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Role of Membrane Architecture in Development of Sensitivity to Cephalosporin Group of Antibiotics

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Abstract
Due to efficient adaptation process the spread of resistance among microbial pathogens is always a major problem to therapy. The resistance towards an antibiotic can be either biochemical or genetic. The molecular orientation of bacterial cell wall is responsible in determining drug resistance. The role of cephalosporins for e.g.: ceftriaxone & cefazolin was studied in relation to its toxicity to B. subtilis (Gram positive) & E. coli (Gram negative).In the presence of cefazolin an abnormally high level of protein release was observed when intact cells as well as membrane vesicles. In the presence of cefazolin protein release was observed to been enhanced in contrast to ceftriaxone. The protein export in case of intact bacterial cells was higher than membrane vesicles suggesting the involvement of membrane proteins in drug sensitivity/resistance. The extent of protein released was also found to be modulated when both the cells were subjected to temperature treatment. However, maximum protein export was seen when gram positive& gram-negative cells were subjected to EDTA concentrations. In contrast availability of Mg^{2+} ions in the medium resulted in slight fall in protein release indicating stabilization of membrane vesicles as well as bacterial cell wall, which might have resulted in lowered protein export due to involvement of transport system. From this study it can be concluded that outer membrane orientation determines the therapeutic value of cefazolin & ceftriaxone.
Keywords: Ceftriaxone sodium, Cefazolin sodium, Ethylene diamine tetra acetate, Proton motive force, Minimum Inhibitory Concentration

1. Introduction

Man has always lived in an environment that abounds with living organisms. Micro-organisms can be defined as living creatures so small that individuals cannot be seen without the aid of microscope. Bacteria are the simplest organisms to which the term cell can be applied. They are the smallest form of organic life that is visible under a compound microscope. Because of their ubiquitous nature, a number of studies have been carried out on these organisms.

Gram-positive and gram-negative bacteria are basically classified on the basis of their cell wall architecture. In bacterial cells the main cellular components has been divided into cytosole, cytoplasmic membrane and cell wall. The cytosole surrounded by cytoplasmic membrane and outer envelope is known as cell wall (Giesbrecht et. al., 1976).The cell wall of Gram-positive bacteria is a peptidoglycan having additional molecules of techoic acid, trichromic acid, polyphosphates and carbohydrates. (Hancock., 1997; Salton., 1994). The peptidioglycan layer of bacterial cell consist of disaccharide N-acetyl Muramic acid (β-1-4)-N-acetylglucosamine (MurNAC-GlcNAc) (Ghuysen and Strominger. 1963a; 1963b). The length of the Glycine molecule varies from 5-30 subunits depending upon the bacterial species. (Henz et.al., 1993; Snowder and Perken; 1990; and Glauner., et .al., 1987). In majority of bacterial cell D-lactyl moiety of each muramic is amide linked to short peptide component of peptidoglycan. (Tipper et.al., 1967; Munoz et .al., 1966 and Ghuysen et.al., 1965). The wall peptides are cross linked with other peptides that are attached to a neighbouring glycan strand (Tipper and Burman., 1969; Tipper and Storming., 1968), thereby generating a three dimensional molecular network that surrounds the cell and provides desired exoskeletal function.

Gram- positive and gram- negative bacteria are basically classified on the basis of their cell wall architecture. The envelops of different microorganisms differ considerably. A gram negative bacterium has more complex structure as compared to its counterpart, i.e., gram positive bacteria. In bacterial cells the main

cellular components has bee divided into cytosole, cytolasmic membrane and cell wall. The cytosole surrounded by cytoplasmic membrane and outer envelope is known as cell wall (Giesbrecht et. al., 1976). The cell wall surrounds the inner cytoplasmic membrane, maintaining its shape and protecting mechanically fragile cytoplasmic membrane from rupture due to high internal osmotic pressure generated by the cytoplasm.

Many antibiotics exert their action either in or outside the cytoplasm membrane, and only those, which have targets within the cytoplasm, must find means of penetration of the cell interior. Whereas, the fungal cell envelope is a complex structure in which the extension zone (hyphen tip) is bound by a wall made up of chitin or cellulose micro-fibrils embedded in the matrix of proteins. Glycans and glycoproteins may also be incorporated. The inner layer of the secondary wall is made up of chitin, overlaid by a proteinaceous layer, outside which is a glycoprotein reticulum, again overlaid by a layer of mixed α and β-glucans (Gooday and Trinci., 1980). The fungal membrane contains high proportions of carbohydrates in addition to phospholipids and sterols such as cholesterol and ergo sterol. The relative proportions vary according to the growth phase, which in turn affects the response of the organism to antimycotic drugs.

In some bacteria during the life cycle cell can lose these outer walls and deficient forms thus generated are referred to as L-forms or L-Phase variants (L for Lister Institute, where they were discovered). L-forms can be induced artificially by treatment with lysozyme or penicillin that disrupts the cell wall integrity. When gram-positive cell is exposed to either of these two chemicals cell wall synthesis is completely hampered / disrupted and become protoplast. However when a gram-positive cell is exposed to such chemicals it loses its peptidoglycan, but retains its outer membrane and spheroplast is formed.

Since bacterial cells are prokaryotic in their cellular organization therefore the cell membrane provides a site for various functions such as energy reaction, nutrient processing and biosynthesis. Apart from this, the major action of cell membrane is to regulate transport of nutrients, as well as, waste from external environment to cell interior. The cytoplasmic membrane of bacterial cell is selectively permeable with special carrier mechanism for passage to various molecules. The glycocalyx and cell wall act as a barrier for the passage of large molecules but they are not the primary transport apparatus. The cell membrane is also involved in secretion of discharge of metabolic product into extra cellular environment. In addition, bacterial membranes are important site for a number of metabolic activities. In these cells, most of the enzymes of respiration and other energy processing activities are located on the membrane. Enzyme system located on the cell membrane also synthesize structural macromolecules to be incorporated into the cell envelop. These membranes are also responsible for secretion of enzymes and toxins into the environment.

