Radiation Responses Of Foodborne Microorganisms: Recovery From Potentially Lethal Radiation Damage

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Lisa Michelle Lucht

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Food Science Department

April 1997

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RADIATION RESPONSES OF FOODBORNE MICROORGANISMS: RECOVERY FROM POTENTIALLY LETHAL RADIATION DAMAGE

BY

LISA MICHELLE LUCHT

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

MASTER OF SCIENCE

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ABSTRACT

The ability of common foodborne microorganisms to effect repair of radiation-induced potentially lethal damage(PLD) was examined. Microbial cultures in stationary growth phase were harvested, re-suspended in phosphate buffer at 0°C, and irradiated. A two stage incubation protocol was used to determine the ability of irradiated microorganisms to recover from PLD. In the first stage (recovery step) replicates of appropriate dilutions of irradiated cultures were plated onto agar plates and incubated for 20 h at specified temperatures (ranging from 2 to 37°C, respectively) which are suboptimal for growth. Duration of incubation for the irradiated controls was equivalent to the sum of the two separate incubations for the groups subjected to the recovery step. The ratio of survivors assayed with and without a recovery step defined a recovery factor (RF), which reflects the ability of a particular culture to repair potentially lethal radiation damage. RF as a function of radiation dose was determined for different species of microorganisms which are of significance to the food industry. Results of the survey indicated some microorganisms, which included *Escherichia coli*, *Salmonella typhimurium* and *Brocothrix thermosphacta*, show an enhanced survival attributable to the repair of PLD during the recovery step. Optimal resuscitation temperatures ranged from 14 to 22°C. The RF appeared to be dose dependent, with larger doses of radiation

giving rise to larger RFs. *S. typhimurium* irradiated to a dose of 1.5 kGy, and allowed to recover for

20 h, at an optimum temperature of 22°C, exhibited an RF of 161, and *E. coli* irradiated to a dose of 0.52 kGy had an RF of 375. Various other microorganisms including *Listeria monocytogenes*, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Saccharomyces cerevisiae* exhibited an RF of less than 2, regardless of incubation temperature used during the recovery step.

The kinetics of repair and fixation of potentially lethal radiation damage in *E. coli* was also assessed using a two or three-stage incubation protocol. The recovery of *E. coli* irradiated with either gamma rays or high energy electrons was compared. Radiation and heat injury in *E. coli* and *Listeria monocytogenes* (Scott A) were compared and *E. coli* was irradiated in various media to examine the effect of the suspending matrix on the recovery phenomenon. Under these conditions the recovery process appeared to demonstrate biphasic kinetics and continued for at least 48 hours, post irradiation. The magnitude of the recovery factor was exponentially related to dose. *E. coli* was able to effect recovery under the given conditions, irrespective of the irradiation suspension media tested. Under conditions non-permissive for recovery, potentially lethal damage was fixed irreversibly within about 90 minutes. *E. coli* recovered equally well from potentially lethal damage following either gamma ray or high energy electron

irradiation. A comparison of radiation and heat injury in *E. coli* and *L. monocytogenes* using UV absorbance indicated that cellular membrane damage did not appear to greatly contribute to potentially lethal injury caused by irradiation.

Results of these studies demonstrated that some common food-borne microorganisms are capable of effecting significant recovery from potentially lethal radiation damage. This has implications for determinations of efficacy of irradiation in reducing levels of these microorganisms in foods.

ACKNOWLEDGEMENTS

I wish to thank my advisors Dr. Joseph Borsa and Dr. Greg Blank for their expert guidance, support and encouragement. I would also like to thank Atomic Energy of Canada Limited (AECL) for the opportunity to use their facilities to carry out this research. The technical expertise and assistance of all the members of the Radiation Applications Research Section, especially Dr. John Barnard, Wayne Stanley, Minda Chung and Walter Kremers was greatly appreciated.

I thank my parents for instilling in me an appreciation of science and the ambition to pursue my goals and I thank my husband Duane and son Jordan for their love, patience and understanding.

This thesis has been arranged into three main sections consisting of a review of literature and two manuscripts for publication entitled *Recovery of Microorganisms from Potentially Lethal Radiation Damage* and *Recovery of Escherichia coli from potentially lethal radiation damage: Characterization of a recovery phenomenon.* General conclusions are given at the end of the manuscript.

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INTRODUCTION

Food irradiation is the exposure of a food commodity to ionizing radiation for the purpose of achieving a technical benefit. Uses of ionizing radiation include: delay of ripening and sprouting of fruits, vegetables and mushrooms; disinfestation of fruits and herbs and spices, thereby reducing dependence on harmful chemicals such as ethylene oxide and methyl bromide; killing pathogenic organisms such as *Salmonella* and *Escherichia coli* (O157:H7), thereby enhancing the safety of these foods; and extending the shelf-life of products by killing spoilage bacteria (CAST, 1986).

The use of ionizing radiation as a food preservation method is only now beginning to reach significant commercial scale. Food irradiation first began emerging into the commercial and industrial sector in the U.S. in 1992, with the first food irradiation facility opening in Florida. Large scale marketing studies carried out in a number of countries have shown that irradiated food products outsell unirradiated products to informed consumers (Loaharanu, 1994). For example, retail stores in the U.S., selling irradiated products, have reported increased sales (Pszczola, 1993). Marketing studies also suggest that the majority of consumers would prefer to buy irradiated products if they were deemed safe (Bruhn, 1995; Resurreccion et al., 1995). In some cases irradiated products, such as strawberries outsell unirradiated by a margin of 20:1 (Pszczola, 1993). New

acceptance of this process by consumers, and the demand for safer food products has attracted the attention of the food industry in North America, which previously yielded to consumer activism and the fear of public controversy (Lagunas-Solar, 1995). Recent changes in regulations (United States Department of Agriculture and Food Safety Inspection Service) (Loaharanu, 1994) regarding surveillance for pathogenic microorganisms in the meat and poultry industry may hasten adoption of this technology by meat producers in North America. It is already being adopted in the agri-industry for the purpose of quarantine (Loaharanu, 1994). In addition radiation is an excellent alternative to chemical fumigants used for disinfestation which are currently restricted and which are scheduled to be banned in the near future (Loaharanu, 1994).

Irradiation has been shown to be safe and wholesome, as well as an excellent method of inactivating pathogens and spoilage bacteria in foods. However, as in all sub-sterilizing processes, a fraction of the irradiated microorganisms may be in an *injured* but *recoverable state* (Ray 1986, Abiss, 1983; Busta, 1976, 1978;). Under such circumstances, a fraction of the injured microorganisms is able to recover from potentially lethal damage (Ray 1986; Abiss, 1983; Busta, 1976, 1978) which is defined as damage which is lethal unless repaired. In the majority of cases recovery occurs only under suitable conditions. In general, they exhibit more exacting nutritional and physical

requirements for growth, and are more sensitive to their environment than are their uninjured counterparts (MacLeod et al., 1966). This selectivity may include the inability to grow on minimal media, or in the presence of selective agents such as dyes or bile salts, as well as having longer lag times (Mackey and Derrick, 1982) and a smaller colony size (Teufel, 1983). The practical significance of a recovery phenomenon is that it may lead to overestimating the extent of microbial inactivation under certain treatment conditions. Therefore the possibility of a recovery process should be considered when determining the efficacy of irradiation in regards to microbial inactivation.

It is well documented that physical processes such as heating, freezing, drying and irradiation will produce injured or damaged microorganisms (Mossel and Van Netten, 1984). However, the mechanisms of damage will differ as may the manifestation of injury and the conditions for recovery. Recovery from potentially lethal radiation damage warrants investigation since it is an area of research that has not been adequately examined and may have important implications for food processing. In the following review of literature some of the factors contributing to the effects of ionizing radiation on microorganisms, and their recovery from potentially lethal radiation damage are examined.

REVIEW OF LITERATURE

Sources of Radiation Used in Food Irradiation

The sources of ionizing radiation permitted for use with food irradiation are gamma rays (from Co^{60} or Cs^{137}), high energy electrons (up to 10 MeV) and X-rays (up to 5 MeV), (Lagunas-Solar, 1995). High energy electrons are machine generated in an electron accelerator. The main operational difference between processing with gamma rays and electron beams (e-beams) is the *dose rate*. With e-beams, the instantaneous dose rate is several orders of magnitude higher than with gamma rays (Saunders et al., 1993). However, in contrast with gamma rays there is no arbitrary limit of what depth can be penetrated, while 10-MeV e-beams can only penetrate about 5 cm in unit density material ($1 g/cm^3$), (Saunders et al., 1993). Gamma rays transfer energy primarily by Compton scattering collisions with atomic electrons. In electron irradiation, energy is also dissipated as a result of electrons colliding inelastically with other electrons causing ionization and free radical formation. However, electron beams normally dissipate their energy over a shorter path length because the probability for electron-electron and electron-nuclear scattering is much higher than for Compton scattering (Saunders,1993).

Radiation Chemistry Basics

Both gamma rays and electron beam irradiation affect biological organisms in that critical molecular bonds are broken when energy is deposited either by primary radiation or by secondary reactions. Direct action is that chemical change which results from deposition of energy, sufficient to disrupt covalent bonds, directly in the affected molecule (Urbain, 1986). In contrast, indirect action results from the reaction of the molecule of interest with radiation-induced reactive species (generally radiolytic products of water). In the radiolysis of water, principal products include the hydroxyl radical ($^*OH$), the hydrogen atom ($^*H$), the hydrated electron (e^-_{aq}), and molecular products including H_2, H_2O_2 and H_3O^+ (Urbain, 1986).

The radiolysis of water consists of:

$$H_2O \rightarrow 2.7\ ^*OH + 2.7\ e^-_{aq} + 0.55\ ^*H + 0.45\ H_2 + 0.71\ H_2O_2 + 2.7\ H_3O^+$$

The bacterial cell is about 75 percent water (Kelner et al., 1955), thus it would be expected that indirect effects, mediated by the radiolysis of water, play a large role in the inactivation process. The hypothesis that indirect action plays a significant role in cell inactivation is well established (Ewing and Kabala, 1987, Kelner et al., 1955) and have been reported to account for 53-82% of the overall radiobiological effect (Davis, 1973). Lethal changes in critical molecules result largely from the reactions between these critical molecules and reactive radiolytic products

resulting from interaction of radiation with water molecules, although it has been reported that organic radicals can also participate (Blok and Lohman, 1973). Some studies have also suggested that $^*OH$ radicals are responsible for the majority of the damage within the cell (Samuni and Czapski, 1978; Ewing and Kubala, 1987). Overall, the relative importance of each radical or reactive species in the final outcome will be related to the composition of the material being irradiated and to the conditions of irradiation (Urbain, 1986), these are discussed in section 5.

The conditions of storage after irradiation can also affect or modify the damage, through biochemical repair mechanisms. They will not however, affect the radiation chemistry which is essentially instantaneous (Urbain, 1986). The reactive species formed during irradiation participate in a cascading set of reactions, termed an "action cascade". In this action cascade, chemical reactions occur in micro to milli seconds and lead to biochemical reactions which take place within seconds to hours. This in turn leads to physiological and biological consequences which may take even longer times to manifest (Urbain, 1986). In dry or frozen systems where free radicals are either present in reduced yield or else are immobilized, direct effects will play a relatively larger role in the inactivation of microbes (also refer to sections 5.1 and 5.2). It is also possible that damage inflicted on a specific molecule is not lethal because the site is not critical and/or

can be repaired by the cell. This damage is distinguished from potentially lethal damage which will result in cell death under certain conditions.

