General Discussion and Summary

Generally media used in laboratory provide ample amounts of essential elements like sugars, nitrogen, phosphorus and other vital components which are considered fundamental for the bacterial growth. Natural environments generally contain only trace amounts of nutrients as compared to nutrient concentrations in culture media used in bacteriological laboratories. It is also rare to find a true eutrophic (= copiotrophic) environment in the earth's biosphere. Bacteria, adapted for growth under low-nutrient (sometimes extremely low) conditions are called as oligotrophs (Van der Kooji and Hijen 1983; Hattori 1984; Suwa and Hattori 1984; Wainwright *et al.*, 1991). The bacteria that grow in oligotrophic condition have a number of important implications in biotechnology, bio-medical fields, and environment (Ohta and Hattori, 1983; Wainwright *et al.*, 1991; Tada and Inoue, 2000; Nagarkar *et al.*, 2001; Zhang and Huang, 2005). As for example, a soil oligotrophic bacterium was used as tool in monitoring heavy metal pollution (Tada *et al.*, 2001). Similarly HuiXia *et al.* (2007) isolated an oligotrophic bacterium SGB-5 from biological soil crust underlayer in the Xinjiang Gurbantunggut desert which was having water retention as well as sand-fixing properties. Researchers have observed that these organisms generally fail to grow on rich nutrient media used in laboratory for cultivating the bacterial cultures. However, most of the oligotrophic bacteria changed their nature on subsequent cultivation and could grow on rich-nutrient media. Scholars have devised several methods (for details please see oligotrophic bacteria: A general introduction) to isolate these bacteria from environmental and non-environmental settings; for example, some workers used media like R2A (Reasoner and Gerldreich, 1985), diluted Nutrient or Luria Bertani or Luria broth (Tada *et al.*, 1995; Oh *et al.*, 2009; Kumar *et al.*, 2010), and Ravan medium (Watve *et al.*, 2000), etc. Crude aqueous samples itself (e.g. river water/ acid mine drainage/ sea water etc.) collected from diverse niches were also used as media (Yanagita *et al.*, 1978) to recover oligotrophic bacteria from that environment. Studies exploring the phenomena of resistance to different groups of antibiotics by the oligotrophic bacteria revealed that these bacteria were often resistant to different antibiotic(s), and quite often the isolates were found to resist two or more antibiotics called multiple-antibiotic-resistant (Nikitin *et al.*, 1988; Zlatkin *et al.*, 1991; Tada *et al.*, 1995; Kimura *et al.*, 1995; Miyake *et al.*, 2003; Riesenfeld *et al.*, 2004; Oh *et al.*, 2009; Kumar *et al.*, 2011). In a recent study conducted on oligotrophic bacteria isolated from Lechuguilla Cave, New Mexico, it was found that majority of recovered oligotrophic bacteria were resistant to antibiotics; even some strains were resistant to 14 different antibiotics used in the study (Bhullar *et al.*, 2012). Studies have also shown that most of the antibiotic resistance genes were plasmid-borne (Kimura *et al.*, 1995; Tada *et al.*, 1995). Very recently, two large plasmids, pREV1 and pREV2 (about 150 and 250 kb, respectively) from oligotrophic bacterium *Ancylobacter vacuolatus,* were shown not only to carry resistance genes for chloramphenicol and trimethoprim but also have been predicted to play a significant role in bacterial survival in low-nutrient condition(Zlatkin *et al.*, 2012). The genome sequence analysis of *Minibacterium massiliensis* (bacterium recovered from 0.22μm filtered ultra-pure water in a hospital setting and was found resistant to chemicals used for water disinfection), a *beta-proteobacteria,* revealed several antibiotic resistance genes on their genome including resistance to penicillin and streptomycin as well as genes coding for virulence factors (Audic *et al.*, 2007). *M. massiliensis* also contained a unique genomic island that encodes resistance to specific heavy-metal ions and metalloids that are generally used for water disinfection.