The cytoplasmic membrane lies directly outside the cytoplasm. It acts as a selective permeable barrier between the cytoplasm and the cell environment and is also the site at which may important and in primarily comprises of phopholipids and proteins. The membrane proteins are enzymes and carrier proteins, the latter mediating the specific transport of nutrients and ions. The membranes proteins constitute about 20% of the total bacterial proteins, which are mainly lipophilic on their surface. The electron transport system, the cytochromes, quinones, iron-sulphur proteins and flavin adenine dinucleotides are embedded in the cytoplasmic membrane, which acts membrane (transmembrane proteins) or they may be exposed on only one face. The cytoplasmic membrane of Bacillus subtilis contains predominantly derivative of ethanolamine in the inner layer and glycerol in the outer layer. During metabolism, protons are extruded to the exterior of the bacterial cell, the net result being acidification of the cell exterior, which also becomes positively charged relative to the cell interior. This combined potential, the concentration or osmotic effect of the proton and its electropositivity, is the electrochemical potential of the proton (ΔμH+) which can be quantified and expressed in terms of electrical units (mV). It is the potential of the protein motive force (ΔpH) which derives ancillary activities (Russell and Chopra., 1990). Energy transduction can occur (1) in membranes or (2) in non-membranous components of the cell. In these two processes two different forms of convertible energy currencies are used. These are $\Delta\mu H^+$ and ATP in (1) and (2) respectively.

According to Fig. 1, the transmembrane differences in the electrochemical potential of H^+ ions (ΔμH or protonic potential) holds a central position in the energy transduction pattern occurring in 'protonic' membranes. $\Delta\mu H^+$ consists of electrical, chemical and concentrational (osmotic) components, that is, the electric potential difference (ΔΨ) and the pH difference (ΔpH, Skuhachev. 1980).

2. Method

The present study was conducted at the department of Microbiology, Barkatullah University, Madhya Pradesh, India. Institutional ethical board approved the study and the informed consent was obtained from all the subjects before the commencement.

2.1 Organism and Culture Conditions

Bacillus subtilis NCIM 2063 strain used for the present study is a non pathogenic Gram- positive rod, and obligate aerobe (Plate 01: a; b). It is known to form protective end spore thereby providing tolerance to extreme environmental conditions. It was obtained from National Chemical Laboratory (NCL) Pune, India. The bacterial culture was routinely maintained on presterilized NAM at 04^0C. The culture was routinely monitored by single colony isolation method on Nutrient Agar plates for confirmation of purity of culture.

Chemical as well as reagents used in the present experiments were of reagent grade. However, antibiotics preferred for the experimental purpose for eg. Ceftriaxone (CT) sodium and Cefazolin (CZ) sodium were procured from Lupin Laboratories Ltd. M.P., India under the common generic names during experimentation Bacillus subtilis cultures were grown in Erlenmeyer flask containing presterilised growth medium. Incubation was done at 37^0C ± 0.1 in thermostatically controlled orbital shaker (Lab India, Model, 3521) under aerobic condition with plate rotation of 180 revolutions per minute. The pH of the media was adjusted by pre addition of 1 ML^{-1} HCL or 1 ML^{-1} NAOH solution. Glucose (0.5% w/v) was added to presterilised growth medium as carbon source; however carbohydrates such as Sucrose, Fructose etc. are also preferred in other experimentation.

2.2 Inoculum Preparation

Fresh colonies were picked up and suspended in 1 ml of the Dye's minimal medium. The population size of the inocula was estimated regularly by the optical density method as at 540 nm to avoid sporulation.

2.3 Determination of MIC

The minimum inhibitory concentrations (MIC) of the β-lactam were determined by the optical density method. The MIC was defined as the lowest antibiotic concentration inhibiting 50% of the visible bacterial growth under optimal conditions.

2.4 Growth studies

Erleneyer flasks (250 ml) were prepared to contain 50 ml of the sterile media. These flasks were inoculated with 0.25 ml aliquotes taken from an overnight growth culture. (0.341~O.D.) in the same media. Incubation was done at 37± 1 ^{0} C in the thermostatically controlled orbital shaker (Labline, Model no. 3215) under aerobic conditions with a platform rotation of 180 rev. min^{-1} Growth recorded at 550 nm using Shimadzu (1610) spectrophotometer. Aliquotes were aseptically removed from the flasks at hourly intervals and placed in a 1 cm path length cuvette of the spectrophotometer. Growth inhibition studies of B.subtilis was also carried out by subjecting the bacterium to adverse condition of growth such as varying temperature, pH, EDTA, varying Mg^{2+} ion concentrations. The behaviour of the bacterium was determined both in the presence and absence of antibiotic stress condition, measured spectrophotometrically (Shimadzu – 1610) at 540 nm against a medium blank.

3. Results

The Growth response of Bacillus subtilis under varying physiological conditions show different growth pattern. The capability of structural transformation under stress conditions in the form of endospore makes the observation somewhat critical. In order to avoid transition between sporulation and vegetative growth the inoculum size was considerd as important factor in determining the MIC50 for the CT and CZ antibiotics. To maintain proper inoculum cell were harvested in early log phase inspite of late log phase. The MIC50 in case of CT observed was 1.5 ppm in contrast to CZ, which shows MIC50 at 18 ppm. When Bacillus subtilis cell were inoculated in presterilised Dyes medium they showed differential growth tendency in the presence and absence of CT and CZ (Fig. 2). In the presence of antibiotic stress the log phase, which was seen to be 7 hrs under controlled conditions, was extended followed by stationary phase. Prominent log phase was observed when cell were grown in the absence of CZ, along with observations of optical density in relation to increasing cell density protein content of the culture at similar time intervals was made and presented as illustrated in (Fig. 2).

In all the cases CT was found to be more effective than CZ at MIC50. The increased sensitivity of Bacillus subtilis in case of CT might be due to high sodium concentration as structural component, which was lacking in CZ. The sodium content of CT and CZ appears to be 83.0 mg.g-1 and 48.3 mg.g-1, respectively. The increased sensitivity of bacteria in the presence of CT can be reflected as facilitation of β-lactam antibiotics across the bacterial membrane.

