Chapter 7

Biological Activity of Crystal

7.1 Introduction

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid your body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well.The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940s, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials.However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents. The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe to survive, change, and become

resistant to the poisons and/or heavy metals.Antimicrobial nanotechnology is a recent addition to the fight against disease causing organisms, replacing heavy metals and toxins and may some day be a viable alternative.Infections that are acquired during a hospital visit are called "hospital acquired infections" or nosocomial infections. Similarly, when the infectious disease is picked up in the non-hospital setting it is considered "community acquired".A wide range of chemical and natural compounds are used as antimicrobials. Organic acids are used widely as antimicrobials in food products, e.g. lactic acid, citric acid, acetic acid, and their salts, either as ingredients, or as disinfectants. For example, beef carcasses often are sprayed with acids, and then rinsed or steamed, to reduce the prevalence of E. coli O157:H7.Traditional healers long have used plants to prevent or cure infectious disease. Many of these plants have been investigated scientifically for antimicrobial activity, and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal. So, it is worthwhile to study plants and plant products for activity against resistant bacteria.

7.2 Escherichia coli

Escherichia coli is a Gram-negative, rod-shapedbacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most E. colistrains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls[1,2]. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2,[3] and by preventing the establishment of pathogenic bacteria within the intestine[4,5].E. coli and related bacteria constitute about 0.1% of

gut flora[6].andfecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination[7,8]. The bacterium can also be grown easily and inexpensively in a laboratory setting, and has been intensively investigated for over 60 years. E. coli is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA.Escherichia coliencompasses an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of E. coli and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its medical importance[9]and Escherichia coli remains one of the most diverse bacterial species: only 20% of the genome is common to all strains[10]. In fact, from the evolutionary point of view, the members of genus Shigella (dysenteriae, flexneri, boydii, sonnei) should be classified as E. coli strains, a phenomenon termed taxa in disguise[11]. Similarly, other strains of E. coli (e.g. the K-12 strain commonly used in recombinant DNA work) are sufficiently different that they would merit reclassification.A strain is a sub-group within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of E. coli are often host-specific, making it possible to determine the source of faecal contamination in environmental samples[7,8]. For example, knowing which E. coli strains

are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird.

Role in biotechnology

Because of its long history of laboratory culture and ease of manipulation, E. coli also plays an important role in modern biological engineering and industrial microbiology[12]. The work of Stanley Norman Cohen and Herbert Boyer in E. coli, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology[13].Considered a very versatile host for the production of heterologousproteins[14], researchers can introduce genes into the microbes using plasmids, allowing for the mass production of proteins in industrial fermentation processes. Genetic systems have also been developed which allow the production of recombinant proteins using E. coli. One of the first useful applications of recombinant DNA technology was the manipulation of E. coli to produce human insulin [15]. Modified E. coli cells have been used in vaccine development, bioremediation, and production of immobilisedenzymes[14]. E. coli cannot, however, be used to produce some of the larger, more complex proteins which contain multiple disulfide bonds and, in particular, unpaired thiols, or proteins that also require post-translational modification for activity[12].Studies are also being performed into programming E. coli to potentially solve complicated mathematics problems, such as the Hamiltonian path problem[16].

Model organism

E. coli is frequently used as a model organism in microbiology studies. Cultivated strains (e.g. E. coli K12) are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to thrive

in the intestine. Many lab strains lose their ability to form biofilms[17,18]. These features protect wild type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using E. coli as a model bacterium[19], and it remains the primary model to study conjugation. E. coli was an integral part of the first experiments to understand phagegenetics[20], and early researchers, such as Seymour Benzer, used E. coli and phage T4 to understand the topography of gene structure[21]. Prior to Benzer's research, it was not known whether the gene was a linear structure, or if it had a branching pattern.E. coli was one of the first organisms to have its genome sequenced; the complete genome of E. coli K12 was published by Science in 1997[22].The long-term evolution experiments using E. coli, begun by Richard Lenski in 1988, have allowed direct observation of major evolutionary shifts in the laboratory[23]. In this experiment, one population of E. coli unexpectedly evolved the ability to aerobically metabolize citrate, which is extremely rare in E. coli. As the inability to grow aerobically is normally used as a diagnostic criterion with which to differentiate E. coli from other, closely related bacteria, such as Salmonella, this innovation may mark a speciation event observed in the lab.By combining nanotechnologies with landscape ecology, complex habitat landscapes can be generated with details at the nanoscale[23]. On such synthetic ecosystems, evolutionary experiments with E. coli have been performed to study the spatial biophysics of adaptation in an island biogeography on-chip.

7.3 Bacillus megaterium

Bacillus megaterium is a rod-shaped, Gram-positive, endospore forming, species of bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillus form.Bacillus

megaterium is a rod shaped bacterium and one of the largest eubacteria found in soil. Groups of the bacteria are often found in chains where the cells are joined together by polysaccharides on the cell walls. Bacillus megaterium is able to survive in some extreme conditions such as desert environments due to the spores it forms. Where there are favourable conditions the spores can survive. Sometimes this particular bacteria can be found on common surfaces that are frequently touched.Bacillus megaterium produces penicillin amidase used for making penicillin. It produces enzymes for modifying corticosteroids, as well as several amino acid dehydrogenases.