Freshwater is the fundamental requirement to sustain terrestrial life. It is a finite resource, essential for agriculture, industry and even human existence; without which sustainable development is not possible. Rivers are the main sources of fresh water. In past few decades the water quality extensively deteriorated due to addition of various kinds of pollutants and nutrients

through the agencies like sewage, industrial effluents, agricultural runoff etc which brings a series of changes in the physicochemical and other characteristics of water (Olimax and Sikorska, 1975; Piecznska *et al.*, 1975; Tiwari and Mishra, 1986; Vollenweidre, 1986; Milway, 1987; Reddy and Venkateswar, 1987). Deterioration of the fresh water quality is a global problem (Mahananda *et al.*, 2005). In developing countries, 90% of the sewage is being discharged directly into rivers, lakes, coastal waters without any treatment (World Resource Institute, 1996) (www.wri.org). Like that of the predecessors in this field of investigation, we pertained to the following hypothesis: "oligotrophic bacteria may be potential reservoir of novel antibiotic-resistance genes in the aquatic (riverine) environment".

With an overall aim to reveal phenotypic and genotypic data of the oligotrophic bacterial population of a city-waste polluted river, Mahananda, of northern West Bengal, India, in the light of genomics of Integron associated gene cassettes, five major objectives were set in this study: (I) to provide detailed descriptive information about the nature of antibiotic resistance in culturable oligotrophic bacteria from the water samples of river Mahananda; (II) to study the diversity of the oligotrophic bacteria of Mahananda river; (III) to apply molecular systematics in ascertaining taxonomic status of the isolates; (IV) to explore the incidence of resistance integrons in oligotrophic bacterial population; and (V) to explore the molecular diversity of the antibiotic resistance gene cassettes. The study describes result of investigations aimed to uncover diversity of the culturable oligotrophic bacterial isolates, their antibiotic resistances phenotype, and antibiotic resistance determinants associated with class 1 integrons.

Culturable oligotrophic bacteria and fraction of their population resistant to antibiotics used were enumerated on nutrient-poor-agar (NPA) medium and NPA amended with defined concentration of each antibiotic tested. A new protocol (chapter-1) for determination of antibiotic resistance cut-off points in case of oligotrophic bacteria by selecting proper reference strain was developed. Two international standard methods (CLSI and EUCAST) available for determining sensitivity/resistance are basically suited for copiotrophic isolates (that are grown in nutrient-rich media); hence for assessing antibiotic sensitivity of oligotrophic bacteria, sensitive reference strains from amongst the isolates were chosen for calculating LD_{50} (half lethal dose where half of the bacteria were killed in respect control devoid of any antibiotic). The break points for antibiotic resistance were set as five times greater of the calculated LD_{50} for resistance determination (elaborated in chapter 1). A total of ninety composite water samples in three consecutive years (2007-2009) [each sample is a mixture of three grab samples collected per sampling site per month(except July and August, being avoided due to heavy downpour)] from river Mahananda were analyzed. The geographical location(s) of sampling sites, SS I, SS II, and SS III, are as follows: 26°44′22.62″ N, 88° 25′21.92″E (SS I), 26°44'23.20"N, 88°25'22.89"E (SS II), and 26°38′42.44″ N, 88°24′19.67″E (SS III). The oligotrophic bacterial load of river Mahananda at three sites of sampling ranged from 1×10^3 to 5.9×10^4 CFU/mL. From the pool of total oligotrophic (obligate and facultative) bacteria, facultative ones were selected by replica plate method for further study. It was observed that facultative oligotrophic bacteria could survive on rich nutrient plate for longer period (more than 6 months if the plate is stored under suitable condition preventing desiccation of agar medium) compared to copiotrophic bacteria (model copiotrophic bacterium, *Escherichia coli* failed to survive beyond 20 days on Luria agar plate); owing to their adaptability to resist more adverse condition. This phenomenon also explains why oligotrophic bacteria attain adaptive fitness over copiotrophs in nutrient-scarce environment. The ability to switch from oligo to copiotrophic condition and vice-versa is an important phenomenon of facultative oligotrophs which makes them sustainable in fluctuating nutrient conditions which often prevail in an environment. 76.2% of the total facultatively oligotrophic bacteria isolated from river Mahananda were resistant to one or more than one antibiotics and 23.8% were sensitive to all the antibiotics tested. Within antibiotic-resistant facultatively oligotrophic bacteria, 47% were SAR (single-antibiotic-resistant) and 53% were MAR

(Multiple-antibiotic-resistant). Surveillance programme in this study has enabled to understand the size of the problem and suggest strategies for control of antibiotic resistance. The results of this study presented in chapter 1 revealed high frequency of antibiotic-resistance in oligotrophic bacteria. Reports of such studies are very rare as compared to copiotrophic bacteria reported from several laboratories throughout the world. Hence, the present study has broadened the complexity of the problem.