It is already known that in evolutionary pattern of transport system presently Na+ ion offers greater advantage over H+/OH- antiport system. As far as variation in protein concentration is concerned the cellular protein content was measured by Lowery's method and plotted with respect to time (Fig. 2). The protein content was seen at late log phase as 45.2 and 40.3 µg/ml in contrast to control conditions in the presence of CT and CZ respectively. The growth of microorganisms when subjected to varying temperature condition undergoes

modifying metabolic processes. It has been seen that the synthesis of fatty acids is directly influenced by temperature as observed in case of Bacilli (Fujii and Fulco., 1977). Inspite of those bacterial cells are seen to repair the initial heat injury leading to lysis of bacterial cells (Condons et. al., 1996). The inhibitory effect of heat stress are seen to result from the damage of proteins and the permeabilisation of membranes, particularly the cytoplasmic membrane, H+ gradient and lowering in the intracellular pH (Magger and Ferriera., 1993; Benschoter and Ingram., 1986 and Weitzel et. al., 1987). Bacillus subtilis showed highly reduced growth rate i.e. inhibition up to 92% under controlled conditions at 250C in the absence of antibiotics (Fig. 3). CT and CZ induced inhibition to bacterial cell, which was found to be 87.48% and 88.24% respectively at 250 C temperatures. In contrast at higher temperature of 500 C, 88.28%, 81.62% and 90.26% inhibition of growth was recorded. 100% survival of Bacillus subtilis was considered at 370C. However, 42% and 44% growth inhibition in case of CT and CZ was recorded respectively.

Furthermore the growth as well as sensitivity of Bacillus subtilis was observed to be effected in the presence of various pH range (Fig. 4). At pH 5.0 β-lactam untreated Bacilus subtilis showed 62% reduction in contrast to 86% and 84% inhibition of growth in presence of CT and CZ respectively.

At alkaline pH 8.0 the growth inhibition was found to be 52% and 12% respectively. The percent inhibition was more pronounced in case of CT as compared to CZ at both pH conditions.

The integrity of bacterial membranes lies in the availability of ionic strength of the medium. Chelating agents are known to modify property of membrane thus altering movement of molecules from outside to cell interior, variation in Mg2+ ions. Concentration is known to affect the permeability of bacterial cell membrane. When cells are grown in the presence of chelating agent in laboratory as well as natural conditions the property of exchange of material across cell membrane gets altered. A chemical change in the cell surface occurs concomitantly, since most of the lipopolysacharide (LPS) is released during treatment (Leive. 1968). When Bacillus subtilis is grown in the varying concentration of EDTA – a chelating agent growth performance was found to be gradually reducing as no growth was observed beyond a concentration of 0.08mM (Fig. 5). In the absence of EDTA as well as β-lactam antibiotics the growth of bacteria at 370C was found to be 100%. In the presence of CT and CZ the percentage survival of Bacillus subtilis was reduced in the presence of CT in contrast to CZ with increase in concentration on EDTA i.e. 0.5mM the survival was highly reduced in the combination of CT and CZ. Increasing concentration of EDTA in combination with drugs was found to promote drug sensitivity. The appearance of susceptibility to β-lactams as mediated by chelating agent (EDTA) under laboratory conditions supports the significance of hydrophobic interactions between phospholipids and the lipid 'A' moiety of LPS that undoubtedly contributes to the integrity and stability of bacterial membranes. The decyclation of phospholipids in the outer membrane weakens these hydrophobic interactions, perhaps to permit sufficiently, the release of LPS and leads to increased sensitivity to the β-lactam antibiotics. The permeability change induced due to EDTA treatment has been shown to be non-specific, resulting in increased permeability to several micro and macromolecules (Leive, 1968).

4. Discussion

The growing culture of bacterial cells are known to pump H+ ions into the bacterium where negatively charged groups are located resulting in a weak competition between H+ and cations. The Mg2+ ion content of the medium binds with the outer cell surface and induces cell wall biosynthesis either directly or indirectly. However, translation and transcription in cells, is also given by the Mg2+ ion, as it is a structural component necessary for maintaining structural integrity of ribosomal subunit (Tissieres., 1959). In total cellular metabolism shows deviation from normal routine when proteins are not made available. In the present study Mg2+ ions alone were found to modulate survival of Bacillus subtilis in Dye's minimal medium at 370C (Fig. 6). Mg2+ ions with increasing concentrations upto 10mM were found to be effective. The observation indicates facilitation of growth in combination with Mg2+ ions in the presence of CT and CZ. Therefore it can be established that Mg2+ ion when present in combination with therapeutic drugs modify the sensitivity to the bacterium. The treatment of β-lactam can be potentiated in combination of Mg2+ ions to make more success while treatment. The fact that structure of gram - positive bacterial walls has a high affinity for metal ions as they are negatively charged at normal pH conditions (Urratia – Meera et al., 1992). Mg2+ ions in combination with EDTA have been found to have protective behaviour towards treatment of β-lactams (Fig. 7). The growth performance of Bacillus subtilis in combination with EDTA + Mg2+ ions in the presence of β-lactams shows reduced inhibition of 0.25mM EDTA + Mg2+ ions.

5. Conclusion

Bacillus subtilis survives in the environmental condition exposed to various prevailing stress condition for eg. Salt, pH, Temperature etc. Therefore a complex behaviour of organism is seen under stress conditions (Volker,

1994). In the present study observations reflects that the therapeutic significance of β-lactam antibiotics viz. CT and CZ is influenced by laboratory condition might be true for pharmaceutical applications. Alkaline pH was sensitive than acidic pH of 5.0. In case of CT 86% and 52% inhibition of growth of Bacillus subtilis was found at pH 5.0 and 8.0 in contrast to only 12% inhibition at pH 8.0 in case of CZ. As compared to CZ, CT was found to have lower MIC50 with Bacillus subtilis although both are β-lactam groups only differing in 1 level of generation as reflected by structured modifications. Higher temperature conditions for e.g. 500C showed more sensitivity that optimum temperature of 370C. At l lower temperatures of 250C the normal growth of Bacillus subtilis was reduced in contrast to optimum temperature. Drug sensitivity in case of EDTA- a chelating agent was found to be pronounced when Bacillus subtilis was grown in Dye's medium. However, Mg2+ ions when applied in combination with EDTA protected the chelating behaviour of EDTA thus making cell more sensitive to treatment. The Mg2+ alone was found to potentiate the effect of CT and CZ in combination when added to the growth media. The study reflects the capability of Bacillus subtilis in determining the coexistence of cells under various stress conditions. Although application of β-lactams starting from first generation (CZ) to appearance of third generation (CT) shows present day need of control of several infections caused by gram positive and gram-negative counterparts. In the current observation establishment of phenomenon with respect to non-pathogenic isolate give a model for interpretation of further observations.

