Migal Galilee Technology Center

Survival of Bacteria in River Water

A literature review

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1. Introduction

1.1 The River Yarkon

The River Yarkon runs for 17 km from the springs at Rosh Hanikra through the city of Tel Aviv to the Mediterranean Sea. Over the latter part of its course (4.5km) the water is brackish, mixing with seawater and is subject to tidal and differential current flows. The remaining part is freshwater, although its chemical characteristics and general water quality are influenced by the origin of the water. Due to the heavy water demand on the mountain aquifer that originally served as the source for the waters of the Yarkon, little remains to supply the river. Depending on the rainfall, the springs still contribute a fraction of the flow, however in years affected by very low rainfalls, such as the current one (1999), this contribution is negligible. The main sources for the river Yarkon are currently the wastewater effluent from two large wastewater treatment plants serving the cities and surrounding regions of Hod Hasharon and Kfar Saba. Wastewater treatment plant (WWTP) effluents are only as good as the level and efficiency of treatment of the water in the plant. With respect to microbiological quality, neither of the two WWTP's is operating adequately and consequently, large quantities of bacteria are discharged into the River Yarkon. The chemical quality of the river is also determined by the composition of the WWTP effluents as well as by the chemical, physical and biological; processes occurring in the river along its course. During most of 1998 the river was closed for secondary contact recreation sports (i.e. sports which do not involve immersion in the water). This ban was levied because the level of fecal indicator bacteria exceeded the maximum permissible levels of 1000 per 100ml (a level normally limited to primary contact sport). Currently in 1999 the level of fecal bacteria has been sufficiently low as to permit these activities. However, it is necessary to understand those factors that control and influence the level of fecal bacteria in the river in order to permit adoption of suitable management practices to prevent occurrence of high coliform counts. This literature review was commissioned in order to help clarify why the river Yarkon can maintain a high bacterial population and what factors prevent this from happening.

2. Microorganisms in water

2.1 Microbial Pollution of Natural Water

Microbial pollution has become one of the leading issues affecting water quality. Although the emphasis for many decades was placed on the problems associated with chemical pollution, it is now widely acknowledge that waterborne microorganisms can show significantly greater threats to public health. In the United States, the Centers for Disease Control and Prevention (CDC) have estimated that up to 900,000 cases of illness and, an as yet unconfirmed estimate of 900 deaths occur as a result of infection from water-borne microorganisms. Amongst these microorganisms are various types of bacteria, including *E. coli* O157; protozoa, such as *Cryptosporidium* and *Giardia*; and viruses, such as hepatitis A. The diseases caused by these waterborne microorganisms vary from diarrhea to respiratory infections, heart disease and neurological disorders.

Most of the water borne diseases affect humans following ingestion and are associated with drinking water supplies. In other instances, infection and disease has occurred as a result of recreational use of water contaminated with the pathogenic microorganisms.

2.2 Sources of Microbial Pollution

The sources of these microbial contaminants are from animal and human fecal wastes. These are the primary habitat for these microorganisms and serve as the principal source. There are some pathogens, such as *Legionella* and *Pfiesteria* which occur naturally in water and which multiply as a result of changes in environmental conditions, particularly following influx of nutrients. Those pathogens, more intimately associated with humans and animals, tend not to reproduce outside of the human host and their survival depends upon environmental factors prevailing in the water body. Some pathogens such as viruses are only active when they are in their hosts and exist in a totally inactive form in water.

Human fecal contamination of the environment is usually prevented by directing sewage water to some form of pre-treatment before discharge into a river or other aquatic body. This may take the form of a septic tank or by collection through a network of sewers and transporting the sewage to a

wastewater treatment plant. Depending on the efficiency of the wastewater treatment plants, the numbers of pathogenic bacteria should be reduced following treatment, but inevitably, a residual fraction can be released into the receiving water with the purified wastewater. In addition, where a receiving water is also used as a sink for urban runoff and sewer overflows, these can significantly impact on the microbial quality of a natural water body. Groundwater contamination from septic tanks is a very serious problem in most parts of the world, including the United States, where, 175 billion gallons of waste water could theoretically contaminate groundwater resources from the 25 million septic tanks in the country. Testing for virus contamination (e.g., rotavirus, hepatitis A virus and coxsakieviruses) in groundwater has indicated that as many as 20% of the groundwater resources are contaminated from fecal sources.

Microbial pollution also originates from animal wastes. Both dairy and beef cattle are reservoirs of the Shiga toxin producing *E. coli* O157 that can result in serious enteric infections in man. In addition, cattle are reservoirs of *Cryptosporidium*, *Giardia* and other pathogenic microorganisms. Chickens are reservoirs of *Salmonella* and *Campylobacter*. The fate of wastes from farm animals has to be carefully controlled to prevent contamination of water resources and the consequent threat to public health.