Mechanism of Radiation Inactivation of Microorganisms

For cell death, the critical target of radiation damage within the cell is generally regarded as being the DNA (Ginoza, 1967; Altman et al., 1970; Ley, 1963). Since there are usually only a few copies of DNA, its integrity is vital for reproduction (Diehl, 1990). Because of it's unique nature, relative to other critical sites such as ribosomes or mitochondria, damage to DNA has far greater impact on the cell. Evidence which supports this conclusion includes: the observation that organisms with large genomes tend to be more sensitive to radiation; the correlation of loss of viability with DNA damage; the observation that highly resistant organisms often have highly effective DNA repair mechanisms (Davies, 1976); as well, experiments have shown the incorporation of labeled bases into newly synthesized DNA segments following irradiation indicating lost or broken segments (Ley, 1973). Single strand (Busta, 1978; Durban et al., 1974) and double strand breaks (Brynjolfsson, 1977) have been detected in DNA following irradiation. Brynjolfsson (1977) developed a mathematical model to predict microbial kill by radiation, based on known radiation effects on DNA and features of the genetic structures of the microorganisms. The author suggested that repair of radiation damage plays a dominant role in the radiation sensitivity of an

organism, and that the genetic material in resistant microbes exists in multiples (providing redundancy). The mechanism of radiation resistance was studied in one of the most radiation resistant bacteria, *Micrococcus radiodurans*, (Kitayama et al., 1977). The extreme radiation resistance of this organism has been explained by its efficient DNA repair capability including the capacity for rejoining of DNA double-strand scissions. In addition to damaging DNA, gamma radiation has been reported to target the cell wall, with minor effects including leakage of cellular components through the membrane (Mossel and Van Nettan, 1984). In contrast, damage to the cell wall plays a relatively larger role in potentially lethal damage caused by other physical processes such as heating and freezing (Mossell and Van Nettan, 1984). The physical conditions during irradiation, the physiological condition of the cell, and the activity of different repair mechanisms will all effect the overall radiation sensitivity of a particular microorganism. The radiation sensitivity of an organism is described by its D_0 value, which is the decimal reduction dose, (the dose required to reduce the population by one log cycle). The D_0 value is calculated as the negative inverse of the slope of the regression line of the radiation survival curve (where surviving fraction is plotted as a function of dose).

Conditions Affecting Radiation Sensitivity of Microorganisms

Temperature

The temperature employed during irradiation can significantly affect the radiation sensitivity of bacteria (Grecz, 1965; Ma and Maxcy, 1981). This may be attributed largely to the influence that temperature has on the amount of available water in the system. Available water influences radiation sensitivity through the production of free radicals which exert a damaging effect on cellular constituents (Huhtanen et al., 1989). The formation of ice for example, restricts the mobility of free radicals, reducing the effectiveness of irradiation (Anellis et al., 1973). It has been estimated that freezing abolishes at least 50% of the indirect effect (Davies, 1976). This accounts for higher radiation resistance or D_o values in frozen systems. For example, Hanis et al., (1989) found that the natural microbiota in chicken was more radiation resistant at -15°C compared to +10°C. However, in this study inoculated organisms did not show this effect, which was attributed to the presence of contaminant organisms suppressing the growth of the inoculant. Bruns and Maxcy (1979) found that the main effect of sub-freezing temperatures during irradiation on *Micrococcus radiodurans* and *Moraxella-Acinetobacter* was an extension of the shoulder in the inactivation curve. Similar to a frozen system, dry systems also lack available water. This extension of the shoulder region was

also observed in air dried samples (Christensen and Holm, 1964), providing further support for the theory that available water affects radiation resistance. The greatest effect of temperature has been reported to occur in the transition from sub-freezing to above freezing temperatures, where below freezing, the amount of available water decreases. For example, the D_o value of *Streptococcus faecium* in phosphate buffer decreased from 2.4 kGy at -30°C to 0.09 kGy at 5°C (Anellis et al, 1973). The radiation sensitivity of *E. coli* (O157:H7) has also been reported to be strongly influenced by temperature during treatment (Thayer and Boyd, 1993). In this study the largest increase in resistance to radiation occurred between 0°C and -5°C, where the D value increased from 0.27 kGy to 0.44 kGy. In contrast, salmonellae species have been reported to be less sensitive to temperature effects during irradiation (Thayer and Boyd, 1990,1991). *Staphylococcus aureus*, suspended in mechanically deboned chicken meat, was also not markedly sensitive to temperature effects during irradiation (Thayer and Boyd, 1992).

Available Water

The water content of the food being irradiated has been reported to affect the radiation sensitivity of suspended microorganisms due to the influence of indirect effects, ostensibly caused by free radical formation (see section 3) (Kelner, 1955; Shamsuzzaman et al., 1993; Ma and Maxcy, 1981). In this respect, desiccated and dried fungal spores of *Aspergillus terreus* were found to be more

resistant to X-rays than when suspended in an aqueous system (Kelner, 1955). Shamsuzzaman et al., (1993) also found *Clostridium sporogenes* spores irradiated in non-aqueous (fats and oils) suspensions had D_0 values in the range of 4.0-11.2 kGy compared to a D_0 value of 2.8 kGy when spores were irradiated in phosphate buffer. The influence of water content was shown by Ma and Maxcy (1981) when lyophilized cultures were allowed to absorb water in a humid atmosphere for 1 hour. Specifically, irradiation (6 Mrad) resulted in a 3 log reduction in *Moraxella-Acinetobacter* cells when subjected to a humid environment compared to only a 1 log reduction while in a lyophilized state.

Composition of Suspending Matrix

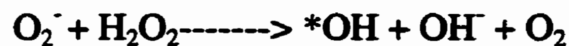
The composition of the matrix in which the microorganisms are suspended during irradiation will affect both the direct and indirect action of the treatment (Jay, 1992). Matrix components have been reported to compete with microorganisms for interaction with radiolytic products of water, giving rise to a protective effect (Urbain, 1986). Often, for this reason, as the complexity of the matrix increases the D_0 value of the microorganisms suspended in that matrix also increases. This is well illustrated in studies using menstrua with and without organic matter. For example, the effect of menstruum on the radiation sensitivity of *L. monocytogenes* was examined in water, phosphate buffer and trypticase soy

broth (TSB) with glucose (Huhtanen et al, 1989). When treated with a 1.0 kGy dose, 5.82 log colony forming units per mL (CFU/mL) were assayed following irradiation in TSB. However, no survivors were detected in water; and counts were just detectable in the phosphate buffer. Patterson (1989) examined the sensitivity of four strains of *L. monocytogenes* irradiated in phosphate buffer and on poultry meat. The D_0 values on poultry meat ranged from 0.481-0.540 kGy. The D_0 values were slightly lower in phosphate buffer ranging from 0.394-0.467 kGy. Moustafa et al. (1989) found that *L. monocytogenes* strains V7, California and Scott A had D_0 values of 1.0, 0.58 and 0.51 kGy respectively, in ground beef compared to 0.50, 0.41 and 0.34 kGy in tryptic soy broth. *Salmonella. seftenberg* was reported to have a D_0 value of 0.504 kGy liquid egg versus 0.130 kGy in a buffer solution (Diehl, 1990). Shamsuzzaman et al., (1989) reported that D_0 values for *Salmonella typhimurium* were greater in complex media. D_0 values of *S. typhimurium* strain K1-2B in phosphate buffer, nutrient broth and on chicken drumsticks were 0.21, 0.40 and 0.32 kGy, respectively. *S. typhimurium* (ATCC 13311) had D_0 values of 0.20, 0.57 and 0.53 kGy, for the same suspending matrices. Dempster (1986) reported that the resistance of *Yersinia enterocolitica* and *Pseudomonas putrifaciens* to radiation increased when the menstruum was changed from water to slurried beef; however, this was not observed for *Staphylococcus aureus*. These reports indicate that media/menstrua have a

significant influence on the radiation sensitivity of microorganisms, and that the quantitative aspects of this influence differs among species, and even strains.

Presence or Absence of Oxygen During Irradiation

The presence of oxygen enhanced the indirect effects of radiation (Stapelton et al., 1952). There are at least two pathways through which oxygen affects the radiodamage. First, oxygen is an efficient scavenger of e_{aq}- yielding O_2^- radicals which can enhance the indirect effects of radiation; and second it has a high affinity for radical sites formed in irradiated biomolecules (Samuni and Czapski, 1978), giving rise to organic peroxides and hydro-peroxides, preventing reconstitution of damaged sites through recombination. Damage due to O_2^- radicals results from direct attack on vital biomolecules, and/or to the formation of secondary $^*OH$ radicals, possibly through the Haber-Weiss reaction (shown below), (Samuni and Czapski, 1978).



$$O_2^- + H_2O_2 \text{-------}> {}^*OH + OH^- + O_2$$

A study by Hollaender et al., (1951) showed a 4 log reduction in *E. coli* cells in an oxygen saturated broth system when given a 60 kiloroentegen x-ray dose; when the broth was purged of oxygen no reduction in cell numbers was observed. Similar research has shown bacteria to be more sensitive to radiation in an aerated system, (Kelner, 1955). Early studies showed that the systematic removal of oxygen resulted in an increased resistance to radiation. It is generally

recognized that anoxic conditions will reduce the effectiveness of irradiation because oxygen contributes radicals which impact on the inactivation of microorganisms. However, under conditions applicable in food systems where the atmosphere has been modified or the amount of oxygen has been reduced, some studies have indicated oxygen may not contribute significant effects. For example, six strains of *Salmonella* irradiated in air or under vacuum showed no significant difference in D_o values (Thayer et al., 1990). Research by Patterson (1988) found *Streptococcus faecalis* and *S. aureus* were unaffected by atmosphere while *Pseudomonas putida, S. typhimurium, E. coli, Moraxella phenylpyruvica* and *Lactobacillus* sp. were more affected, as evidenced by a decrease in their D_o value, when irradiated in atmospheres lacking oxygen. In general these studies showed that a vacuum or CO_2 atmosphere during irradiation had the most lethal effect. Thayer and Boyd (1992) found the D_o value of *S. aureus* to be slightly lower, but not significantly so , *in vacuo*. Given that the solubility of oxygen in water at 20°C is only about 8 ppm the small amount of oxygen that one would expect to be present in food may not significantly effect the microbial lethality during irradiation.