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References

Abraham, E.P., and Newton, G.G.F. (1961).The structure of Cephalosporin C.Biochem.J.79:377-393.
Abraham, E.P (1990). Selective reminiscence of β-Lactam antibiotics: Early research on Penicillin and Cephalosporins. Bioassays.12:601-606.
Abrahams, J.P., Leslie, A.G.W., Lutter, R., Walker, J.E (1944).Nature .370:621-628.
Abee, T., and Wouters.J.A (1999). Microbial stress response in minimal processing.Inter.J.Food.Micro.50:65-91
Adam, D (1999).Bacillus subtilis spore coat .Microbiol. Mol.boil .Rev 63:1-20
Aguilar, P.S., Cronan, J.E. Jr., and Diego, de.M. (1998).A Bacillus subtilis gene induced by cold schock encodes a membrane phospholipid desaturase.J.Bacteriol. 180:2194-2200
Alloing, G., Trombs, M.C., and Claverya, J.P. (1990).The amilocus of the Gram-positive bacterium Streptococcus pneumonic is similar to binding protein-depedent transport operons of Gram-negative bacteria.Mol.Microbol.4:633-644.
Bartholomew, J.W. (1962). Variables Influencing results and précised definition of steps in gram staining as a mean of standardizing the result obtained stain technol .37:139-155
Baquero, F. (1997). Gram-Positve resistance: Challenge for the development of new antibiotics. J. Antimicrobe. Chemother.39 (Supp 1; 16).
Beecher, D.J., and Macmillan, J.D.(1991)characterization of the component of hemolysin BL from Bacillus cereus.Infect.Immun.59:1778-1784.
Benschoter, A.S. and Ingram, L.O (1986) .Thermal tolerance of Zymomonas mobilis: temperature induced changes in membranes composition .Appl.Envor.Microbiol.51:1278-1284
Blackwell, K.J., Singleton, I., and Tobin, J.M. (1995). Metal cation uptake by Yeast: a review.App.Microbiol.Biotechnol.43:579-584
Booth, I.R., Cairney, J., Sutherland, L., and Higgins, C.F. (1988). Entricbacteria and osmotic stress: an integrated Homeostatis system .J.Appl.Bacteriol.Symposium Suppl.355-395
Bush, K (2004). Antibacterial drug discovery in the 21st century.J.Clin.Microbiol.Infect. Vol.10:p.10
Bush, K (1999), β-Lactamases of increasing clinical importance.Currt.Pharm.Des. 5(11):839-845.
Carper S.W., and Lancaster, J.R.Jr. (1986). An electrogenic sodium-translocating ATPase in Methanococcus voltae.FEBS Lett200:177-180
Curtis NSC Orr D Ross GW Boulton, M.G. (1979). Affinities of and cephalosporin for the penicillin binding protein of E. coli K-12 and their antibacterial activity .Antimicrob agents Chemother.16:533-539
Davies, P.L., and Bragg, P.D.(1972). Properties of soluble $Ca^{2+}$and Mg^{2+} activated ATPase released from Escherichia coli membranes.Biochim.Biophys.Acta.266:273-284
Decker, K., Persist, R., Reidl, J., Kossmann, M., Brand, B., and Boss, W. (1993). Maltose and Maltoriose can be formed endogenously in Escherichia coli form glucose and glucose-1-phosphate independently of enzymes of the maltose system.J.Bacteriol.175:5655-5665

Devlin, T.M. (1992). In textbook of biochemistry with clinical correlation .III edition Wiley –liss.A John Wiley and sons Inc Publication.

Dimroth, P., Kaim, G., and Matthey, U.(1998).The motor of ATP synthase Biobhim .Biophys .Acta 1365:87-92

Einasdottir, O. (1995). Fast reaction of Cytochrome oxidase.Biochim Biophys.Acta 1229:129-147.

Farber, J.M., and Pagotto, F (1992).The effect of acid schock on the heat resistance of Listeria monocytogenes.Letters in app.Micro.15:.197-201.

Furgusan, S.J. (1991). The function and synthesis of bacterial C type cytochrome with particular reference to Paracoccus denitrificans and Rhodobacter capsulatus. Biochim.Biophys.Acta .1058:17-20

Foster, J.W., and Hall, H.K.(1991). Inducible pH homeostasis and the acid tolerance response of Salmonella typhimurium. J.Bactriol.173, 5129-5235.

Gadd, G., Mowll, J.L., and White, C. (1986). Methods for assessment of heavy metals toxicity towards fungi and yeasts.Toxic assess.169-185.

Gale, E.F. (1963).Mechanism of antibiotics action .Pharmacol.Rev.15:481.

GaleE.F., Cumdliffe,E., Reynolds,P.E., Richmond,M.H., and waring,M.J.(1981).Inhibitors of bacterial and fungal cell wall synthesis.In the molecular basis of antibiotics action .2nd edn.London:John Wiley and Sons. pp 49-174

Guirard, B.M., and Snell, E.E. (1962). In: The Bacteria, edited by Gunsalus, I.C., and Stanier, R.Y. Academic press, London

Hadas, H., Einav, M., Fishnov, I., and Zaritsky, A. (1995).Division-inhibition capicity of penicillin in Escherichia coli is growth rate dependent.Microbiol.141:1081-1083

Hancock, I.C. (1997).Bacterial cell surface carbohydrate: structure and assembly.Biochem.Soc, Trans.25:183-187.