Many studies have addressed the fate of pathogenic bacteria in aquatic systems and the results have helped to understand better how to manage water resources. Many of the studies have been carried out under laboratory conditions and only a few in natural systems themselves. Both freshwater and seawater have been examined with respect to the fate of pathogenic microorganisms and the range of organisms investigated has varied from viruses, protozoa to various bacteria, including *E. coli* and *Vibrio*.

2.3 Bacterial life cycle

In a discussion of the fate of bacteria in natural and technical systems, the fate of bacteria was described according to the schematic shown in Fig 1 (Mason *et al.,* 1986). According to this model, bacteria when present in a growth environment such as the digestive tract of mammals, are able to use soluble carbon sources for growth and replication. Under such idealized conditions, this process can continue unabated. However, many factors influence the extent to which this continues, including the availability of

sufficient food, the prevalence of optimal physical and chemical conditions and the maintenance of the bacteria in the physical growth environment. The digestive tract of mammals works, however, like a semi-batch reactor with a discontinuous input of fresh food and a discontinuous output of waste material including excess bacteria. Once outside of the host, the bacteria are no longer in the correct physical, chemical conditions and they are no longer receiving a supply of food, and so they begin to undergo physiological changes that will be discussed subsequently. Two possible fates await bacteria, the first is that they die by lysis, i.e. explosion due to high pressure inside of the cell and failure of the membrane potential to maintain an intact and strong barrier against lysis. The second possibility is that they enter a dormant state, where they are no longer capable of reproduction and where their metabolic activity is reduced to a basic minimum. In the natural environment where a complex interaction between numerous micro- and macro-organisms exists to form a unified carbon cycle and food chain, bacteria are also subject to death as a result of physical action such as exposure to UV light, dehydration or by chemical action such as exposure to a bacteriostatic chemical. In addition they are subject to predation by a wide range of micro- and macro-organisms at higher levels in the food chain.

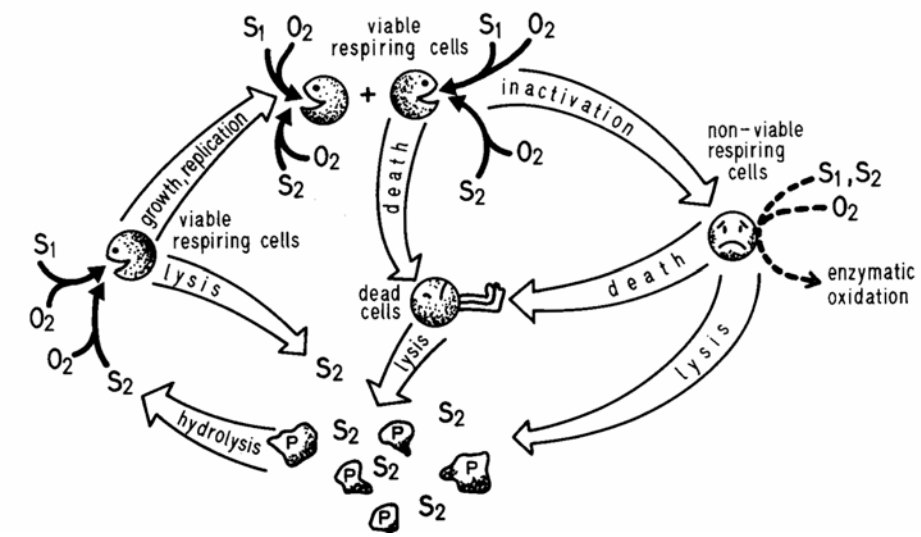

Fig 1. Different physiological forms of bacteria and their interrelationships. This scheme is based on an original model which was developed to describe the fate of bacteria in a closed laboratory culture system (Mason *et al.,* 1986). It can be also be applied to describe the different forms of bacteria which are present in the environment since the non-viable respiring cells are equivalent to the viable but nonculturable bacteria (VBNC) as described in the text.

2.4 Fate of Bacteria in the Environment

The fate of bacteria outside of their natural hosts and therefore outside of their normal growth environment requires an understanding of the

physiological mechanisms involved in the transition from a growth environment to a non-growth environment. Physiologically, there are three "states" that bacteria can be found in and the ability to detect, enumerate and assess the public health relevance depends on the nature of the physiological state in which the microorganisms are in at the time of sampling. These three states are a) growing, b) transient and c) 'dormant'. The first and last are the two extremes, in between which is the transition state, the nature of which determines the longer term survival of bacteria. The growing state will not be discussed here in any detail since this is a standard textbook state and has only limited relevance to the subsequent survival. This is not to say that it should be underestimated since there is a lot of evidence to show that especially for laboratory systems, the pre-starvation growth environment affects the transition and long term survival of bacteria (García-Lara *et al.,* 1993; Gauthier *et al.,* 1989). However, for the case of the Yarkon River, this is too specialized a subject to have much relevance and so will not be discussed further.