Dose Rate

Depending on the source of radiation, dose rates can vary enormously. An average dose rate for gamma rays is typically about 4 kGy/hr (Saunders et al., 1993) but can vary from 0.06-6.0 kGy/h (Hayashi, 1991). In contrast, 10 MeV electron beams have an average dose rate of 2.2×10^4 kGy/hr for a 50-kW beam (Saunders et al., 1993). Instantaneous dose rates in pulsed sources can be in the 10^6-10^7 kGy/hr range. Nevertheless, it does not appear that there is any significant dose rate effect on microorganisms (Lucht et al., 1997; Ley, 1963; Tarpley et al.,1953; Dewey,1969; Edwards et al.; 1954; Goldblith et al., 1953). It is generally recognized by the medical sterilization industry that dry microbiological systems do not exhibit a dose rate dependence (AAMI, 1992). In wet systems some slight dose rate effects have been reported (AAMI, 1992). In this instance, it has been suggested that the required dose for sterilization may be higher when using a high dose rate because of possible localized oxygen depletion in and around microorganisms in wet systems (AAMI, 1992). A high dose-rate may also produce higher concentrations of radicals, with a corresponding increase in radical-radical recombination reactions, which would reduce the effectiveness of the irradiation. In a complex system, such as food, a dose-rate effect is not expected, because *pseudo* first-order reactions with the main components of the food will predominate at almost any dose rate (Hayashi, 1991). A review by Ley

(1963) cites numerous studies in which results are contradictory with regards to dose rate; some authors suggest that a low dose rate is more efficient in killing bacteria, while others cited, in the same article suggest high dose rates as more efficient. Some researchers have argued that at very low dose rates, sublethally damaged biomolecules may be repaired during the course of a long period of low dose rate irradiation (Hayashi, 1991). In this case one might expect an increase in radiation resistance using a low dose rate. Saunders et al. (1993) reported no effect of dose rate on lethality efficiencies of gamma compared to e-beam under wet and dry conditions in a study which included *Clostridium sporogenes* spores, *L. monocytogenes* and *E. coli.* Lucht et al. (1997) also reported no significant dose rate effects (gamma versus e-beam) on spores of *B. subtilis*, *B. stearothermophilus*, *Aspergillus fumigatus*, *B. pumilus*, and cells of *Candida albicans*, *Cryptococcus albidus*, *Mycobacterium fortuitum* and *Staphylococcus aureus*. Vincent et al., (1990) also found no significant effect of dose rate on the inactivation of *Bacillus*, *Salmonella*, *Staphylococcus* and *Clostridium* species when e-beam and gamma rays were used for inactivation in simulated food systems. Tarpley et al. (1953), Dewey (1969) and Edwards et al. (1954) similarly reported no effect of dose rate in gamma and e-beam irradiated bacteria. Studies carried out on bacteria using two gamma sources having dose rates of 5.7 and 90.6 Gy/min also showed no dose rate effect (Lucht and Stroes-Gascoyne, 1996).

Work by Furata et al (1987) reported that *B. pumilus* sensitivity to gamma irradiation versus e-beam irradiation was not significantly different. Goldblith et al. (1953) compared the effects of gamma rays and 3 MeV electrons produced in a Van de Graaff accelerator on thin samples of *Bacillus thermoacidurans* and *E. coli*. The relative bactericidal effects of the two were essentially the same.

Dependence on Growth Phase

Generally microorganisms in the log phase are more sensitive to almost all types of stress than those in the resting (lag) stage (Mossel and Van Netten, 1984). This has also been demonstrated in regards to irradiation sensitivity. As cells enter the stationary phase of growth a gradual increase in resistance occurs (Kelner, 1955). Thayer and Boyd, (1992) calculated significantly lower D_0 values for *S. aureus* when irradiation was carried out on mid-log phase cells compared to stationary phase cells. Huhtanen et al., (1989) found *L. monocytogenes* cells which were 1.5 and 2.5 hours old (lag phase) had significantly higher D_0 values than was observed for log phase cells (5 or 18 hours).

Characteristics of Potentially Lethally Damaged Cells

A number of reviews have addressed bacterial injury due to stress (Ray, 1986; Mossell and Van Netten, 1984; Abiss, 1983; Hurst , 1977; Busta, 1978 and 1976). Physical stresses which have been demonstrated to cause potentially lethal damage include: heat, cold, drying, freeze-drying, freezing, irradiation and

osmotic shock (Mosell and Van-Netten, 1984). Chemical agents causing stress include detergents, acids, food preservatives, disinfectants and antibiotics (Mossell and Van-Netten, 1984). An organism having potentially lethal damage has conditionally lost its ability to reproduce. It normally exhibits more exacting nutritional and physical requirements for growth; that is, it is more sensitive to its environment. These injured organisms pose potential problems because they may be in a different physiological state from laboratory reference cultures (Busta, 1976) and may exhibit changes in taxonomically relevant characteristics (Teufel, 1983). Injury is expressed as a loss of one or more growth capabilities characteristic of their un-injured counterparts. This includes loss of the ability to grow on minimal media and in the presence of certain selective agents, as well as longer lag times and a smaller colony size. An organism with potentially lethal damage must repair or modify that damage to become fully viable. Recovery from potentially lethal damage can occur under suitable conditions. Pathogenic organisms, in particular, will regain their virulence (Heckly and Blank, 1980; Sorrels et al., 1980; Simon et al., 1963) following recovery from their potentially lethal injury. Therefore injured cells should always be considered and included in viable counts of relevant organisms.

Effect of Post-Irradiation Incubation Temperature on Recovery From Potentially Lethal Radiation Damage

The optimal incubation temperature used to assay survivors following irradiation has been reported to vary with the species and types of vegetative or spore-forming organisms. For injured organisms, the optimum temperature is often lower than for their un-injured counterparts. This phenomenon has been observed in particular with heat-injured spores, including, *B. subtilis, B. stearothermophilus, C. perfringens* and *C. botulinum*, in which lower or more restrictive temperature ranges were optimal (maximum) for recovery (Sofos, 1989). This has also been observed in some vegetative cells following irradiation. For example, Stapelton et al. (1953) found higher counts (approximately 1.5 log) of irradiated *E. coli* when cultures were incubated at 18°C rather than the usual optimal temperature of 35-37°C. *Moraxella* sp. have been shown to have a narrowed growth temperature range following irradiation (Maxcy, 1977). In this case , the organism grew from 5 to 37°C prior to irradiation, however, after irradiation it exhibited a minimum growth temperature range of 20-25°C. The author reported a similar effect on *Salmonella typhimurium*. Higher counts of *Cl. perfringens* spores were reported at 26°C compared to 35°C, following heat or irradiation treatment, but not ethylene oxide treatment (Roberts, 1970). In addition, Futter et al., (1970) found several species of *Clostridium* had a narrowed

and lower optimal incubation temperature following heat, gamma radiation or ethylene oxide treatment. Chowdhurdy (1976) found spores of *Cl. botulinum* had a sharp optimum germination temperature of 40°C following irradiation. This differed from untreated spores which were able to germinate over a temperature range of 20-45°C. Similarly, *B. subtilis* spores showed an optimum recovery temperature of 30°C following heat treatment, while untreated spores could be grown in a temperature range of 15-50°C (Prentice et al., 1974). *E. coli* and fungal spores of *Penicillium notatum* and *Aspergillus niger* damaged by phenol also exhibited lowered optimum recovery temperatures (Chauhan et al., 1966). When growth was delayed by the addition of chloramphenicol (inhibitor of protein synthesis) a higher recovery of irradiated *E. coli* was reported (Gilles, and Alper, 1959). The authors attributed this effect to a metabolic disturbance which may have been counteracted by the chloramphenicol. It is likely that by slowing down protein synthesis either by reducing the temperature or by the addition of chloramphenicol the net effect was to allow time for some cells to effect repair. Interestingly, incubation of microorganisms on selective enrichment media at temperatures higher than 35-37°C has been recommended by several investigators as a general method for the recovery of injured organisms. For example, salmonellae selective enrichment media is incubated at 42-43°C (Andrews,1986). Studies have shown that the number of Salmonella positive samples in dried milk

products was higher when samples were rehydrated at 45°C and pre-enriched at 45°C, as opposed to 25°C and 35°C, respectively (Andrews,1986). This was not the case for pure cultures of freeze-dried *Salmonella typhimurium* (Andrews,1986). Heat-injured (Waterman, 1982) and freeze-stressed (Ray and Johnson, 1984) *Campylobacter jejuni* were reported to repair better when incubated at 42°C. However, this effect may also be due to a reduction in competitive microbiota at elevated temperatures rather than enhanced resuscitation at elevated temperatures.

Growth on Selective Media

One of the most significant characteristics of injured organisms is their temporary inability to grow on selective media. This is significant because selective media are commonly employed for the enumeration of viable bacteria, including pathogens, as a means of assessing the efficacy of a food process. The inability of some injured organisms to grow on selective media has been associated with lesions in the cellular membrane (Hurst, 1977). There is some evidence that the outer membrane of the gram negative cell wall serves as the barrier to the penetration of detergents (Hurst, 1977). Lesions in the cellular membrane can be detected using UV absorbance, since intracellular materials (nucleic acids, proteins) which absorb UV radiation in the 260-280 nm range leak out of the cell and are detected in the supernatant. Membrane leakage has been

detected after heating, freezing, irradiation, drying, osmotic shock, exposure to detergents and antibiotics (Mossel, 1984). The number of injured organisms in a treated population is commonly enumerated as the difference in viable count obtained on non-selective media from the number able to grow on selective media (Busch and Donnelly, 1991).

In the majority of cases, the number of bacteria in a treated population able to grow on selective media are lower than the number able to grow on non-selective media. For example, in studies using differential media following irradiation, Tiwari and Maxcy (1972) found that counts of *Salmonella typhimurium* were slightly lower on a selective medium, violet red bile (VRB) compared to counts on plate count agar (PCA). *For S. typhimurium* the proportion of injured cells did not increase with increasing dose (severity of treatment). Similarly, numbers of irradiated *E. coli* were slightly lower on another selective medium, brilliant green agar (BGA) as compared to PCA (Tiwari and Maxcy, 1972). In contrast, Licciardello et al. (1970) examined the recovery of *Salmonella oranienburg* and *S. typhimurium* when irradiated in chicken meat, and found that Salmonella grew almost equally well on a number of selective and non-selective media. Results showed viable numbers did not differ by more than 0.5 log on any of the media evaluated. A study examining a radiation resistant *Moraxella-Acinetobacter* isolate found that viable numbers were considerably lower on PCA

having a reduced water activity (Aw) (6% glucose) as compared to unsupplemented PCA (Bruns and Maxcy, 1979). The same organism yielded higher recovery (up to 2.5 log higher) on PCA compared to tryptic soy agar (TSA) even though both are considered non-selective media. In a comparison of peptone agar and a synthetic glucose salts medium, Cook and Widdowson. (1967) found higher counts (up to 2 log) of irradiated *E. coli* on the former medium. Very low doses of irradiation did not produce this effect, suggesting that low doses did not produce high numbers of injured cells. This study also found that amino acid supplements did not increase recovery while high concentrations of phosphate (165 mM) decreased recovery of irradiated *E. coli* by about 2 log cfu/mL at the highest irradiation dose (0.5 kGy). Briggs and Yazdany (1970), examined the effect of irradiation on the salt tolerance of various bacterial spores. It was thought that sensitivity to NaCl would reflect the injured fraction of spores. The authors reported that *B. pumilus* spores were not sensitized to salt after 1.6 kGy, sensitization was observed, however after an 8.1 kGy irradiation dose. *B. stearothermophilus* spores were sensitized after a treatment of 1.6 kGy while *B. subtilis* and *B. subtilis var. niger,* irradiated up to 8.1 kGy, had only a slight sensitivity to NaCl in the recovery medium. Okazawa et al.(1978) reported that *B. subtilis* spores following irradiation showed no increase in NaCl sensitivity (up to 15% (w/v).