Haque, H., and Russell, A.D. (1976).Jape. Bacteriol.40.89

Haque, H., and Russell, A.D. (1974).Antimicrob.Chemother.5.447

Hardaway, K.L., and Buller, C.S. (1979).Effect of Ethylenediaminetetraacetate on phospholipid and outer membrane function in Escherichia coli.J.Bacteriol.137:62-68

Hecker, M., Scumann, W., and Volker, U. (1996).Heat shock and general response in Bacillus subtilis .Mol.Microbiol

Hederstedt, L., and Andersson, K.K. (1986).Electron Paramagnetic–resonance Spectroscopy of Bacillus subtilis cytochorme b558 in E.coli membranes and in succinate dehydrogenase complex from Bacillus subtilis memebrane J.Bacteriol.167

Keilin. D., and Hartree, E.F. (1939).Cytochrome and cytochrome oxidase
Proc.R.Soc.Lundon.B.Biol.Sci.B127:167-191.

Kemper, M.A., Urrutia, M.M., Beveridge, T.J., Koch, A.L., and Doyle, R.J. (1993).Proton –motive force may regulate cell wall-associated enzyme of Bacillus subtilis .J. Bacteriol.175:5690-5696.

Katida, M., Hoshimoto, M.,Kudo,T., and Harikoshi, K.(1994).Properties of two different Na^+/H^+ antiport system in alkaliphilic Bacillus sp. strain C-125J.Bacteriol.176:6464-6469

Lee, J.K., Movahedi. Harding, S.E., and Waites, W.M (2003). The effect of acid shock on the sporulating Bacillus subtilis cells.Jour.of.App.Microb.94:184-190.

Leive, L. (1968).Studies on the permeability changes produced in coliform bacteria by ethylene diamine tetraacetate.J.Biol.Chem.243:2373-2380

Lendenmanm, U., Thomas, E., and Egli.T. (1995). is Escherichia coli growing in glucose–limited chemostat culture able to utilize other sugar without lag? Microbiol.141:71-78

Maity, H.P., and Krishnamorty, G. (1995). Absence of kinetic barrier for transfer of proton from aqueous phase to membrane – water interface.J.Biosci.20:573-578.

Makman, R.S., Sutherland, E.W. (1965).Adenosine 3', 5'- phosphate in Escherichia coli J.Biol.Chem.240:1309-1314

Maloney, P.C., and Wilson, T.H. (1985).The evolution of ion pumps.Bioscience.35:43-48

Navarre, W.W., Dasdler, S. and Schneewind, O., (1996).Cell wall sorting of lipoprotein of Staphylococcus aureus.J.Bacteriol

Navarre, W.W. and Schneewind, O., (1999).Surface protein of Gram –positive bacteria and mechanisms of their targeting of the cell wall envelope .Microbiol and Mol Biol.63:174-229.

O'Sullivan, J., and Sykes, R.B. (1986). β- lactam antibiotics. In: H.Pape and H. J. Rehm (ed). Biotechnology, a comprehensive treatise in 8 volumes, Vol. 4, VCH Verlagsgesellschaft, Weinheim, Germany. 247- 281

Padan, E., Zilberstain, D. and Rottenberg, H (1976). The Proton electrochemical gradient in Escherichia coli cell. Eur.J.Biochem. 63: 533-541

Padan, E., Zilberstain.D, and Shuldenir, S. (1981). pH homeostasis in bacteria . Biophys. Acta. 650: 151-166
Russel, A.D. and Chopra.I. (1990). In.: Understanding antibacterial action and resistance. Pp 19-227. Ellis Horwood Series in pharmaceutical technology, England (U.K.).
Saier,M.H.Jr., and Reizer, J (1990). Shuffling during evolution of the proteins of the bacterial phosphotransferase system. Res.microbial.141: 1033-1038.
Tsiomenko, A.B., and Tuimetova, G.P.(1995).Secretory yeast proteins of thermal shock : A novel family stress proteins? Bokhimiya.60:837-842.
Ullmann, A., and Danchin, A. (1983). Role of cyclic AMP in Bacteria. Adv.Cyclic AMP Res. 15:1-53.
Von Heine, G. (1989). The structure of signal peptides from bacterial lipoproteins .Protein Eng. 2: 531-534.
Woodson, K., and Devine, K.M. (1994). Analysis of ribose transport operon from Bacillus subtilis. Microbial.140:1829-1838.
Yamada, J., Oishi, K., Tatsuguchi, K., and Watanable, T. (1979). Interaction of trialkyltin chloride with inorganic phosphate and phospholipids. Agric. Biol. Chem.(Tokyo).43 : 1015-1020.
Zilberstein, D., Agmon, V., Schuldiner, S., and Padan, L. (1984). Escherichia coli. Intracellular pH, membrane potential and cell growth. J. Bactriol. 158:246-252.