The transition-state occurs because bacteria move from an environment with a large supply of nutrients to one where the supply is very low or non-existent. When the nutrient availability is very low, these are referred to as oligotrophic conditions. In the absence of any food, these are referred to as starvation conditions. In order to survive, a bacterial cell has to undergo a range of metabolic changes to convert it into a long-term survival form. The concept of "feast and famine" has actually been considered to be the normal mode of life for bacterial cells, moving from a nutrient rich environment to a nutrient poor environment under many natural conditions in the environment. Naturally, a bacterium has no way of "knowing" how long such a state is likely to last and so the transition is one of complete dedication to what can only be assumed to be a 'long-term" survival strategy. Accordingly, sequential activation of a range of genes causes different proteins to be activated and others suppressed (see chapter 4). This results in a reduction in metabolic activity, to strengthen the physical defense of a bacterial cell and to protect the sensitive genomes and other structural components of the cell.

2.5 Dormancy in Bacteria in the Environment

The transition to a state of a 'dormant' cell is less well defined for the enteric bacteria. For bacteria such as *Bacillus* sp., where the existence of a morphologically defined spore is well characterized, this final status is clear. However, the pathogenic enteric bacteria tend to be Gram-negative, non-spore-forming bacteria and do not differentiate into such morphologically distinct forms. Both a spore and the 'dormant' state of non-sporulating bacteria are reversible states from which active bacteria can eventually redevelop once favorable conditions return to the environment of the cells. However, like a spore, it cannot be assumed that a dormant bacterial cell will be able to form colonies on an artificial agar medium (see chapter 3). As such our knowledge of the existence of such forms has been delayed for a long time by the absence of suitable techniques and concepts to detect and identify this physiological form.

The problem of dormancy in non-differentiating bacteria is a fundamental one since, in the absence of suitable techniques to demonstrate that such bacteria are potentially alive, they are assumed to be dead and therefore of no further relevance in the system being investigated (Kaprelyants *et al.,* 1993). However, the important word in the pervious comment is "suitable" and we are only slowly beginning to appreciate the importance of correctly assessing this class of bacteria in the study of the state of bacteria in natural systems. The dormant state is often characterized by a dramatic change in cell size. This miniaturization has been well characterized in several marine *Vibrio* and *Pseudomonas* strains. During the miniaturization process the periplasmic space becomes enlarged (Marden *et al.,* 1987; Novitsky & Morita 1976), the surface becomes rougher, outer membrane vesicles are formed and the surface becomes more hydrophobic. The last feature can result in increased adhesion both to surfaces and to other cells. In addition, appendages, including fimbrae-like structures have been observed which may assist in mediating adhesion and cell aggregation (Dawson *et al.,* 1981; Kjelleberg & Hermansson 1984). The size difference can be quite pronounced. For *Vibrio* cells, a growing cell typically has a volume of ca. 6 μm^3. Starved cells can be as small as 0.05 μm^3.(Moyer & Morita 1989). Similarly, *Pseudomonas* sp. S9 can decrease from 2 μm^3 to less than 0.1 μm^3 within 24 hours after the transition from a nutrient rich to an environment without nutrients. This miniaturization is due to a reductive cell

division, without biomass increase. This means that ongoing rounds of cell division are first completed and endogenous material in the cell is degraded including storage compounds.

The transition into a dormant state is an active process requiring that the vegetative cells expend energy. There remains some debate as to whether these ultramicrobacteria are actually 'alive' since in order to prove this there needs to be some way to show that the condition is reversible and living cells can arise from these dormant structures. Experimental evidence to support this is not very prevalent. For example in a study of resuscitation of ultramicrobacteria taken from soil, only a small proportion of the total population were capable of forming colonies on agar. (Bakken & Olsen 1987). In another study a population of ultramicrobacteria from seawater were shown to consist of two cell types, one which could be revived to form normal colonies and one which remained in the ultramicrobacteria state and were unable to multiply further (Torella & Morita 1981). There have been many suggestions that these bacteria really are incapable of further rounds of multiplication although this conclusion is dependent on the fact that no appropriate methods had been discovered to resuscitate these bacteria (Morita 1988).

3. The viable but non-culturable controversy and its implications for water quality assessment

The aquatic environment is not the primary environment for most of the pathogenic bacteria and as such they tend to be unable to grow or reproduce under such conditions. Already in 1975, it was seen that when natural cultures of *E. coli* and *Streptococcus faecalis* were exposed to a natural aquatic environment within membrane filter chambers, a significant fraction of these cells lost their ability to form colonies on selective media but were able to form colonies on a non-selective medium (Bissonnette *et al.,* 1975). This was interpreted to mean that many of the cells became injured due to the environmental stress of being exposed to an aquatic environment.