The lack of sensitivity to high NaCl concentrations, shown by irradiated microorganisms, relative to other destructive modalities (heating, freezing) may be explained by the mechanism of damage. During irradiation damage to cells is mainly focused to DNA and to a lesser extent to the membrane. Selective media tests of the type described here detect membrane damage. Also, it is possible that the proportion of sublethal to lethal damage is lower in irradiated cells relative to that in cell populations exposed to other (sub)lethal processes. This generalization was noted by Mossel and Van Netten (1984) in a review which noted that different types of injury resulting from the same severity of stress (lethality) varied markedly. Irradiation was suggested to be the least injurious (caused the lowest proportion of sub-lethal damage for a given amount of lethality) and heating caused the greatest proportion of sub-lethal injury. The ranking of sub-lethal injury inducing processes was irradiation < freezing < drying < heating, with species specific exceptions (Mossell and Van Netten, 1984).

Length of Lag Phase Following Irradiation

One method of assessing the extent of injured cells is by examining the length of the lag phase. Mackey and Derrick (1982) found that given equivalently lethal treatments, the greatest increase in lag time resulted from heat injured *S. typhimurium* cells, followed by freeze injured cells. Cells given equally damaging treatments of irradiation or desiccation had the shortest lag times, which extended

from 0.5 hours for untreated cells to approximately 5 hours for cells given the severest irradiation treatment. Lag times were calculated as a mathematical fit of the growth curves using the Gompertz function. Maxcy (1977) found considerably longer lag times (nearly 24 h) for *S. typhimurium*, *E. coli* and *Moraxella* sp. following irradiation. However, in this case lag times were estimated from growth curves.

Summary

The literature suggests radiation effectively inactivates microbes through two main mechanisms. The first is via direct action wherein damage to critical targets results from deposition of energy directly to target molecules. The second mechanism is indirect action brought about through the damaging effect of free radicals produced from the radiolysis of water. Indirect action appears to be the main mechanism which inactivates microorganisms in wet systems, including many foods. Accordingly conditions affecting available water will impact on radiation sensitivity of microorganisms. The presence of oxygen, or lack of, does not appear to have a major impact on inactivation of microbes in food, although it does in other systems. The effect of dose rate is not significant in the vast majority of circumstances. The growth phase of microbes will affect their radiation sensitivity, with the stationary phase being most resistant. Non-sterilizing doses of radiation will leave a fraction of cells in a damaged but recoverable state. Relative

to other destructive processes the ratio of sub-lethal to lethal injury caused by irradiation may be lower, as indicated by growth on selective media and lag times of injured cells. Cells with potentially lethal radiation injury exhibit some of the same characteristics as cells injured through other physical processes such as heating and freezing. These characteristics include more exacting physical and nutritional requirements for growth, the inability to grow on selective media, and having a narrowed optimal temperature range for growth. The ability to grow on selective media may be affected following irradiation, however, this is not as pronounced as in cells injured by other modalities, such as heating. Some studies have found cells injured following irradiation grow equally well on certain selective differential media. Certain non-selective media can recover radiation damaged cells better than others. There is a corresponding increase in sensitivity to the environment with severity of treatment. Conditions for recovering injured cells may be optimized by selecting the appropriate media and incubation temperature. While only a limited number of organisms and conditions have been examined with reference to their ability to recover from potentially lethal radiation damage, the available data indicates that the recovery phenomenon is an important consideration in food processing applications.

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Recovery of Foodborne Microorganisms from Potentially Lethal Radiation Damage

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Keywords: Recovery, foodborne, microorganisms, irradiation, damage.

Running title: Recovery of irradiation injured microorganisms

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ABSTRACT

The ability of common foodborne microorganisms to effect repair of radiation-induced potentially lethal damage (PLD) was examined. Microbial cultures in stationary growth phase were harvested, re-suspended in phosphate buffer at 0°C, and irradiated. A two stage incubation protocol was used to determine the ability of the irradiated microorganisms to recover from PLD. In the first stage (recovery step) replicates of appropriate dilutions of irradiated cultures were plated onto agar plates and incubated for 20 h at specified temperatures (ranging from 2 to 37°C) which are suboptimal for growth. This was followed by a second incubation of 24 h at the optimum growth temperature of the organism being examined in order to allow for colony development. Duration of incubation for the irradiated controls was equivalent to the sum of the two separate incubations for the groups subjected to the recovery step. The ratio of survivors assayed with and without a recovery step defined a recovery factor (RF), which reflected the ability of the particular culture to repair potentially lethal radiation damage. RF as a function of radiation dose was determined for different species of microorganisms which are of significance to the food industry. Results of the survey indicated some microorganisms, which included *Escherichia coli*, *Salmonella typhimurium* and

Brocothrix thermosphacta, show an enhanced survival attributable to the repair of PLD during the recovery step. Optimal resuscitation temperatures ranged from 14 to 22°C. The RF was dose dependent, with larger doses of radiation giving rise to larger RFs. *S. typhimurium* irradiated to a dose of 1.5 kGy, and allowed to recover at an optimum temperature of 22°C, exhibited an RF of 161, which was the largest observed. Various other microorganisms including *Listeria monocytogenes*, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Saccharomyces cerevisiae* exhibited an RF of less than 2, regardless of incubation temperature during the recovery step. Results of these studies demonstrated that some common food-borne microorganisms are capable of effecting significant recovery from potentially lethal radiation damage under conditions relevant to the food industry. This has implications for determinations of efficacy of irradiation in reducing levels of these microorganisms in foods.

Introduction

Substerilizing levels of food processing can leave a fraction of the surviving microbiological population in a damaged but repairable state (15,16,17). This type of damage is sometimes referred to as potentially lethal damage (PLD) (5,15). Potentially lethal damage becomes irreversibly lethal if the injury is not repaired or modified within a limited period of time, post-treatment. Bacterial injury resulting from food processing and/or preservation techniques has been extensively reviewed (15).

In general injured microorganisms may lose their ability to proliferate under certain conditions in which the uninjured cells can thrive. In particular, they may exhibit more exacting nutritional and physical requirements for growth and appear more sensitive to their environment as compared to non-injured microorganisms (13). Overall, injury may be expressed as a loss of one or more growth capabilities and may include the loss of ability to grow either on minimal media or in the presence of selective agents such as dyes or bile salts (13). Injured microorganisms may exhibit an extended lag phase prior to growth. In addition, they may form smaller colonies (12). From a food processing perspective, recovery of injured microorganisms may be a significant phenomenon because it may contribute to an under estimation of the surviving population and could over estimate the efficacy of a process. Further, injured microorganisms which can

recover their proliferative potential may pose a problem in regards to identification, due in part, to differences in their physiological state and changes in taxonomically relevant characteristics (5,12,17). A recovery protocol should therefore be included when evaluating the efficacy of a process treatment, especially in foods that are supportive for the resuscitation and/or repair of injured pathogens.

While it is well documented that physical processes such as heating, freezing, drying and irradiation give rise to injured microorganisms, the mechanisms of damage with these treatment modalities differ, as may the manifestation of injury and the conditions for recovery. During the past few years irradiation treatment of foods for the purposes of pathogen control and extension of shelf-life has gained increased acceptance (11,14,19). However, recovery protocols for microorganisms subjected to potentially lethal radiation damage are lacking (15).

The objective of the present investigation was to characterise the occurrence and magnitude of the recovery phenomenon for irradiated microorganisms exhibiting potentially lethal radiation damage. In this respect the response of various spoilage and pathogenic microorganisms was examined following irradiation experiments. Recovery from potentially lethal radiation damage was assessed by incorporating a resuscitation protocol whereby injured cells were

incubated at suboptimal growth temperatures prior to enumeration. The protocol used here was initially described by Stapleton *et al.* (18) when investigating the survival of irradiated *Escherichia coli.*

MATERIALS AND METHODS

Cultures and maintenance

Salmonella typhimurium (ATCC 13311), *Escherichia coli* (ATCC 11775), *Yersinia enterocolitica* (ATCC 23715), *Pseudomonas fluorescens* (ATCC 13325), *Aeromonas hydrophila* (ATCC 7965), *Brocothrix thermosphacta* (ATCC 11509), *Saccharomyces cerevisiae* (ATCC 2360) and *Bacillus subtilis* (ATCC 6063) were obtained from the American Type Culture Collection (Rockville, MD). *Listeria monocytogenes* (Scott A and strain 81-861) were obtained from Dr. J. Farber (Health Canada, Ottawa, ON, Canada). *Listeria innocua*, *Listeria ivanovii* and *Staphylococcus aureus* were obtained from the Food Development Center (Portage La Prairie, MB, Canada). Cultures were maintained on tryptic soy agar (TSA; Difco, Detroit, MI) slants at 4°C with the exception of *S. cerevisiae* which was maintained on potato dextrose agar (Difco).

A. hydrophila and *P. fluorescens* inocula were prepared using tryptic soy broth (TSB, 10 ml, Difco) containing yeast extract (0.6%, Difco) and incubated at 30°C for 24 h. All remaining inocula with the exception of *S. cerevisiae* were similarly prepared, however, incubation was at 37°C for 24 h. *S. cerevisiae* was

grown in yeast and mold broth (Difco) at 30°C for 24 h. All inocula were subcultured at least twice prior to use.

Irradiation of microorganisms

Cultures were grown in their respective broths for 24 h as previously described and centrifuged (10,000 g; 10 min at 4°C). Resultant pellets were washed twice and resuspended in an equal volume of Butterfield's phosphate buffer (BPB).

For gamma irradiation (Gamma cell 220; AECL, Pinawa, MB, Canada) culture aliquots (0.5 ml) contained in sterile screwcap test-tubes (16 x 125 mm) were supported in an aluminum disc assembly with holes at the circumference. The assembly was packed in a 2-L beaker of ice water. Target irradiation doses ranged from 0 to 3 kGy. The gamma dose rate was 1.22 kGy/h. Irradiation treatments were all performed in duplicate. Absorbed radiation doses were confirmed by using radiochromic dye films (GAF) which were enclosed in test-tubes and irradiated with the treatment tubes. Dosimetry standards used were calibrated against international standards.

Survival curves

Following irradiation, the suspensions were serially diluted using BPB and surface plated in duplicate onto basal yeast extract agar (BYEA). Basal yeast extract agar consisted of (g/L): KH_2PO_4, 1.0; $(NH_4)_2HPO_4$, 4.0; $MgSO_4$, 7.0;

sodium citrate, 0.5; dextrose, 10.0; yeast extract (Oxoid, Nepean, ON), 20.0; and agar (Oxoid), 15.0; adjusted to a pH of 6.8 (18). Incubation was at 30°C for 48 h for *A. hydrophila*, *P. fluorescens* and *S. cerevisiae*. The remaining cultures were incubated at 37°C for 24 h. Curves were constructed by plotting survivors (colony forming units; CFU/ ml) versus actual radiation dose, and fitted by linear regression. Radiation sensitivity was expressed in terms of D_{10} values. A D_{10} value is defined as the dose required to reduce a given population by 90% of its initial value, and was determined from the reciprocal of the slope for the straight-line portion of the survival curve.