Figures

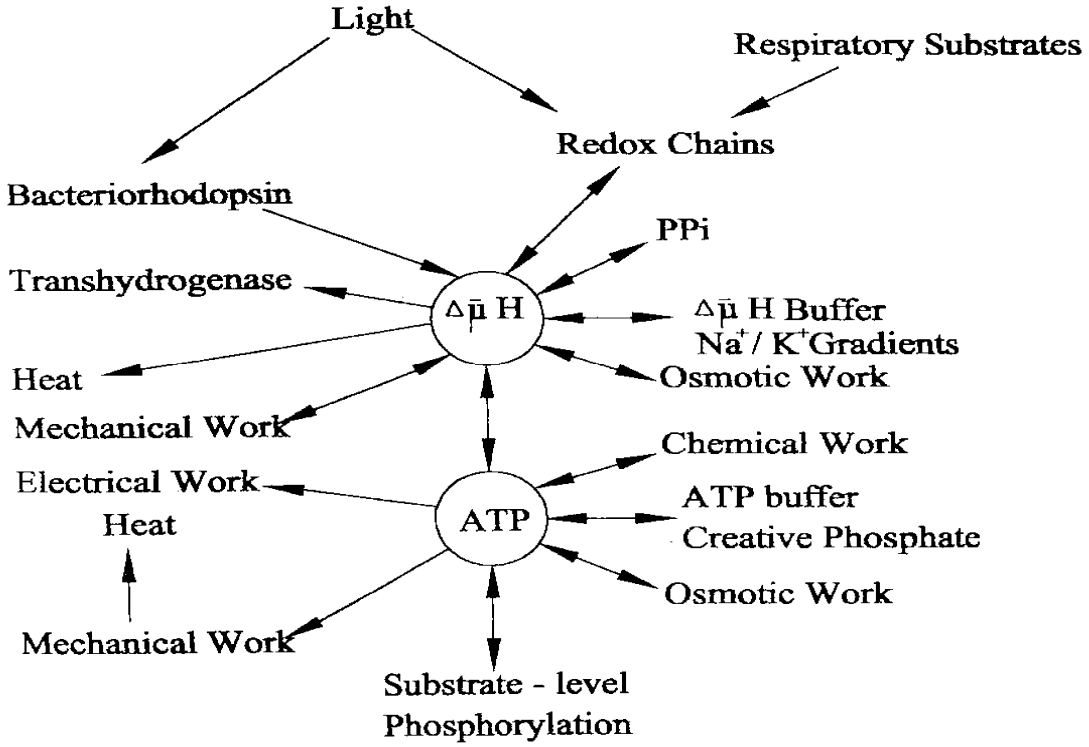

Figure 1: Transmembrane differences in the electrochemical potential of H^+ ions (ΔμH or protonic potential)

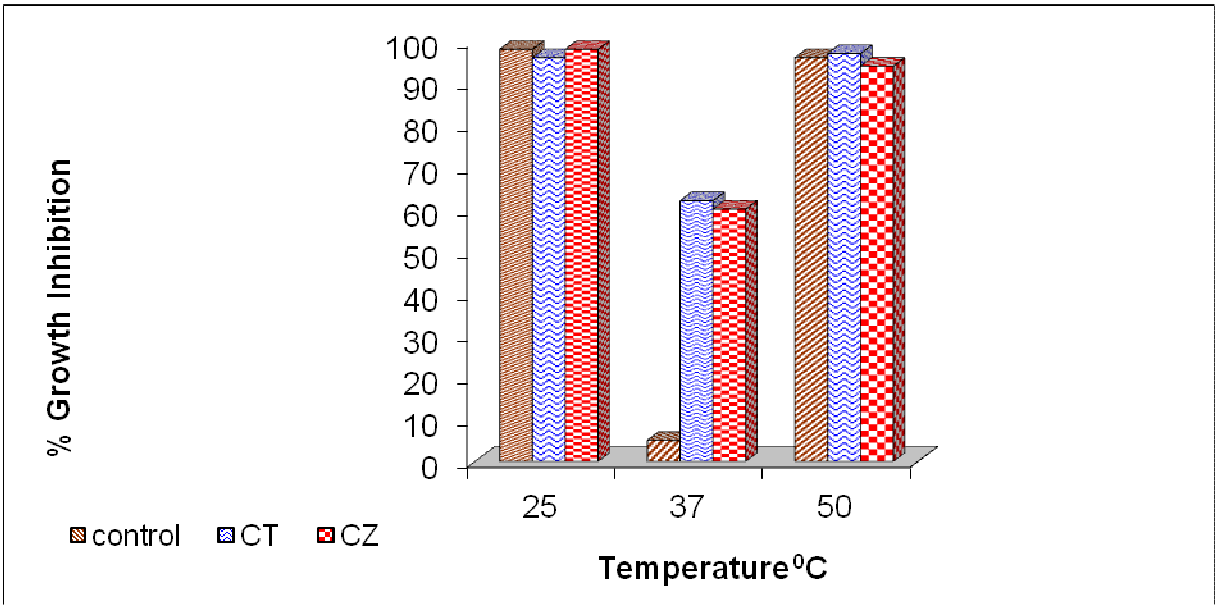

Figure 2: Percentage growth inhibitions of Bacillus subtilis at varying temp. (25, 37, 50 ^{0}C) in presence and absence of CT and CZ at pH 7.0

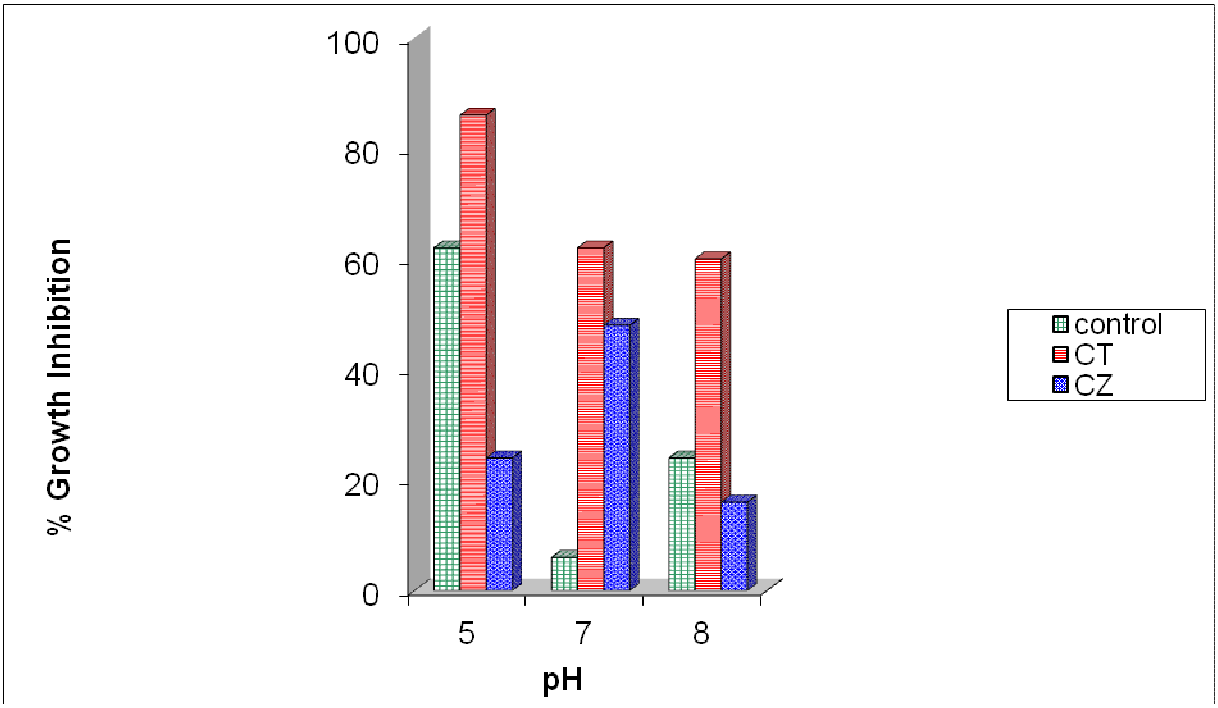

Figure 3: Percentage growth inhibition of Bacillus subtilis at pH (5, 7, and 8) in presence and absence of CT and CZ.