A major part of this research (Chapter 2, 4 and 5) was motivated by a desire (ought to be unbiased) to understand the gene cassette diversity associated with class 1 integrons irrespective of their origin, in hosts, be it sensitive, single or multiple antibiotic resistant. There are several classes of integrons have been reported till date, of which the most documented and well characterized are the class 1 integrons (Martinez-Freijo *et al.*, 1998, Martinez Freijo *et al.*, 1999, Chang *et al.*, 2000, Schmitz *et al.*, 2001, White *et al.*, 2001, Gebreyes *et al.*, 2002; Thungapathra *et al.*, 2002; Jones *et al.*, 2003; Lindstedt *et al.*, 2003; Chen *et al.*, 2004; Nandi *et al.*, 2004; Betteridge *et al.*, 2011; Nardelli *et al.*, 2012; Stalder *et al.*, 2012). The following three different segments (Fig 2.1) that constitutes a class 1 integron are: (i) 5' conserved segment (CS) containing an *intI* gene that codes for integrase and an attachment site (*attI*) recombination site; (ii) the 3' CS containing a combination of antiseptic resistance gene (*qacE*), sulfonamide resistance gene (*sulI*), and one or two additional open reading frames, ORF5/and or ORF5 and ORF6 that are hypothesized to act as transposition sites of the progenitor *ISCR1* element (Toleman *et al.*, 2006) for development of complex class 1 integrons; and (iii) a variable region between 5' CS and 3' CS (Hall, 1997; Hall and Collis, 1998). Variable region is the part of class 1 integron platform where integration and excision of gene cassette (a mobile non-replicating DNA element usually bear antibiotic resistance gene but other types of genes inserted within gene cassettes also have been reported) catalyzed by *intI1* encoded integrase (Collis and Hall, 1992) (Fig. 2.2 and 2.3). Selection of a total of 2188 facultatively oligotrophic bacterial colonies/isolates was random (without any human biasness), because research randomizer tool was used which generated a random set from non-selective plates serving as an universal set of numbers (each isolated colonies bearing a number) irrespective of the knowledge of the phenotype (sensitive, single or multiple-antibiotic-resistant). These randomly selected isolates were examined for the presence of class 1 integrons, using a highly reproducible PCR strategy (CS-PCR). PCR assay showed presence of class 1 integrons in eighty nine Gram negative isolates and one Gram positive isolate. Thus 4.1% (90/2188) isolates were found to carry class 1 integron in their genome, and amongst them 18 (22%) were sensitive to all the twelve antibiotics, 07 (7.8%) were SAR (single-antibiotic-resistant), and 65 (72.2%) were MAR (resistant to two or more antibiotics) (Fig 2.4A). The amplified amplicon lengths of variable region (as the methodology itself enables to amplify the variable region of the class 1 integrons) varied from 0.15 to 3.45 kb. Cloning and sequencing of amplified class 1 integron borne gene cassettes revealed that amplicon of size ~1.0 kb was predominating and was detected in 24.4% (22/90) of the total integron positive isolates; however very short sequence like 153 bp only were also detected from two isolates, MB62 and MB63 which did not carry any gene cassette within variable region and was designated as empty class 1 integron. The finding of empty class 1 integron is as a clear indication that the isolate are ready to capture a gene cassette of desired function for better adaptability and competency in the environment where they are living. Sequence analyses and cassette characterization showed that ~29% isolate carried gene cassettes, bearing ORFs not related to any of the reported antibiotic resistance and ~71% isolates were having the gene cassettes encoding antibiotic resistance. The most common carriages in gene cassettes bearing antibiotic resistance genes were observed for aminoglycoside adenyltransferase gene cassettes such as *aadA, aadA1, aadA2, aadA4,* and *aadA5* conferring resistance to streptomycin/ spectinomycin antibiotics followed by dihydrofolate reductases (type-A: *dfrA1, dfrA5, dfrA7, dfrA12, dfrA16, dfrA17,* two novel *dfrA* genes, *dfrA28* and *dfrA30* and type-B: *dfr-IIe,* a single gene cassette) conferring resistance to trimethoprim. Only very recently, Bhullar *et al.*

(2012) reported that aminoglycoside antibiotic resistance was common in oligotrophic bacteria isolated from Lechuguilla cave. In this study, bacterium MB35 revealed the largest amplicon of length ~3.5 kb containing array of four gene cassettes, *blaIMP-9-aacA4-oxa10-aadA2*. Amongst dual antibiotic-resistance gene cassettes in variable region of class 1 integrons of the isolates, frequency obtained for *dfrA-aadA1* type (10%) was highest followed by *dfrA17-aadA5* (~8%). The homology modeling of DfrA1 and nucleotide binding domain of AadA1 protein derived from MB47 produced three dimensional structure(s) similar to dihydrofolate reductase and nucleotide binding domain respectively of *Haemophilus influenzae*.