In 1982, it was found that that cells of *Vibrio cholerae* and of *Escherichia coli* could be detected using direct methods of enumeration, but they rapidly lost their ability to form colonies on culture media, during long term starvation studies in seawater (Xu *et al.,* 1982). Subsequently, this same property has been found for a wide variety of other bacteria including *Salmonella*, *Shigella* and *Legionella*. Bacteria in the so-called viable but nonculturable (VBNC) state can take up substrates (Fig 1) and carry out metabolic reactions but have lost the ability to form colonies on agar. Many researchers have questioned both the fundamental basis for such a classification and the physiological existence of such bacteria.

The main problem arises from the fact that the physiological state of VBNC is derived from a methodological deficiency and not necessarily reflected by a true physiological state (Mason *et al.,* 1986). Two lines of thought still exist with one group who believe that VBNC bacteria really represent truly unculturable cells which will never become culturable. This implies that such bacteria, if they are pathogenic, will also fail to elicit disease if taken up by a suitable host in sufficient number. For example, a recent study of the infectivity of non-culturable *Salmonella typhimurium* indicated that pathogenicity was lost concomitantly with the ability to multiply despite the fact that cellular viability according to membrane and genome integrity and respiratory activity were still existent (Caro *et al.,* 1999). In another study, it was shown that *E. coli* added to sterile river water at 37ºC and to sterile artificial seawater at 20 and 37ºC declined in number, as determined using agar plate counts, by 3 to 5 orders of magnitude over a period of 60 days. At the same time, the total bacterial count as determined by direct staining with acridine orange indicated that the bacterial number actually remained unaltered (Fig 2) (Bogosian *et al.,* 1996). Therefore, either the bacteria had failed to grow on the cultivation media because they had entered into the VBNC state or they were simply dead. The authors used a wide range of different techniques to elicit either a growth or a metabolic response from these cells but were unable to do so, and accordingly declared their cells to be dead.

The second line of argument proposes that the VBNC state is a true physiological state representing a pseudo-dormant state akin to that found with some Gram-positive bacteria. Addition of nutrients to a population of predominantly non-culturable cells results in re-growth of the small percentage of culturable cells in that population and prevents true interpretation of the

effects of the nutrient addition on the VBNC bacteria. In fact one series of experiments have shown that addition of nutrients to *Vibrio vulnificus* that were in the VBNC state, actually prevented their reactivation and regrowth (Whiteside & Oliver 1997). Similarly, *Legionella pneumophila* can only be resuscitated from the VBNC state when it is in the presence of the natural host for this bacterium, an amoebae called *Acanthamoeba castellanii*. And a report concerning the resumption of growth by *Enterococcus faecalis* has also been published recently (del Lleo *et al.,* 1998). Therefore, the controversy remains one that revolves around a methodological deficiency and not a physiological one. The presence of dormant cells appears to be almost certain. The question remains as to what extent they are a potential problem in the environment remains unanswered.

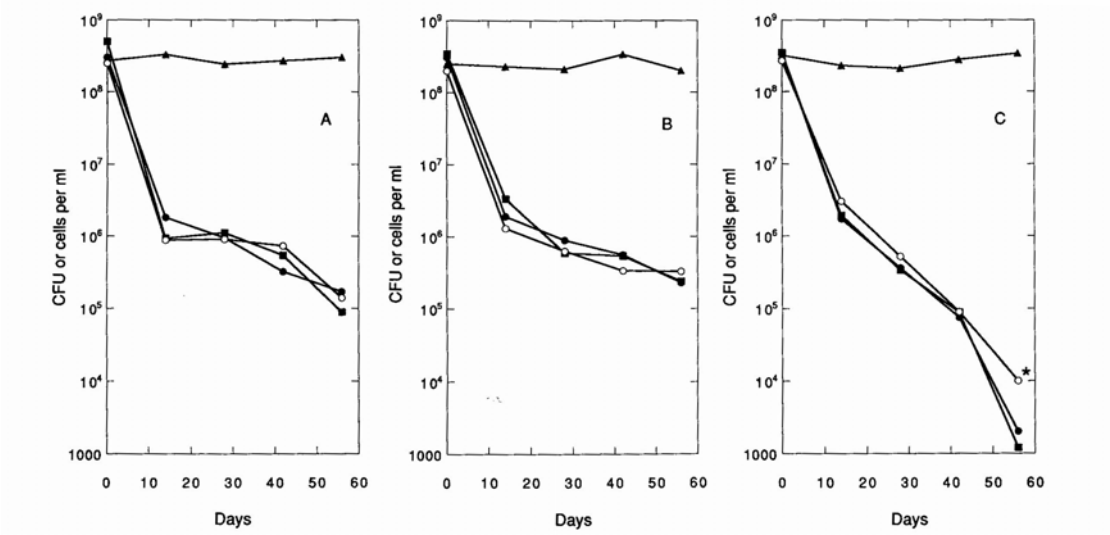

Fig. 2. Decline of E. coli cells in sterile river water at 37ºC (A) and sterile artificial seawater at 20ºC (B) and 37ºC (C). Counts of CFU per milliliter of water by plate counts on LB agar (●), E. coli viable cells per milliliter by MPN estimate (■) and DVC (o), and total cells per milliliter by AODC (▲) are shown. From (Bogosian *et al,* 1996). The data indicate the considerable number of VBNC as indicated by the difference between the viable cell number determinations as compared to the total cell count.