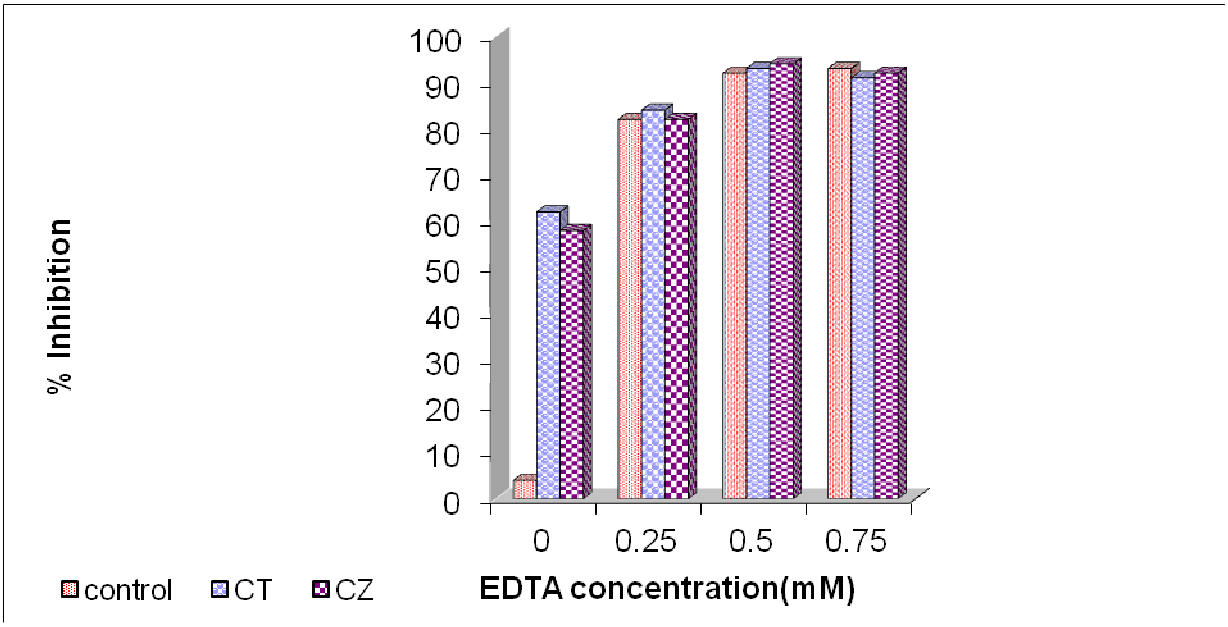

Figure 4: Percentage growth inhibition of Bacillus subtilis at varying EDTA concentration (0.25, 0.5, and 0.75) in presence and absence of CT and CZ at pH 7.0

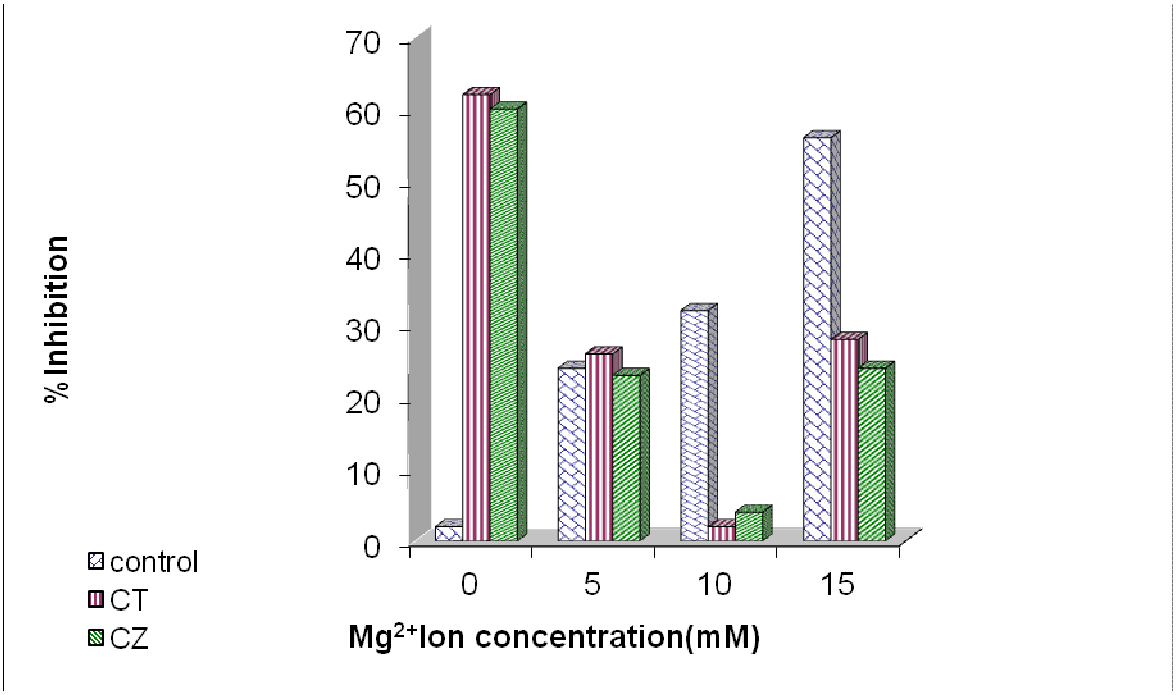

Figure 5: Percentage growth inhibition of Bacillus subtilis at varying Mg^{2+} concentration (0, 5, 10, 15 mM) in presence and absence of CT and CZ at pH 7.0.