Besides antibiotic resistance gene cassettes, a number of gene cassettes (~29% of the total) bearing ORFs for unrelated function than the antibiotic resistance were observed. The analyses of gene cassettes bearing ORFs of unrelated function and comparison with the existing data exhibit that most of the ORFs were coding for hypothetical proteins. The CS-PCR product sequencing of OB05 and MB83 showed that the derived sequences was 75% identical (nucleotide-nucleotide) to the part of the genome of *Acidovorax avenae* subsp. *citrulli* (Ac. No. CP000512). The feature present in that part of *Acidovorax* genome included a portion of ribosomal large subunit pseudouridine synthase B. However the predicted polypeptide of the ORF generated from OB05 DNA sequence did not produce any significant similarity with existing sequences of the protein database. Sequence analyses of the amplicons of MB05 and MB70 revealed the presence of a truncated ORF of 64 amino acids that showed 73% identity with the methyl transferase protein from *Ralstonia eutropha* (Ac no. AAZ62060). A translated polypeptide from the sequence derived from bacterium MB09, signifying type I site-specific deoxyribonuclease, HsdR family, exhibited nearly 42% identity with a protein of the same family, found in *Thiomicrospira crunogena* (Ac. No. YP390604). Partial sequencing of the CS-PCR product obtained from MB12 showed the presence of a single ORF (*livM1*) of length 154 amino acids (Fig 2.8A). BlastP analysis (Table 2.4) of this putative polypeptide product exhibited best scores with ABC type branched chain amino acid transport system with 72% identity at amino acid level to the ABC transporter permease of bacterium *Aromatoleum aromaticum* EbN1 (Ac. No. NC_006513). The multiple sequence alignment of LivM1 derived from amplicon sequence of bacterium MB12 with its neighboring ABC transporter proteins is shown in Fig. 2.8B. The transmemebrane (TM) regions prediction revealed the presence of four transmembrane regions in the putative LivM1 protein (ID: CAQ53856) of MB12 (Fig. 2.8C and 2.8D). The predicted transmembrane (TM) regions were at positions (AA position), 2-20, 50-74, 87-110, and 119-138. The conserved domain search for putative translated product obtained from translated ORF of MB12 integron sequence revealed that the predicted protein was one of the members of two TM subunits which play role in uptake of branched chain amino acids (Fig 2.8E). Mapping of signature motifs (Fig 2.8F) revealed that 19 of 19 [TM_PBP1_LivM_like, a type of transporters which consist a PBP (periplasmic binding protein), two TMs (trans-membrane subunits) and two cytoplasmic ABCs (ATP-binding cassette)] residues which were recognized on the TM-ABC transporter protein sequences of other bacterial species were also found conserved on ABC protein derived of MB12. It was assumed that in these types of transporters probably play vital roles in the import of solutes from environment. Other workers have also reported unrelated genes inserted as cassettes within class 1 integron (Nemergut *et al.*, 2004; Gillings *et al.*, 2008a, 2008b). Finding of these transporters in class 1 integrons has a greater significance since oligotrophic bacteria thriving in low nutrient milieu may require improved transporter molecules to concentrate and channelize nutrients from outside to the interior of the cells. The theoretical pI and instability index of the putative ABC transporter of MB12 was computed 9.3 and 19.13. The protein was predicted as stable protein with an estimated half life of 10 h (*in vivo* in respect to *E coli*) with a high aliphatic index of 127.21. MB16 was found to carry gene cassette carrying *appA* gene encoding bacterial extracellular solute binding protein. ProtParam computing showed that the extracellular binding protein derived from bacterium MB16 was stable and its instability index was computed 18.94. The GRAVY (grand average hydropathicity),

aliphatic index and theoretical pI was computed -0.087, 78.5, and 9.26 respectively. Psortb, a tool for subcellular localization prediction showed that the translated product of *appA* gene was periplasmic with localization score of 9.44.