4. The Stress Response and Survival Enhancement

The stress response in bacteria has been the subject of intensive investigation. The response involves a physiological adaptation by the stressed cells, involving enhanced synthesis of a range of proteins that function to protect the cell against the harmful effects of the stress and to promote its survival. The earliest studied, and perhaps the best understood

stress, is that of a heat shock. Since the original phenomenological description of the over-expression of certain proteins, today an vast array of specific proteins that function as emergency response, protection and repair agents have been characterized. Heat affects a cell in several different ways, including membrane fluidity, protein folding, and protein synthesis fidelity etc.. Many of the heat-shock proteins are involved in the repair of damaged, unfolded or misfolded proteins (Gottesman 1996; Lindquist & Craig 1988). Many of these proteins also carry out the same or similar functions in an unstressed cell (Bukau 1993; Thomas & Baneyx 1996).

A generalized scenario for how stress affects cells and how they respond is shown in Fig 3. In this scheme, the response of a cell is shown to promote its survival. Should the stress continue, the cell enters either a physiologically “injured” state or a metabolically disturbed state. The fates of such cells are either cell death or an eventual return to a metabolically active form.

Analogous to the effects of heat, changes in other growth-environmental conditions have been shown to result in enhanced expression of a range of specific proteins. These also function either to protect a cell against the specific detrimental effects of the change in conditions or to provide it with a means to maintain metabolic processes under newly imposed conditions. In the case of survival of enteric bacteria in freshwater or saline water environments, it is necessary to understand how a bacterial cell reacts physiologically when it is taken from a nutrient rich environment such as in mammalian intestines, to a fresh or saline water environment. Studies of such changes have resulted in the identification of a regulator of central importance in the metabolic maintenance of a bacterial cell, the product of the *rpoS* gene, σ^s. Initially found to be stimulated during entry into the stationary phase of batch growth, (Lange & Hengge-Aronis 1991a; Lange & Hengge-Aronis 1991b). it has now been shown that this transcription sigma factor controls the expression of a large number of genes involved in the cellular response to various different stresses. Included in these are starvation, osmotic stress, acid shock, cold shock, heat shock, and oxidative DNA damage (Hengge-Aronis 1996; Hengge-Aronis *et al.,* 1993; Lee *et al.,* 1995; Sledjeski *et al.,* 1996). Over 50 genes have been identified as being under the control of σ^s (Loewen *et al.,* 1998). Under non-stressed “ideal growth” conditions σ^s is highly unstable (Schweder *et al.,* 1996). Under carbon starvation, an increase

in the σ^s content of a cell occurs as a result of increased stability of the sigma protein (Zgurskaya *et al.,* 1997). Mutants of *rpoS* are sensitive to long term starvation and do not develop stationary phase induced tolerance to heat or hydrogen peroxide (Lange & Hengge-Aronis 1991a).

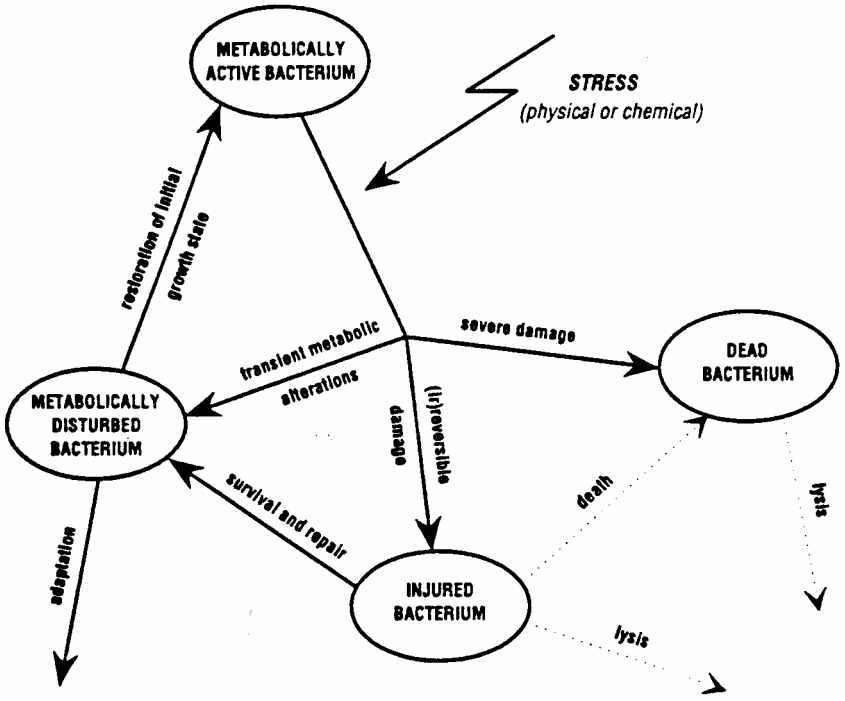

Fig. 3 "Stress cycle": Generalized scheme of effects of adverse environmental conditions on bacteria (from Heitzer et al., 1992).