7.4 Materials and Method

The biological activity of ligand (Schiff base) and derived crystals were checked with to their impact on the growth of bacteria (Escherichia coli and Bacillus megaterium) at Department of Biology, Hemchandracharya North Gujarat University, Gujarat, India.

7.5 Collection of Culture

The microbial strains Escherichia coli (ACCT 1642), Bacillus megaterium were collected from IMTECH, Chandigarh, India.

7.6 Maintenance of Culture

The culture of bacteria Bacillus subtilis and Escherichia coli were grown on nutrient agar with following composition

Peptone	10 g/ml.
Meat extract	03 g/ml.
NaCl	05 g/ml.
Agar agar	30 g/ml.
pH	7.4

The volume was made to 1000 ml with distilled water. The mixture was mixed and heated to boiling to dissolve the medium completely. Sterilization was carried at 15 lbs pressure (121^0 C) for 15 minutes.

7.7 Inoculum Preparation

One loop full growth of Bacillus subtilis and Escherichia coli were inoculated in 50 ml of Nutrient broth containing all the component of N agar shown above except agar in 250 ml of Erlenmeyer flask and incubated overnight on rotary shaker adjusted at 120 rpm, 37^0C.

7.8 Antibacterial Assay

The synthetic heterochelates were explored in the range of (0.1 t0 10 µg/ml) for its antimicrobial potential against E. coli and Bacillus megaterium by disc diffusion method [13]. Besides that antimicrobial activity of Schiff base was evaluated in the similar range (0.5 to 10 µg/ml). Various metal salts like $NiCl_2.6H_2O$ and $CuCl_2.2H_2O$, were also examined for their antimicrobial activity in the range of (2000 t0 10000 µg/ml).

7.9 Results and Discussion

The effect of synthesized crystals on the growth of E. coli and B. subtilis was investigated. Antibacterial activity was investigated in selected range of 2000 to 10000 (µg/ml). Antimicrobial activity was recorded in variable manner for all the metal salts included in present study for a given set of concentrations. Highest activity was recorded for Ni(II) salt followed by Cu(II) salts respectively for E. coli while in case of B. megaterium again Ni(II) oxide salt showed highest activity followed by Cu(II) chloride salts. The zones of inhibition were comparatively greater in case of the *E. coli* in comparison to *B. megateriuma*s per the results presented in Table: 7.1 and 7.2. The sub component of crystal which is

Schiff base was also investigated for the antibacterial activity in the range of 0.1 to 10.0 (µg/ml). Inhibitory action recorded for Schiff base was at from 0.5 µg/ml and onwards in range of 4 to 18 mm for *E. coli* while in case of *B. megaterium* inhibition was recorded 0.25 µg/ml and onwards in range of 7 to 13 mm. in Table: 7.3 and 7.4. In case of *E. coli* for the synthesized crystals, antibacterial activity was recorded at the lower concentration of 0.1 to 0.25 µg/ml, which were non inhibitory in case of individual application for Schiff base. Highest activity was recorded for compound containing Ni(II) salt followed by other crystals. The results indicate that the highest activity was due to incorporation of Ni(II) in the crystals. From Table: 7.5 and 7.6 that is for antimicrobial activity of crystals against *E. coli* and *B. megaterium*respectively. A perusal of Table: 7.5 and 7.6 reveals that at all concentration of crystals (0.1 to 10 µg/ml), Cr(VI) crystals shows maximum inhibition as compared to other crystals.The difference in the antimicrobial pattern can be due to variation in the cell wall structure of gram positive Bacillus megaterim and gram negative E. coli.

Table: 7.1Antibacterial Activity of Metal Salts against *E. Coli*

Metal Conc. (µg/ml)	Zone of inhibition (mm)	
	$NiCl_2 \cdot 6H_2O$	$CoCl_2 \cdot 6H_2O$
2000	9	8
4000	8	10
6000	10	10
8000	18	13
10,000	32	19

Table: 7.2Antibacterial Activity of Metal Salts against *B. Megaterium*

Metal Conc. (µg/ml)	Zone of inhibition (mm)	
	$NiCl_2 \cdot 6H_2O$	$CoCl_2 \cdot 6H_2O$
2000	7	-
4000	-	-
6000	8	9
8000	9	12
10,000	13	8

Table: 7.3Antibacterial Activity of Schiff Base against *E. Coli*

Concentration (µg/ml)	Zone of inhibition (mm)
0.10	-
0.25	4
0.50	3
1.00	7
2.50	13
5.00	16
10.00	18

Table: 7.4 Antibacterial Activity of Schiff Base against *B. Megaterium*

Concentration (µg/ml)	Zone of inhibition (mm)
0.50	8
1.00	11
2.50	7
5.00	9
10.00	13

Table: 7.5Antibacterial Activity of Synthesized Crystals against E. *Coli*

Conc. of Crystals (µg/ml)	Zone of inhibition (mm)	
	Ni(II) Crystal	Cu(II) Crystal
0.1	10	7
0.25	11	21
0.5	13	14
1	14	10
2.5	17	14
5	14	12
10	20	10

Table: 7.6 Antibacterial Activity of Synthesized Crystals against *B. Megaterium*

Conc. of Crystals (µg/ml)	Zone of inhibition (mm)	
	Ni(II) Crystal	Cu(II) Crystal
0.1	-	2
0.25	-	-
0.5	14	6
1	17	15
2.5	20	16
5	19	15
10	20	17

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