Evaluation of recovery medium for resuscitation protocol

BYEA was compared to TSA for their ability to support the recovery of injured microorganisms following irradiation, using *E. coli* and *S. typhimurium* as test organisms.

Following irradiation (0 to 0.5 kGy) treatment tubes containing *E. coli* were serially diluted (BPB) and surface inoculated onto replicate plates containing TSA or BYEA, as specified. The culture plates were held at 18°C for 20 h (resuscitation) and then further incubated for 24 h at 37°C to allow colonies to develop. The suitability of BYEA compared to TSA was determined as a ratio of the CFU recovered with the two media.

Following irradiation at a fixed dose (1.0 kGy), *S. typhimurium* cultures were serially diluted (BPB) and surface inoculated onto replicate plates containing TSA or BYEA . The culture plates were incubated at 8, 14, 18, 22 and 30°C for 20 h (resuscitation) followed by additional incubation at 37°C for 24 h (enumeration). Non resuscitated cultures (irradiated and non irradiated) were incubated at 37°C for 44 h. The suitability of BYEA compared to TSA was determined as a ratio of the CFU recovered.

Assay for recovery from potentially lethal radiation damage.

All cultures were grown, harvested and irradiated as previously outlined. The irradiation dose used for each microorganism was initially chosen to reduce the initial population by at least 2 logs (based on the calculated D_{10} values as given in Table 1).

Immediately following irradiation, each culture was serially diluted (BPB) and surface inoculated onto two series of BYEA plates. The series of culture plates first inoculated (control) was immediately incubated at the optimum growth temperature for the organism being studied (*A. hydrophila*, *P. fluorescens* and *S. cerevisiae*, 30°C for 68, 68 and 92 h respectively; all other species were incubated at 37°C for 44 h). A duplicate set of BYEA plates was subjected to a resuscitation protocol to effect recovery of injured cells. In this case culture plates were

incubated for 20 h at specified resuscitation temperatures (2, 5, 8, 14, 18, 22, 26, 30, 35, 37^0 C) and then further incubated (*Aeromonas* and *Pseudomonas*, 30°C, 48 h; *Saccharomyces*, 30°C, 72 h; all remaining cultures, 37°C, 24 h) prior to enumeration. The ratio of CFU/ml assayed with and without resuscitation at each specified temperature was used in calculating a recovery factor (RF). A RF was determined for each microorganism at each treatment dose and each specified temperature.

RESULTS

Survivor profiles for microorganisms irradiated in phosphate buffer at 0°C are shown in Figures 1 and 2. In all cases r^2 values, were greater than 0.91. D_{10} values, calculated using the slopes of the survivor curves, are given in Table 1. Overall the values appeared higher for Gram positive microorganisms; *S. cerevisiae*, the only eucaryote examined, exhibited the highest D_{10} value of 0.62 kGy.

Radiation survival levels for *E. coli* appeared higher on BYEA than on TSA (Table 2). Additionally, the survival ratio on BYEA compared to that on TSA appeared to increase with increasing treatment dose. Only three treatment doses, (Table 2, 0.1, 0.2 and 0.5 kGy), however, resulted in significant differences

Figure 1. Survivor profiles of selected gram negative organisms following irradiation. *E. coli* (11775), a; *E. coli* (O157:H7), b; *S. typhimurium*, c; *A. hydrophila*, d; *P. fluorescens*, e; *Y. enterocolitica*, f.
Each point represents the average of two trials performed in duplicate ± SD.
Appendix tables: 1-6

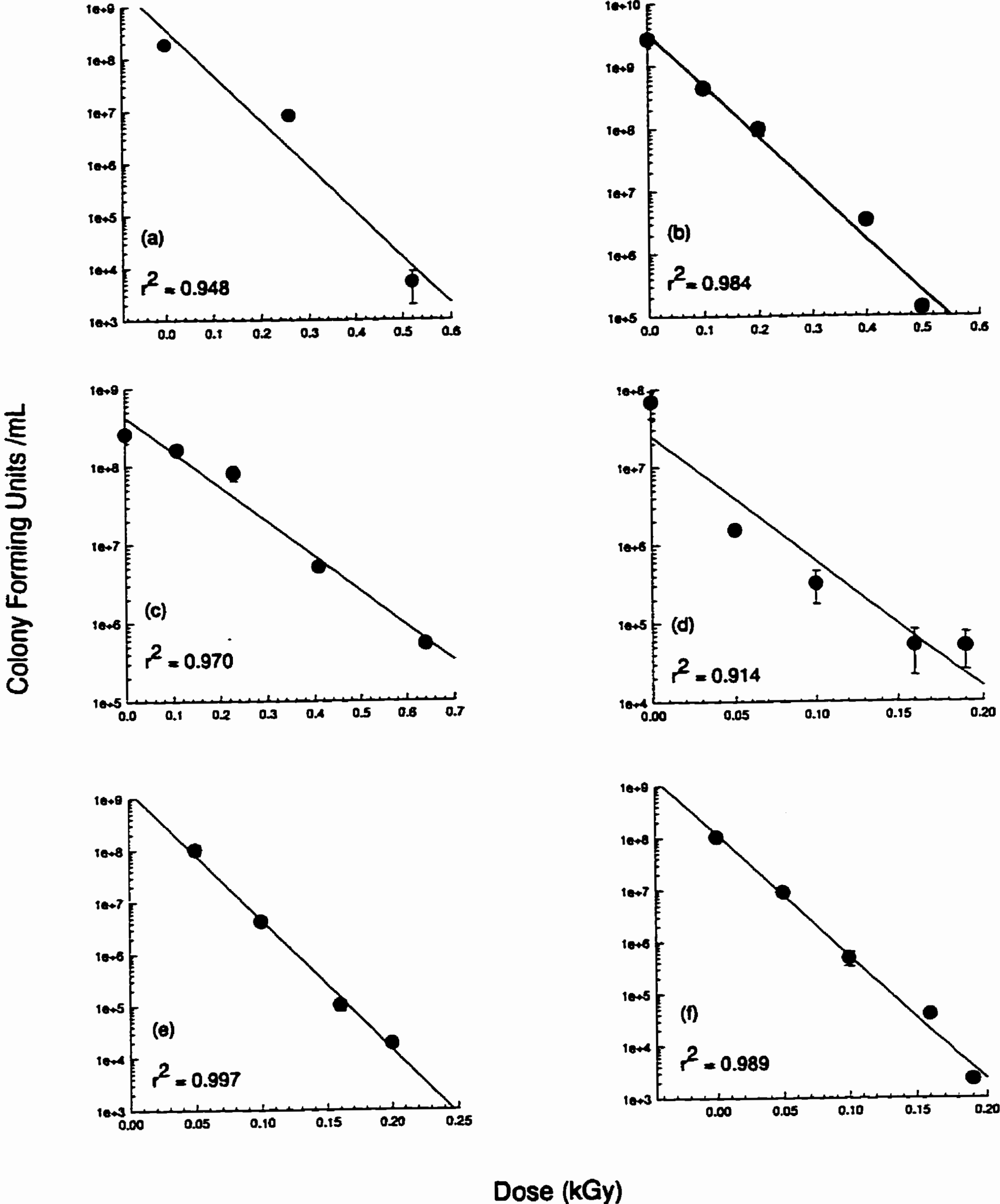

Colony Forming Units /mL
Dose (kGy)
(a)
r^2 = 0.948
(b)
r^2 = 0.984
(c)
r^2 = 0.970
(d)
r^2 = 0.914
(e)
r^2 = 0.997
(f)
r^2 = 0.989

Figure 2. Survivor profiles of selected gram positive organisms following irradiation. *B. thermosphacta*,a; *L. monocytogenes* (81-861), b; *L. innocua*, c; *L. ivanovii*, d;*L. monocytogenes* (Scott a), e; *B. subtilis*, f; *S. aureus*, g; *S. cerevisiae*, h. Each point represents the average of two trials performed in duplicate ± SD. Appendix tables: 7-12.

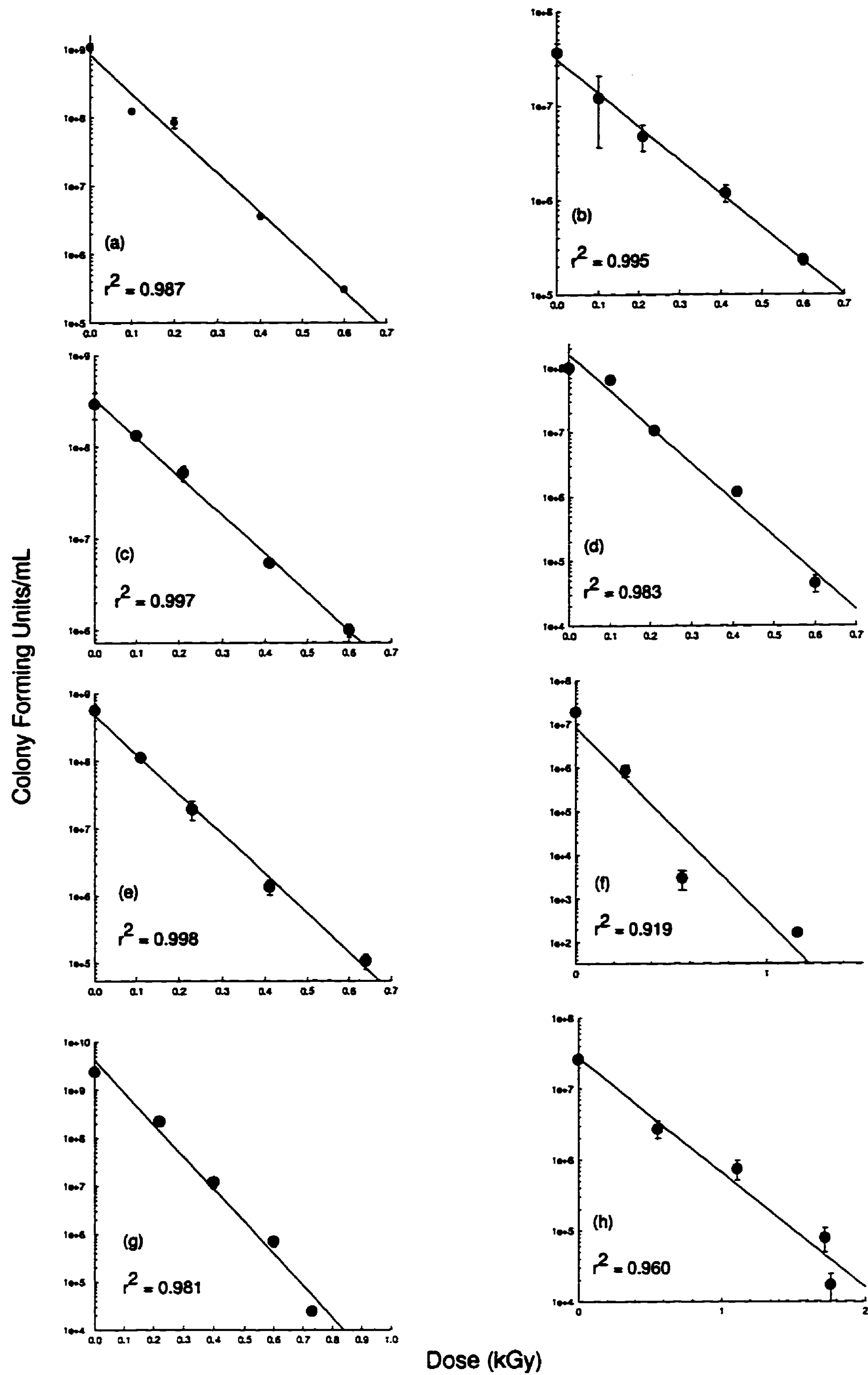

Colony Forming Units/mL
Dose (kGy)
(a)
$r^2 = 0.987$
(b)
$r^2 = 0.995$
(c)
$r^2 = 0.997$
(d)
$r^2 = 0.983$
(e)
$r^2 = 0.998$
(f)
$r^2 = 0.919$
(g)
$r^2 = 0.981$
(h)
$r^2 = 0.960$

Table 1.0 D_{10} values for microorganisms irradiated in phosphate buffer at 0°C.