Sequence analysis of amplicon obtained from MB 19 has shown the presence of a unique hybrid DNA sequence. In the 867 nucleotide long sequence, a continuous stretch of 242 nucleotides (from 3 to 244) produced 96% identity with a vertebrate (*Lepilemur dorsalis)* genomic fragment (Ac. No. AJ244007) and residual 623 nucleotide stretch produced (from nucleotides 245 to 867) 74% identities with *Burkholderia xenovorans* LB400 genomic DNA. This genomic DNA region of *B. xenovorans* coded for the putative reverse transcriptase maturase protein. MB 19 sequence yielded an ORF of 227 amino acids that has shown 65% identity with reverse transcriptase maturase protein from *Burkholderia cenocepacia* HI2424 (Ac. No. YP833935). An ORF of 164 amino acid residues (495 nucleotides) obtained from amplicon sequence of MB 22 shared 81% amino acid identity with phosphoribosylformylglycinamidine synthase (FGAM synthase) from *Acinetobacter baumannii* (Ac. No. YP001712860). The partial sequence of MB 28 (408 nucleotide) was 86% identical with *Klebsiella pneumoniae* subsp. *pneumoniae* MGH 78578 (Ac. No.CP000647) genomic DNA. The amino acid sequence (truncated protein) derived from amplicon of bacterium MB28 was encoding a bifunctional putative transcriptional regulator protein, this truncated protein of 84 amino acids shared homology of 96.3% with the similar protein present in *Klebsiella pneumoniae* (Ac. No.CP000647). The Complete sequence of 704 bp amplicon of MB 40B (Table 2.3B) yielded no significant homology with any nucleotide sequence available in the database. The same sequence yielded a complete ORF of 102 amino acids and BlastP analysis of this protein sequence revealed 58% identity with helicase domain protein of *Verminephrobacter eiseniae* EF01 (Ac. No. ABM585806). The sequences derived from amplicons of OB 12, MB 08, MB41, MB 44, MB 48, MB 51, MB 54, MB55, MB80 and MB81 did not produce any significant homology with any protein sequences available in the database. All of them were characterized as hypothetical proteins. All these polypeptides shared very low level of identity (2.03 to 25.19%) among either with each other or to pre-deposited hypothetical protein sequences. The translated product (Fig. 2.9A) of bacterium MB54 revealed a conserved domain belonging to the UPF0153 superfamily (Fig. 2.9B) when subjected to the BlastP search. However the function of proteins of this family is still unknown; since the proteins of this family contains 8 conserved cysteines hence it was expected that they may constitute a metal binding site and therefore might play a role in metal sequestering like Fe-S cluster as part of oxido-reductase complex. It is apparent that such predicted protein, although putative, in oligotrophic bacteria may play a role in iron acquisition. It is also speculated that there may be other multiple specific systems or mechanisms/and or a systems with multiple functions for transporting microelements and nutrients which are limiting in oligotrophic environment.

Protein homology and CDD search of 178 amino acid long protein derived from the MB 56 isolate was found to carry a conserved protein domain, encoding hemolysin activator/ secretion protein involved in intracellular trafficking and secretion. This hemolysin activator protein of MB56 produced 55% identity with the hemolysin activator protein of *Acinetobacter* sp. (Ac. No. YP045656). Translation of DNA sequence obtained from MB 58 and MB71 yielded a truncated ORF of polypeptide of 181 amino acids. The BlastP analysis of the said truncated polypeptide yielded best score (97% identity) with transposase protein of *Acidovorax* sp. (Ac. No. YP987142). Expression of such genes may enable the bacterium to be an opportunistic pathogen.

Despite other important findings, two novel dihydrofolate reductase genes, *dfrA28* and *dfrA30* were also detected and characterized (chapter 4 and 5). The expression study of *dfrA30* showed that the novel *dfrA, dfrA30* was functional and could resist trimethoprim resistance up to the level 1000 mg/L. The resistance level was too high with respect to the settled breakpoint (sensitive ≤/resistance >is 2/4 mg/L) of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) parameter.