The morphological and physiological changes which occur in stationary phase cells of *E. coli* or *V. cholerae* have been examined in great detail. The stationary phase is equivalent to the transient stage between nutrient rich and starvation conditions for bacteria. Typically, cells become smaller and rounder, storage compounds such as polyphosphate and glycogen accumulate and the DNA becomes more condensed (Ostling *et al.,* 1993). In addition, there are changes in the cytoplasmic membrane, membrane phospholipids are used as sources of carbon and energy and both the fatty acid, and protein composition of the inner and outer membrane change (DiRusso & Nystrom 1998; Farewell *et al.,* 1998). These modifications are effected via a programmed series of events mediated by σ^s.

Little is known about the mechanism that selects for expression of the σ^s-dependent genes. Some of these are induced only under specific conditions such as osmotic stress or carbon starvation. Regulation can

depend upon a co-activator as is the case with the carbon starvation. A complex stress, such as nutrient exhaustion which causes growth to completely cease results in a sequence of events, coordinated through σ^s activity. Such a "global regulator" ensures that a cell, which is unable to grow and reproduce, will have enhanced resistance to other stresses, such as, for example, osmotic shock.

How a bacteria senses a change to starvation conditions or to other environmental stresses is not yet clear. It is known, however, that a range of different small molecules have been implicated as signal molecules in gene regulation. For example, in actinomycetes, butyrolactones regulate both sporulation and antibiotic production (Horinuchi & Beppu 1992). In *E. coli*, accumulation of the compound 1,5-anhydroglucitol during stationary phase has been suggested to act as a metabolic signal for the nutritional conditions (Shiga *et al.,* 1993) and stationary phase supernatant extracts (SSE) have been shown to inhibit expression of genes necessary for cell division (Garcia-Lara *et al.,* 1996). In *Vibrio* sp. strain 14, it was recently shown that when SSE was added to logarithmic phase cells, a significant number of starvation-induced proteins were induced (Srinivasan *et al.,* 1998).

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5. Fate and Survival of Bacteria in Natural Environments

5.1 Sediments

The role of the sediment in enhancing the survival of bacteria in natural waters is well documented. Bacteria will tend to sediment out either attached to particulate material in the water or as cell aggregates. In a study of the survival of various fecal microorganisms in marine and freshwater sediments, Davies and colleagues showed that a complex relationship existed between growth and predation (Davies *et al.,* 1995). Consequently, the sediments themselves may contain 100 to 1,000 times more bacteria than are found in the overlying water. (Van Donsel & Geldreich 1971). Fecal bacteria have been shown to be capable of extended survival times in sediments and there has even been suggestions that growth may occur in the sediment (Gerba & McLeod 1976; Hood & Ness 1982; LaLiberte & Grimes 1982). However, in most of the cases where growth was seen, this occurred under

experimental conditions with the addition of sterile sediment supplements or under conditions where the test bacterial populations were maintained physically separate from biological interactions in the sediment. Accordingly, while the potential for growth of fecal bacteria in the sediment exists, this does not occur in reality due probably to predation in the sediment. Similarly survival of fecal *Streptococci* and fecal coliforms was extended when cylohexamide was added to bottles containing bacteria together with sediment, as a result of the activity of cyclohexamide as an inhibit or of the activity of the bacterivorous population (Davies *et al.,* 1995). Fecal streptococci appeared to survive longer than did the fecal coliforms in both marine sediments and in freshwater sediments seeded with these two bacteria (Davies *et al.,* 1995). By calculating the ratios of fecal coliforms to fecal streptococci a very high correlation was seen between the two fecal bacteria in the water column but a very low correlation in the sediment from water from the Upper Chesapeake bay in USA (Sayler *et al.,* 1975). This confirmed the fact that the different bacteria exhibit very different survival characteristics in the two different aquatic environments. *Enterococci* are known to be very hardy bacteria. They can grow at temperatures between 10 and 45ºC and even survive exposure at 60ºC for at least 30 min. They can grow at a pH of 9.6 and in the presence of 6.5% sodium chloride. (Cabelli *et al.,* 1982; Sherman 1937a; Sherman 1937b). In addition, fecal streptococci can grow in the presence of sodium azide and although present at a concentration 10 to 100 times less than *E. coli* in treated sewage, they survive very much longer.

5.2 Survival in water

5.2.1 *E. coli* as an indicator?