Microorganism	D_{10} value (kGy)
Gram negative:	
E. coli (11775)	0.11
E. coli (O157:H7)	0.12
S. typhimurium	0.22
A. hydrophila	0.06
P. fluorescens	0.04
Y. enterocolitica	0.04
Gram positive:	
B. thermosphacta	0.17
L. monocytogenes (Scott A)	0.17
L. monocytogenes (81-861)	0.28
L. innocua	0.24
L. ivanovii	0.18
B. subtilis	0.23
S. aureus	0.15
S. cerevisiae	0.62

Table 2.0 Evaluation of recovery media for irradiated *E. coli.*

Irradiation Dose (kGy)	[a]Survivors (CFU/mL) on		Survivor ratio (BYEA:TSA)
	BYEA[b]	TSA[b]	
0	1.03 (± 0.65) x 10^9	8.63 (± 3.04) x 10^8	1.19
0.1	[c]4.66 (± 0.65) x 10^8	3.08 (± 0.44) x 10^8	1.51
0.2	[c]9.90 (± 1.38) x 10^7	3.85 (± 0.82) x 10^7	2.34
0.3	5.93 (± 2.94) x 10^6	1.35 (± 0.14) x 10^6	4.39
0.4	9.08 (± 2.97) x 10^5	1.33 (± 0.32) x 10^5	6.83
0.5	[c]2.43 (± 0.39) x 10^4	2.00 (± 1.73) x 10^3	12.15

[a]Incubation at 18°C for 20 h (resuscitation) followed by 37°C for 24 h.
[b]Values are expressed as means of two trials performed in duplicate ± SD.
[c]Means in rows are significantly different ($P<0.05$; Student t-test).
Appendix table 16.

Table 3.0 Evaluation of recovery media and temperature protocol for *S. typhimurium* following 1.0 kGy irradiation.

Sample	Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL) on		Survivor ratio (BYEA:TSA)
		BYEA	TSA	
Non irradiated	37	1.20 (± 0.09) x 10^9	1.55 (± 0.14) x 10^9	0.77
Irradiated but not resuscitated	37	[c]2.10 (± 0.21) x 10^4	4.10 (± 0.70) x 10^3	5.12
Irradiated: and resuscitated	8	1.76 (± 0.27) x 10^4	3.15 (± 0.78) x 10^3	5.58
	14	c9.88 (± 1.00) x 10^4	4.26 (± 0.30) x 10^4	2.32
	18	c2.63 (± 0.19) x 10^5	1.23 (± 0.07) x 10^5	2.14
	22	c2.60 (± 0.15) x 10^5	9.30 (± 0.14) x 10^4	2.80
	30	c6.38 (± 0.84) x 10^4	2.43 (± 0.26) x 10^4	2.81

[a]Followed by additional incubation (24 h, 37°C).
[b]Values are expressed as means of two trials performed in duplicate ±SD.
[c]Means in rows are significantly different ($P<0.05$; Student t-test).
Appendix table 15.

between survivor levels. No advantage for the use of BYEA compared to TSA was observed for the enumeration of unirradiated *S. typhimurium* (Table 3.0) when incubated at 37°C. For irradiated but non resuscitated samples, however, significantly higher numbers of survivors were enumerated on BYEA compared to TSA (ca. five fold increase in survivors). All irradiated cultures, exhibited an increase in survivors when assayed on BYEA. Interestingly, the use of resuscitation temperatures of 18 and 22°C resulted in approximately a 1 log increase in survivors compared to irradiated, but non resuscitated cultures, regardless of the nature of the recovery medium.

Recovery profiles for the various microorganisms as a function of treatment dose and resuscitation temperature are illustrated in Figures 3 and 4. In the case of *E. coli* and *Salmonella* (Figure 3a, b, c), the extent of recovery appeared to be dose dependent, with larger doses giving rise to higher RF values. In addition, the resuscitation temperature range, at which recovery was most pronounced, appeared to narrow with an increase in treatment dose. With *L. monocytogenes*, increasing the radiation dose had an opposite effect compared to *E. coli* in that the RF values decreased (Fig. 4a, b). Thus, this organism experiences enhanced loss of viability under the same conditions shown to enhance survival for *E. coli* or *S. typhimurium*.

The maximum RF and the optimum resuscitation temperature at which this occurred for each microorganism is presented in Table 4. Microorganisms exhibiting a RF > 2 included: *E. coli*, *S. typhimurium*, and *B. thermosphacta*. Among these organisms, the highest RF (161) was observed for *S. typhimurium* irradiated to a dose of 1.50 kGy. The results also indicated that the RF of *E. coli* (11775) had a stronger dependence on dose than did *E. coli* (0157:H7). Overall optimum resuscitation temperatures ranged from 14 to 22°C. All species of listeriae which were examined exhibited a lack of ability to repair PLD. These results indicated that the ability of different microorganisms to repair radiation-induced PLD varied greatly between species.

Figure 3. The effect of resuscitation temperatures on RFs of selected gram negative organisms following irradiation: (a) *E. coli* (11775) (b) *E. coli* (O157:H7), (c)*S. typhimurium,* (d)*P. fluorescens*, *Y. enterocolitica* and *A. hydrophila.*
Each point represents the means of two trials performed in duplicate ± SD.
Appendix tables: 17-22.

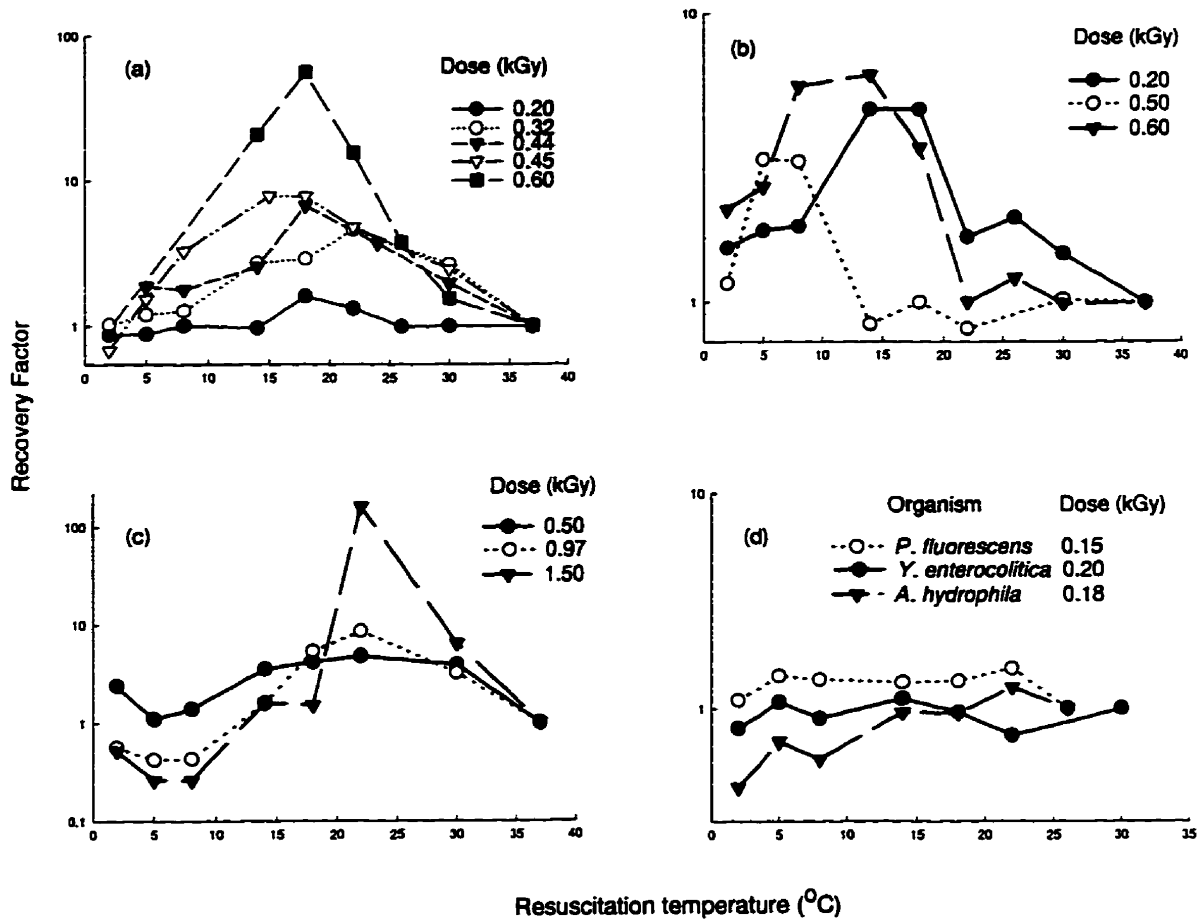
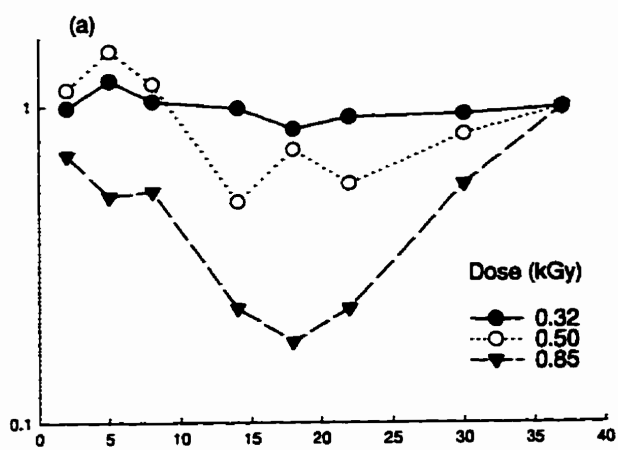
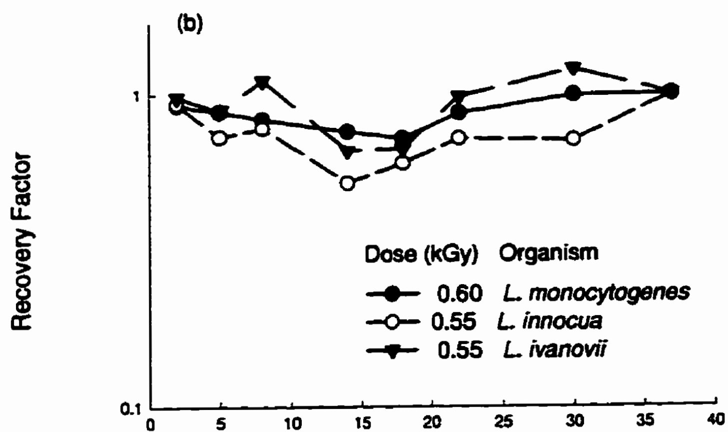

(a)
Dose (kGy)
0.20
0.32
0.44
0.45
0.60
(b)
Dose (kGy)
0.20
0.50
0.60
Recovery Factor
(c)
Dose (kGy)
0.50
0.97
1.50
(d)
Organism
Dose (kGy)
P. fluorescens 0.15
Y. enterocolitica 0.20
A. hydrophila 0.18
Resuscitation temperature (°C)

Figure 4. The effect of resuscitation temperatures on RFs of selected gram negative organisms following irradiation: (a) *L. monocytogenes* (Scott A) (b) *L. monocytogenes*, *L. innocua*, *L. ivanovii* (c) *Brocothrix thermosphacta*, *B. subtilis*, *S. aureus*, *S. cerevisiae*.
Each point represents the means of two trials performed in duplicate ± SD.
Appendix tables: 23-30.