It was observed that the integron load was highest at sampling site II, which may be due to selective pressure of high anthropogenic activity and diverse pollutant concentration at this site. An important relation between calculated resistance index (no. of antibiotics rendered resistant / no. of antibiotics tested) and frequency of the presence of class 1 integron structure. It was found that isolates corresponding to resistance index (RI) between 0.5-0.9 yielded amplicons maximally in the CS-PCR assay. Therefore, isolates with RI 0.5-0.9 has the maximum propensity to carry class 1 integrons supporting recommendation for selection of isolates for short-term snap-shot study aiming at to understand diversity of class 1 integron borne antibiotic-resistance-gene cassettes.

The taxonomic characterization of integron-positive isolates presented in chapter 3 has revealed the diversity of class 1 integron bearing bacteria of the Mahananda River at Siliguri. For classification and identification, phenotypic tests including total protein profiling, and genotypic tests including 16S rRNA gene sequence analysis were done. UPGMA dendogram constructed using binary data generated from phenotypic characters of all the isolates corroborated with the protein profiling data. The 16S rRNA gene sequences of integron positive bacteria fell under two main classes, *Betaproteobacteria* and *Gammaproteobacteria*. Only two isolates belonging to the genus *Comamonas* and *Acidovorax* were identified in class *Betaproteobacteria*. Both the said genera were comprised by single family, *Comamonadaceae*. Other isolates were identified under super class *Gammaproteobacteria*. The representative genera of class *Gammaproteobacteria* were constituted by families, *Moraxellaceae, Pseudomonadaceae, Aeromonadaceae* and *Enterobacteriaceae*. Similar to previous reports published on presence of integrons in copiotrophic bacteria, majority of the identified integron positive oligotrophic bacteria of super class *Gammaproteobacteria* were detected from the family *Enterobacteriaceae*. (Mukherjee and Chakraborty, 2006; Chen *et al.*, 2011; Han *et al.*, 2012; Su *et al.*, 2012). Following genera comprised by family *Enterobacteriaceae*: *Shigella, Kluyvera, Klebsiella, Salmonella, Citrobacter, Serratia, Enterobacter, Proteus, Providencia* and *Escherichia*. Despite of several known bacterial genera, nine isolates, MB25, MB28, MB41, MB44, MB48, MB54, MB81, MB83 and MB12 could not be placed into any of the known genera. On the other hand, the 16S rRNA gene sequences of few isolates (the isolates which could not be assigned any genus) poorly shared with the known genera of different classes. However from phenotypic data, these unidentified isolates clustered with members of family *Enterobacteriaceae* (Fig 3.3). One novel Gram positive bacterium belonging to the genus *Brevibacterium* was finally assigned a status of a novel species employing polyphasic approach including16S rRNA phylogeny, chemical taxonomy, lipid analyses cellular carbohydrate analyses, cell wall amino acid detection, fatty acid methyl ester analysis, DNA base composition and a large number of physiological and biochemical tests. The bacterium was named as *Brevibacterium siliguriense* sp. nov.

Evaluation of antibacterial property of zinc oxide nanoparticles/Quantum dots with surface adsorbed acetate ion (ZnO-Ac) having size 3-5 nm was conducted using a multiple-antibiotic-resistant (resistant to ten antibiotics: cotrimoxazole, ampicillin, gentamycin, netilmicin, tobramycin, chloramphenicol, cefotaxime, kanamycin, streptomycin and trimethoprim) facultatively oligotrophic, and human serum-tolerant isolate of the genus *Klebsiella, K. penumoniae* strain MB45; results of which have been presented in Chapter 6. In recent years, a number of nanosized metals (called as nano-particles) like Ti, Mg, Ag, and Zn have been documented as important alternative candidate of antibiotics in controlling pathogenic bacteria (Joshi *et al.*, 2009). Among the different oxide derivatives of nanosized metals, zinc oxide have been observed as effective drug possessing antibacterial activity (Stoimenov, 2002; Nair *et al.*, 2008; Hanley *et al.*, 2008; Applerot *et al.*, 2009; Joshi *et al.*, 2009) not just because of their stability under harsh processing conditions, but also because they are found as safe materials for human beings and animals (Stoimenov *et al.*, 2002; Fu *et al.*, 2005). Results revealed that growth of *K. penumoniae* strain MB45 was completely arrested at concentration of 500 mg/L ZnO-Ac QDs. Apart from the general direction of the search elucidated in the preceding chapters, the final chapter leaves a ray of hope to curb the menace.