Most of the work that has been carried out on the survival of fecal bacteria has shown that in sterile water bacteria survive for an extended period of time while when non-sterile water is used for the survival study, the fecal bacteria decline more rapidly (Hood & Ness 1982). Moreover, the patterns of survival differ between different bacterial species. For example, *Vibrio cholerae* has been shown to be able to survive longer than *E. coli* under identical conditions (Hood & Ness 1982). *Salmonella* spp. also died off more slowly than did *E. coli* in an *in situ* test in a river estuary where the test bacteria were kept separate from the rest of the biota by sealing them in a

membrane chamber. This allowed free access of the water and solutes but not passage of other forms of biota (Rhodes & Kator 1988). A basic difficulty arises from data such as these since an indicator organisms such as *E. coli,* should fulfil certain basic criteria including: a) specificity to the source of pathogens or fecal contamination, b) sensitivity for detection and c) resistance to negative environmental factors that either equals or slightly exceeds that of other pathogens. The ideal indicator is consequently the pathogen itself, and some have suggested that a bacterium such as *Salmonella* is a better indicator for pollution in freshwater (Cherry *et al.,* 1972; Elliot & Colwell 1985). The once held opinion that pathogens themselves cannot be used because they are present in much lower numbers and the methods to isolate them are more difficult, is not valid today since there are now a large number of methods being developed to specifically detect such organisms. Consequently, a gradual transition to detection of pathogens is being made.

However, the reason for the difference between the survival of bacteria such as *E. coli* and *V. cholerae* is different to the reason for the difference in the survival between fecal coliforms and fecal streptococci. There is now a large body of evidence suggesting that *V. cholerae* has an environmental source. The bacterium (which causes the disease cholera, is known to be transmitted by water. Nevertheless, the locations of cholera outbreaks remain geographically localized to certain specific areas. It has been shown that *V. cholerae* associates with plankton and has been found in this form even in certain coastal rivers and river sources in the United States as well as in England. Due to adequate water treatment systems, the bacteria does not result in disease in these regions but where water treatment is not sufficient, particularly in the developing countries, outbreaks of cholera still occur. The bacterium is associated with the egg cases of small copepods in the zooplankton. Following phytoplankton blooms, zooplankton grazing increases and the numbers of cholera bacteria increase. This increase is usually associated with outbreaks of disease. For example in Bangladesh the outbreaks are usually bimodal, occurring twice a year, coincident with spring and fall peaks in plankton abundance (Colwell & Huq 1999).

5.2.2 Effects of Temperature on Survival

There are many abiotic factors which affect the survival of fecal and other bacteria in freshwater and seawater environments. Amongst these,

temperature appears to be the most important (Faust *et al.,* 1975). For example, in a study of the survival of various different bacteria both *in vitro* and *in situ* there was a marked difference in survival at 16ºC compared to 29ºC (Chao & Wang 1993). At the lower temperature, most of the bacteria studied (including *Aeromonas*, *Pseudomonas*, *Vibrio*, *E. coli*) were not detected in the *in situ* studies but many were still detected in the in vitro studies. In other work prolonged survival has been shown at low temperatures for a range of bacteria including *E. coli* O157 (Wang & Doyle 1998). However, as already mentioned, most of these studies relied on the exclusion of the eukaryotic component of the river water to show extended survival at lower temperatures (Anderson *et al.,* 1983). Nevertheless, in the absence of eukaryotes the decline in numbers of bacteria is greater at higher temperatures. Similar results indicating the very strong influence of temperature have been found by numerous other studies (Flint 1987; Gurijala & Alexander 1988; Rhodes & Kator 1988).

Such survival studies typically involve the use of filter-sterilized water or autoclaved water into which the fecal bacteria of interest are added. Subsequently their survival as a function of time is monitored using standard enumeration techniques. For example, Flint determined that *E. coli* could survive longer than 260 days at temperatures between 4 and 25 ºC with no apparent loss of viability (Flint 1987). When the same experiment was repeated with filtered water (either coarse filtering or through a 0.45μm filter) or with unfiltered water, significantly reduced survival times were seen. Similarly, by tagging *E. coli* with a bioluminescence gene, it was possible to show that it survived in non-sterile water for 9 to 13 days compared with more than 29 days in sterile water. Similar results have also been found for the survival of *E. coli* K12 in seawater, where addition of eukaryotic inhibitors, or suspension in sterile seawater resulted in extending the survival time of the bacterium (Sorensen 1991). In comparison, the non-fecal *Pseudomonas putida* survived in non-sterile water for more than 137 days (Heller *et al.,* 1992). This led to the conclusion that competition with the natural flora of the water was one of the principal factors resulting in the decrease in the numbers of bacteria.

5.2.3 Strain Related Effects

Many of the studies into bacterial survival in natural water have used laboratory strains of fecal bacteria. Such strains have typically undergone many generations of growth under controlled and often over-idealized conditions and may not always reflect the true behavior of a natural strain of fecal bacteria. This was seen to be the case in one such study on the enteropathogenic bacteria in a freshwater river in Bolivia, where natural strains were found to survive much longer than did laboratory strains incubated under similar conditions (Ohno *et al.,* 1997).