(a)
Dose (kGy)
0.32
0.50
0.85
1
0.1
0
5
10
15
20
25
30
35
40


(b)
Recovery Factor
Dose (kGy) Organism
0.60 L. monocytogenes
0.55 L. innocua
0.55 L. ivanovii
1
0.1
0
5
10
15
20
25
30
35
40

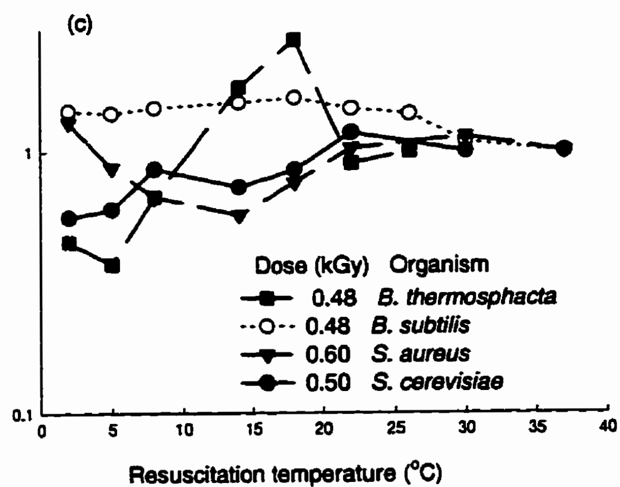

(c)
Dose (kGy) Organism
0.48 B. thermosphacta
0.48 B. subtilis
0.60 S. aureus
0.50 S. cerevisiae
1
0.1
0
5
10
15
20
25
30
35
40
Resuscitation temperature (°C)

Table 4.0 Maximum recovery factors for irradiated microorganisms and corresponding resuscitation temperatures.

Organism	Irradiation dose (kGy)	[1]Maximum RF(± SD)	Resuscitation temperature (°C)
E. coli (11775)	0.20	1.60 ± 0.17	18
	0.32	4.60 ± 0.85	22
	0.44	6.73 ± 1.73	18
	0.45	7.83 ± 1.21	15
	0.60	56.14 ± 12.42	18
E. coli (OH157:H7)	0.20	4.64 ± 1.50	14
	0.20	4.64 ± 1.37	18
	0.50	3.12 ± 0.24	5
	0.60	6.11 ± 0.55	14
S. typhimurium (13311)	0.50	4.81 ± 0.93	22
	0.97	8.57 ± 1.20	22
	1.50	161.29 ± 61.5	22
A. hydrophila (7965)	0.18	1.25 ± 0.19	22
Y. enterocolitica (23715)	0.60	1.11 ± 0.27	14
P. fluorescens (13325)	0.15	1.53 ± 0.48	22
B. thermosphacta (11509)	0.48	2.67 ± 0.73	18
L. monocytogenes (Scott A)	0.32	1.21 ± 0.12	5
	0.50	1.50 ± 0.21	5
	0.85	1.00 ± 0.18	37
L. monocytogenes (81-861)	0.60	1.00 ± 0.30	37
L. innocua (CFPDC)	0.55	1.00 ± 0.12	37
L. ivanovii (CFPDC)	0.55	1.11 ± 0.19	37
B. subtilis	0.48	1.60 ± 0.64	18
S. aureus (25923)	0.60	1.30 ± 0.39	2
S. cerevisiae (2360)	0.50	1.18 ± 0.45	22

[1] Represents the mean of two trials each performed in duplicate ± SD.
Appendix tables: 17-30.

DISCUSSION

Post irradiation environmental conditions, including composition of the medium, (pH, water activity, nutrition) atmosphere and temperature have been reported to affect survivor numbers (15,16,17). Accordingly, this directly affects the resistance (D_{10}) of the organism(s) under investigation and impacts on the efficacy of the process in killing microorganisms. In the current study the use of BYEA for the assay of survivors was based on preliminary studies with *E. coli* and *S. typhimurium*. In both cases, BYEA was shown to support significantly higher levels of survivors. Since both types of media are considered to be relatively non-selective, with similar pH values, it is probable that recovery on BYEA medium was superior due to the presence and/or concentration of factors necessary to effect repair. BYEA contains 2% yeast extract, while TSA contains tryptone and soytone. Yeast extract may contain more readily available factors. Similar studies regarding nutrient effects on recovery of survivors following irradiation have been reported by Bruns and Maxcy (4), and Cook and Widdowson (7). The latter authors working with *E. coli*, reported that survivor numbers, were a function of both dose and type of recovery medium. Interestingly, in the current study the superiority of BYEA appeared dependent on the resuscitation temperature and was maximum at the extremes of the temperature range examined.

The recovery of injured microorganisms, including bacterial spores, has also been reported to vary with the post treatment incubation temperature. For example, recovery of *E. coli* following irradiation increased when incubation was carried out at suboptimal temperatures, specifically at 18°C (18). In contrast, Alper (2) reported that decreasing the post irradiation incubation temperature from 45 to 19°C resulted in lower survivor levels. In the case of bacterial spores, including *Bacillus subtilis,* which were damaged by heat treatment, the use of lower incubation temperatures resulted in increased recovery (13). Changes in the optimal temperature for the recovery of radiation-injured *Cl. botulinum* spores have also been reported (6) ; however, in this case the optimum temperature was 40 °C.

With regards to the resuscitation protocol, the extent of recovery associated with *E. coli* and *S. typhimurium* appeared dose dependent, with larger doses giving rise to higher recovery factors. It would be expected that as the radiation dose was increased the fraction of injured cells would increase proportionally with the number of lethally injured cells. This could result in an increase in the RF. Clearly this phenomenon is not applicable to *L. monocytogenes* (Scott A) since an opposite effect was observed in that lethality was enhanced. Overall, the lack of a resuscitation response shown by *Listeria* spps, *Aeromonas*, *Staphylococcus*, *Yersinia* and *Saccharomyces* appeared to be independent of Gram type. In

addition, there did not appear to be a relationship based on D_{10} values or temperature growth range. Based on results with *E. coli* and *Listeria* sps, however, it does appear that the repair capacity of microorganisms is determined at the genus level. The radiation doses chosen for investigation were partially based on the D_{10} values for each microorganism. In the majority of cases a treatment dose giving a 2-3 log reduction was initially used to evaluate the recovery factor. It is possible that microorganisms which showed no repair ability in the current resuscitation protocol, may respond positively if the treatment dose was increased, although in the case of *L. monocytogenes* (Scott A) this was not realised. Nevertheless further investigation is warranted in this area especially considering that treatment doses up to 3.0 kGy are allowed on meats and poultry (11,14).

The dominant critical target of radiation damage within a microbial cell is DNA (2,3,8,9,10). Incubation of radiation injured cells at suboptimal growth temperatures presumably decreases the rate of DNA duplication preparatory to cell division. This may allow additional time for DNA repair before the damage is made permanent through replication. This is consistent with the finding by Gilles and Alper (8) who reported a higher survival of irradiated *E. coli* when growth was delayed by the addition of chloramphenicol to the assay medium. Suboptimal growth temperatures have been reported to enhance the survival of various food-

borne pathogens (1). Presumably at temperatures suboptimal for growth nucleic acid metabolism favours repair over replication, reflecting favourably on eventual survival. Such suboptimal growth conditions could be particularly prominent in foods containing multiple antimicrobial hurdles since these circumstances appear favourable for repair (12).

In conclusion, suboptimal incubation temperatures were shown to significantly increase survival of various species of radiation injured microorganisms including *E. coli* and *S. typhimurium*. With regards to the recovery of salmonellae, this resuscitation protocol may be particularly relevant in regards to the detection of these organisms on irradiated poultry.

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Recovery of *Escherichia coli* from potentially lethal radiation damage: Characterization of a recovery phenomenon

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Keywords: Recovery, *Escherichia coli*, radiation, damage, recovery.

Running title: Recovery phenomenon in irradiated microorganisms.

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ABSTRACT

The recovery of *Escherichia coli* following irradiation was assessed using a two-stage incubation protocol. In the first stage cells were recovered on basal yeast extract (BYE) agar plates which were incubated at 18°C (resuscitation) for specified time intervals (0 to 48h). In the second stage, the plates were immediately transferred to a 37°C incubator where they were maintained for an additional 24 h and then evaluated. Controls consisted of irradiated cells recovered on BYE agar plates which were directly incubated at 37°C for time intervals equivalent to the total times used in the two-stage incubation protocol. The ratio of colony forming units obtained with and without (control) the resuscitation protocol was used to calculate recovery factors (RF). Depending on the applied dose, the RFs obtained ranged from ca. 2.5 (0.44 kGy) to 15-18 (0.76 kGy) following an optimal resuscitation of 24-28 h. Damage fixation, incurred by incubating treated cells immediately at 37°C for 10 min. prior to their recovery using the two stage protocol, was minimal regardless of dose. Extending the fixation period to 60 min, however, decreased RFs by ca. two-thirds. RFs for cells increased sharply from 1.6 to 375 following irradiation from 0.25 to 0.53 kGy respectively in phosphate buffer at 0°C. In ground beef, RFs increased by ca.

100 following irradiation at the highest dose (1.50 kGy). In contrast, the resuscitation protocol exhibited minimal effects when *E. coli* was irradiated in trypticase soy broth. Survival fractions for *E. coli* following either gamma or electron beam irradiation appeared similar and were higher following the resuscitation protocol. Radiation injury, assessed by survivor growth on BYE agar with and without NaCl and the presence of UV absorbing material from supernatants of treated cells, appeared lower than that observed following heat treatment.

INTRODUCTION

Ionizing radiation is an excellent process method for the reduction and/or elimination of both spoilage and pathogenic food-borne microorganisms (10,13). Similar to other decontamination processes, a fraction of the microbial population may be expected to survive with varying degrees of injury (1,3,4,17,18). In some instances, sublethally injured microorganisms, which fail to undergo repair, perhaps due to the lack of a resuscitation protocol, die. Other injured microorganisms may be better poised to recover from potentially lethal injury particularly if the post-treatment conditions are favourable for their repair.

