyltransferase activity in the rabbit. Lipids, 31(8): 867-870.

Cánovas M, Iborra JL (2005). Whole cell biocatalysis stabilization for L-carnitine production. Biocatalysis and Biotransformation, 23(3/4): 149-158.

Cánovas M, Torroglosa T, Iborra JL (2006). Permeabilization of *Escherichia coli* cells in the biotransformation of trimethylammonium compounds into L-carnitine. Enzyme and Microbial technology, 37 (3): 300-308.

Delpech R (2009). Evolution in an afternoon:rapide naturel selection and adaptation of bacterial populations. J. Biol. Educ. 43(3): 130-134.

Engeman C, Elssner T, Pfeifer S, Krumbholz C, Maier T, Kleber HP (2005). Identification and functional characterisation of genes and corresponding enzymes involved in carnitine metabolism of *Proteus sp*. Arch. Microbiol. 183: 176-189.

Ferreire A, Gray M, Weidman M, Boor KJ (2004). Comparative genomic analysis of the *sig*B operon *Listeria monocytogenes* and in other gram-positive Bacteria. Current microbiol. 48: 39-46.

Franken J, Kroppenstedt, Swiegers JH, Bauer FF (2008). Carnitine and carnitine acetyltransferases. Curr. Genet. 53: 347-360.

Okada Y, Okada N, Makino S, Asakura H, Yamamoto S, Igimi S (2006). The sigma factor RpoN is involved in osmotolerance in *Listeria monocytogenes*. FEMS Microbiol. Lett., 263: 54-60.

Racher KI, Culham DE, Wood JM (2001). Requirements for Osmosensing and Osmotic Activation of Transporter ProP from *Escherichia coli*. Biochemistry, 40(24): 7324-7333.

Rebouche CJ, Seim H (1998). Carnitine metabolism and its regulation in microorganisms and mammals. Annu. Rev. Nutr., 18: 39-61.

Romantsov T, Guan Z, Wood JM (2009). Carpiolipin and the osmotic stress responses of bacteria. Biomembranes, 1788(10): 2092-2100.

Shirazi M, Noorafshan A, Kroup M, Ranideh N (2007). Comparison of the effects of cartopril, tamoxifen and L-carnitine on renal structure and fibrosis after total unilateral ureteral obstruction in the rat. Scandinavian J. Urol. Nephrol., 41: 91-97.

Siaterlis A, Deepika G, Charalampopoulos D (2009). Effect of culture medium and crypotectants on the growth and survival of probiotic lactobacilli during freze drying. Lett. Appl. Microbiol. 48: 295-301.

Van Derlinden E, Bernaerts K, Vanlmpe JF (2008). Dynamics of *Escherichia coli* at elevated temperature effect of temperature history and medium. J. Appl. Microbiol. 104: 438-453.

Vermeulen V, Kunte HJ (2004). *Marinococcus halophilus* DSM 20408T encodes two transporters for compatible solutes belonging to the betaine-carnitine-choline transporter family: identification and characterization of ectoine transporter EctM and glycine betaine transporter BetM. Extremophiles, 8(3): 175-184.

Woolard CD, Indyk HE, Woolard G (1999). Carnitine in milk: A survey content, distribution and temporal variation. Food Chemistry, 66: 121-127.

Xizeng M, Olman V, Stuart R, Paulsen IT, Palenik B, Xu Y (2010). Computational prediction of the osmoregulation network in Synechococcus sp WH8102. BMC genomics, 11(291): 2-15.