Release of genetically modified bacteria has been a concern form several years by many people since the current era of genetic engineering first erupted. Several studies have tried to examine the relationship between survival and the size of the metabolic burden imposed as a result of carrying plasmids by recombinant fecal bacteria. However, there has been no indication that there is any relationship between the number of or size of plasmids in a recombinant cell and its ability to survive in the aquatic environment (Chao & Feng 1990; Flint 1987).

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6. Bacterivory

The most likely cause for bacterial decline in natural water has been described only qualitatively in most reports. Only a small number of studies have been carried out specifically designed to investigate the process of bacterivory, or the consumption of bacteria for food in natural waters. Of these, only a few were found during the preparation for this literature review that specifically addressed the fate of bacteria of fecal origin. Barcina and colleagues were able to show that when *E. coli* was added to river water, the natural balance within the biota was disturbed and the *E. coli* became the preferred object of predation by the protozoa while the autochthonous natural microbiota became the alternate prey (Barcina *et al.,* 1986). In estuarine water samples, the effects of protozoa were most marked during the first two days following addition of bacteria to the water (McCambridge & McMeekin 1980). However, in a river which was continuously being contaminated with fecal bacteria the activity would probable remain high for a longer period of time, given that other physical and chemical parameters were in order.

Several of the studies carried out have shown that during *in situ* experiments f there was an increase in the numbers of microflagellates and plaque forming microorganisms (Gurijala & Alexander 1988; Rhodes & Kator 1988). In addition, it has been possible to show that there is a relationship between the temperature and the development of the protozoan population and this correlates also with the increased survival rates of bacteria at lower temperatures. Although often assumed to be one of the possible causes of decline of bacteria in natural waters, bacteriophages are unlikely to be responsible since most control experiments carried out using filter sterilized water have resulted in extended survival of the fecal bacteria (Gurijala & Alexander 1988). The bacteriophages pass easily through these membranes while the protozoa will be retained. This provides strong evidence to suggest that the protist population are predominantly responsible for the decline of bacteria in natural water.

In the global food web, it is known that a significant fraction of the production of primary producers (20 – 50%) is channeled through bacteria (Cole *et al.,* 1988). Grazing of bacteria by phagotrophic protists, including flagellates and ciliates is one of the most dominant mechanisms for limiting bacterial numbers in most aquatic ecosystems (McManus & Fuhrman 1988; Nielsen & B.W. 1999; Pace 1988). It is therefore not uncommon to see an increase in the numbers of predators such as flagellates as a result of an increase in bacterial numbers. When a river or other surface water is not polluted by sewage but receives an input of nutrients, the population of natural indigenous bacteria grows causing the predators to exert top-down control on bacterial numbers. (Burns & Schallenberg 1998). There is some evidence for selective grazing by nanoflagelates based on bacterial cell size (Gonzalez 1999) but no information is available regarding grazing on selective species of bacteria. Discharge of disinfected effluents may also affect the bacterivorous activity of protozoa. This is particularly the case with chlorine based disinfectants (Muela *et al.,* 1998). Protozoan predators have been shown to be responsible to a greater degree for the removal of *E. coli* cells added to an estuarine environment than are predatory bacteria such as Bdellovibrio (Enzinger & Cooper 1976). In a study designed to investigate the activity of zooplankton as grazers of bacteria, it was shown that the microzooplankton, including the rotifers were more important as bacterivores than were the macrozooplankton, such as the cladocerans (Hwang & Heath 1999). This study was carried out on the freshwater Lake Erie in the USA, and showed

that more than 60% of bacterial productivity was consumed by the zooplankton in the coastal zone while in the offshore sampling sites the zooplankton bacterivory exceed the bacterial productivity.

Daphnia magna Straus has also been shown in *in vitro* laboratory studies to be an avid consumer of certain pathogenic bacteria (Avtsyn *et al.,* 1984). After 3 to 5 days, no bacteria were detected in flasks with *Daphnia magna Straus* to which cultures of *Salmonella*, *Yersinia* and *Shigella* bacteria were added.

7. Conclusions

The crucial factors affecting the survival of bacteria of fecal origin, which appear in the Yarkon River, can be assumed, on the basis of the information in this literature review, to be temperature and predation. The typically high numbers, which are found during the winter months, coincide with the lower temperatures in the river. Whether the low temperature itself directly affects the increase in bacterial survival or indirectly by inhibiting the activity of the protozoa and other zooplankton remains undetermined. The result is that the numbers of bacteria only begin to decline once the temperature has increased and once the zooplankton population has reached a balanced level in the river. The conditions in the River Yarkon are no different from other similar rivers and the principals determined for the survival of bacteria in other rivers are directly applicable to the Yarkon. Better knowledge of the specific interactions between the various biological systems in the river should lead to the ability to better mange the Yarkon River from the point of view of survival of bacterial pathogens in the water.

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