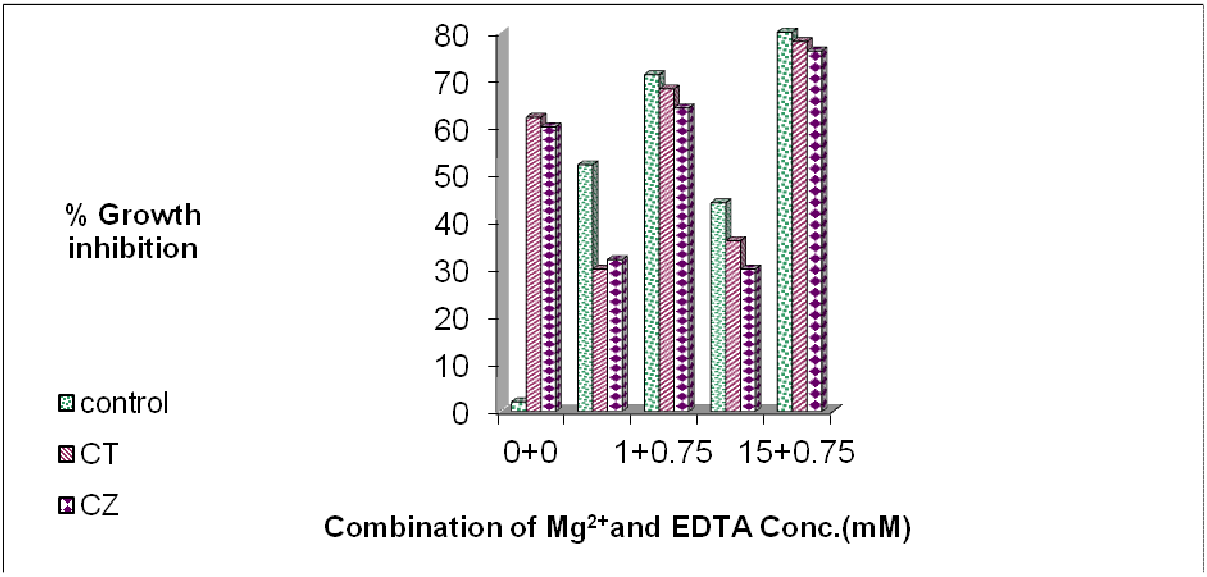

Figure 6: Percentage growth inhibition of Bacillus subtilis at varying combination of Mg^{2+} and EDTA concentration (0+0, 1+0.25, 1+0.75, 15+.025, 15+0.75 mM) in presence and absence of CT and CZ at pH 7.0.

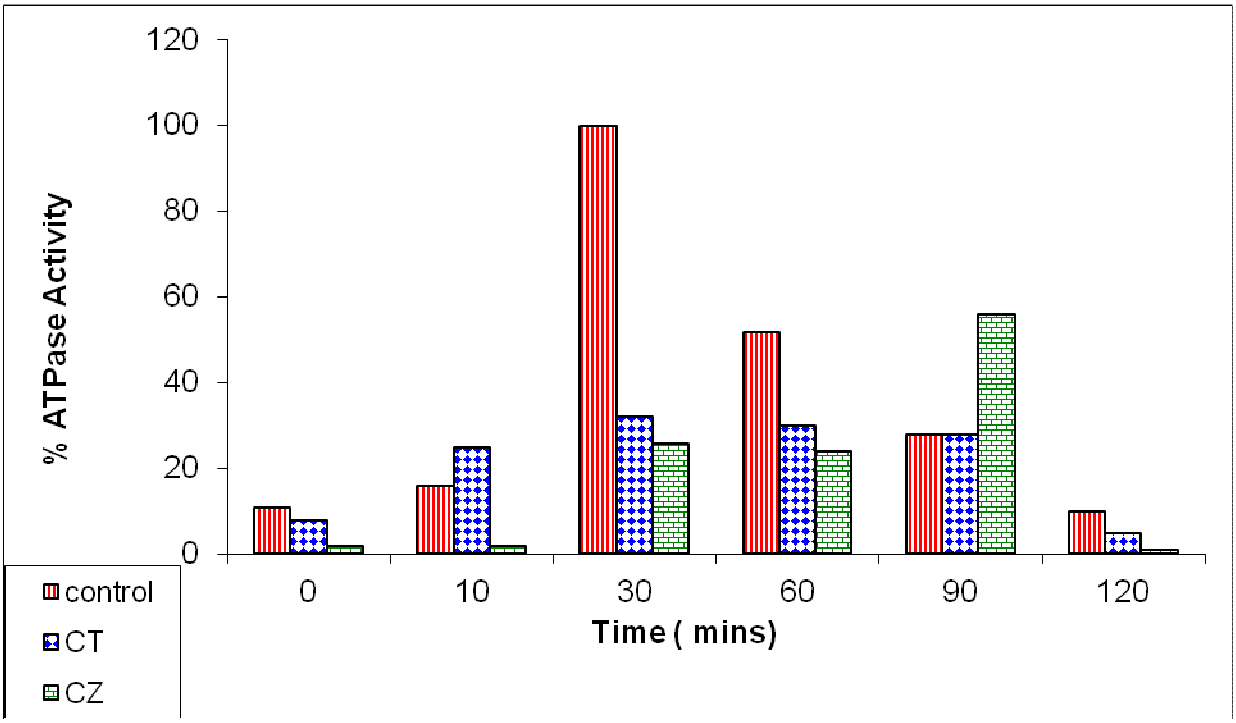

Figure 7: The percentage ATPase activity of B.subtilis in the presence and absence of CT and CZ (MIC_{50},1.5 ppm and 18ppm respectively) at 37+1^{0}C, pH 7.0 100% ATPase activity = 660µg Pi.mg protein^{-1}.min^{-1}

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