The recovery of sublethally injured microorganisms is known to be influenced by various factors including the nature and severity of the treatment (9). In addition, the composition of the food matrix in which the treatment is applied and the type(s) of microorganisms targeted are also important (18,19). The recovery of sublethally injured microorganisms is of great importance in the food industry. Not only does it impact on the efficacy of the treatment but it also underscores the potential of some important microorganisms to avoid detection.

The optimal recovery of injured microorganisms from potentially lethal damage (PLD) has been shown to occur under various specific sets of conditions (1,3,4). Conditions, less than ideal for example, may cause irreparable harm and

fix damage (1,3,4). This invariably culminates in cell death. Although recovery protocols have been examined for a variety of microorganisms in foods, the agents of injury or stress investigated have largely been confined to heat, freezing, refrigeration and desiccation (23). Comparatively fewer studies have examined irradiation as a stress agent in regards to the recovery of sublethally injured microorganisms, despite its increasing use as a decontamination procedure in the food industry (13).

The primary purpose of this study was to assess a recovery protocol for sublethally injured cells of *Escherichia coli* following either gamma or electron beam irradiation. The recovery protocol, based on holding injured cells at suboptimal temperatures, was initially described by Stapelton (22) in work performed with *E. coli.*

MATERIALS AND METHODS

Maintenance of cultures and inocula

Escherichia coli (ATCC 11 775) was obtained from the American Type Culture Collection (Rockville, MD). *Listeria monocytogenes* (Scott A) was obtained from Dr. Jeff Farber (Health Canada). Stock cultures were grown (24h, 37°C) and maintained (7°C) on tryptic soy agar slants (Difco, Detroit, MI). Inocula (10%) were transferred to tryptic soy broth (Difco, 25 ml) containing yeast

extract (0.6%, Difco) at two consecutive 24h intervals before use. Incubation was at 37°C.

Irradiation protocol

Culture broths were centrifuged (10,000 g; 10 min) at 4°C and washed twice with sterile Butterfield's phosphate buffer (BPB). The final pellets were resuspended in equal volumes of sterile BPB, unless otherwise indicated.

For gamma irradiation (Gammacell 220, Atomic Energy of Canada), sterile, screw cap, test- tubes (16 x 125 mm) containing 0.5 ml culture ca. 10^8 colony forming units (CFU/ml) were immersed in a 2-L beaker containing crushed ice. An aluminium disc assembly with holes at the circumference was used to position the test-tubes. For electron beam irradiation (1-10/1 linear accelerator, 10 MeV; Atomic Energy of Canada, Pinawa, MB) samples (0.5 ml, ca. 10^8 CFU/ml) contained in screw cap, test-tubes were placed horizontally in a plastic tray on a layer of crushed ice. The absorbed dose in the range from 0 to 3.0 kGy was determined using radiochromic dye films. These films were included in test-tubes and irradiated along with the sample tubes. All treatments were performed in duplicate.

Damage repair and fixation in E. coli

Damage repair in *E. coli* was examined immediately following gamma irradiation (0.44 and 0.76 kGy). Treated culture aliquots were serially diluted (BPB) and surface spread (0.1 ml) onto two series of plates containing basal yeast extract agar (BYEA). The composition of this medium consisted of (g/L): KH_2PO_4, 1.0; $(NH_4)_2HPO_4$, 4.0; $MgSO_4$, 7.0; sodium citrate, 0.5; dextrose, 10; yeast extract (Oxoid, Nepean, ON), 20.0 and agar (Oxoid) 15.0. The final pH was adjusted to 6.8 (22). The first series was initially incubated at 18°C (resuscitated) for 0, 1, 2, 4.5, 5.25, 24, 28 and 48 h and subsequently incubated for an additional 24h at 37°C to promote colony growth. Inoculated culture plates in the second series were directly incubated at 37°C (no resuscitation at 18°C) for 24, 25, 26, 28.5, 29.5, 48, 52 and 72 h respectively. The ratio of viable colonies recovered with and without the resuscitation protocol was used to calculate the recovery factor. Recovery factors (RFs) were determined for each resuscitation period.

Damage fixation was also examined following gamma irradiation (0.42 and 0.77 kGy). In this protocol treated aliquots were serially diluted (BPB) and surface inoculated onto two series of BYEA plates. One series was subjected to a three stage incubation regime consisting of: 37°C for 0, 10, 30, 60, 90, 155, 180 and 240 min (damage fixation) followed by 18°C for 20h. This second stage allowed for the resuscitation or repair of remaining damage in cells containing

PLD. The last stage consisted of incubating the culture plates at 37°C for 24h in order to promote colony development.

In the second series, culture plates were incubated at 37°C immediately following treatment for 44h plus the corresponding time used for damage fixation in the first series. Recovery factors were calculated as a ratio of CFU recovered from each series at each incubation period for each applied dose.

Efficacy of resuscitation protocol based on treatment medium

The efficacy of the resuscitation protocol for *E. coli* was examined following gamma irradiation using either BPB (0.25, 0.47, 0.53 kGy), TSB (0.25, 0.53, 1.0, 1.5 kGy) or sterile, raw ground meat (0.53, 1.0, 1.5 kGy). The latter product (9.9g) was initially frozen (-20°C) in sterile stomacher bags and irradiated (20 kGy) in order to achieve sterility. *E. coli* (0.1 ml, ca. 10^8 CFU/ml) was inoculated into each bag of meat, which was gently massaged, or into 9.9 ml of liquid (BPB or TSB) contained in (16 x 125) screw cap test- tubes. Following irradiation two series of samples were serially diluted (BPB) and surface plated using BYEA. One series of plates was directly incubated at 37°C for 44h. The second series of plates was resuscitated at 18°C for 20h. This was followed by incubation at 37°C

for 24h. Recovery factors were calculated for *E. coli* at each treatment dose in each treatment medium. All treatments were performed in duplicate.

Comparison of recovery between gamma and electron irradiated E. coli

E. coli, suspended in BPB (0.5 ml, ca. 10^8 CFU/ml), was gamma irradiated (0, 0.1, 0.2, 0.3, 0.5 kGy) as previously described. Following treatment two series of samples were serially diluted (BPB) and surface plated using BYEA. One series of samples was directly incubated at 37°C for 44h. The second series was resuscitated at 18°C for 20h then further incubated at 37°C for 24h. Recovery factors were determined for each treatment dose. This procedure was also repeated using electron beam irradiation (0, 0.25, 0.26, 0.32, 0.47 kGy) as previously described. All treatments were performed in duplicate.

Comparison of heat and radiation injury

L. monocytogenes, suspended in BPB (0.5 ml, ca.10^8 CFU/ml) was either gamma irradiated (0,0.1,0.2,0.4,0.6 kGy; as described previously) or heat treated at 55°C. In the latter case, samples placed into screw cap test-tubes were heated in a thermostatically controlled water bath. Following heating (0, 10, 20, 30, 40 and 50 min) the test-tubes were cooled rapidly on ice. Cultures, following either heat or irradiation were serially diluted (BPB) and survivors enumerated by surface

plating using BYEA with and without the incorporation of NaCl (4%, w/v). This protocol was used to assess sublethal salt-sensitive injury (19). The difference in CFU's on media with and without NaCl was used to represent the number of injured microorganisms incurred as a result of treatment. Incubation was at 37°C for 48h.

This experiment was similarly repeated using *E. coli* as the test organism. Although the heat treatment was also performed at 55°C (0, 10, 20, 30 and 40 min) the irradiation dosages were somewhat increased (0, 0.10, 0.20, 0.30, 0.50 and 1.0 kGy). Survivors were again enumerated on BYEA with and without the incorporation of NaCl (2%, w/v) following incubation at 35°C for 48h.

UV Absorption Assay for Membrane Damage

To assess possible differences in damage between heat and irradiation, *L. monocytogenes* and *E. coli* (5.0 ml, ca. 10^8 CFU/ml BPB) were individually inoculated into a series of screw cap (16 x 125 min) test-tubes and heated at 55°C in a thermostatically controlled water bath. Following heating (0, 15, 30, 45 and 60 min) the test-tubes were rapidly cooled on ice and the contents centrifuged (10000 g, 10 min). The resultant supernatants were monitored at 258 nm using an ultraviolet spectrophotometer (HP 8451A). The presence of UV absorbing

material in the supernatant was similarly assessed following gamma irradiation (0, 0.27, 0.50, 0.93 and 1.88 kGy).

RESULTS

Kinetics of Repair and Fixation

Resuscitation of *E. coli* at 18°C for one hour following treatment resulted in RFs of ca. 2.5 (Figure 1). Extending the resuscitation period to 24-28 hours further increased recovery especially for cells treated at the higher dose. In the latter case RFs of ca. 15-18 were realised.

Damage fixation in *E. coli* following irradiation at 0.42 kGy appeared minimal if the initial fixation period was not longer than 10 min (Figure 2). Further increases in the fixation period, however, resulted in a sharp decline in survivors. Following 90 min of fixation, for example, RFs decreased to ca. 1.0. Increasing the treatment dose to 0.77 kGy resulted in a similar damage fixation pattern. In both cases, RFs for *E. coli* decreased by ca. two-thirds within 60 min of fixation.

The influence of treatment medium on the efficacy of the resuscitation protocol for *E. coli* is shown in Figure 3. With phosphate buffer, recovery levels appeared to increase sharply with increases in treatment dose. Doubling the treatment dose from 0.25 to 0.53 kGy, for example, increased RFs from 1.6 to 376.

RFs for *E. coli* in ground beef also increased following the resuscitation protocol, albeit less sharply. In this case a tripling of the treatment dose only resulted in ca. 100 fold increase in recovery. RFs for *E. coli* in TSB appeared the lowest. A doubling of the irradiation dose from 0.25 to 0.53 kGy for example increased RFs by only 5 fold.

The survivor profiles for *E. coli* following either gamma or electron beam irradiation are shown in Figure 4.0. Survivor fractions for *E. coli* following either gamma or electron beam radiation appeared similar and were higher following the resuscitation protocol. As shown in Figure 5, heat treatment of *L. monocytogenes* at 55°C resulted in a progressive decrease in survivors with time. Differences between the surviving fraction of cells on BYEA and BYEA- NaCl, which may be interpreted to indicate the degree of salt-sensitive injury caused by treatment, appeared greater than those observed with radiation. Overall, heat treatment appeared to cause more potentially lethal salt-sensitive damage than did irradiation, for equivalent reductions in the surviving fraction. With *E. coli* (Figure 6), it appeared that irradiation produced little damage of the type which could be detected by the use of selective media. In contrast, injury induced by heat, as indicated by differences in surviving fractions between BYEA and BYEA-NaCl, was very much apparent. Supernatants from cell suspensions of either *E. coli* or *L. monocytogenes* following radiation (0 to 1.88 kGy) contained minimal

Figure 1. Kinetics of repair of potentially lethal radiation damage in *E. coli*. All points represent the means of two determinations, each performed in duplicate ± SD.
Appendix table 32.

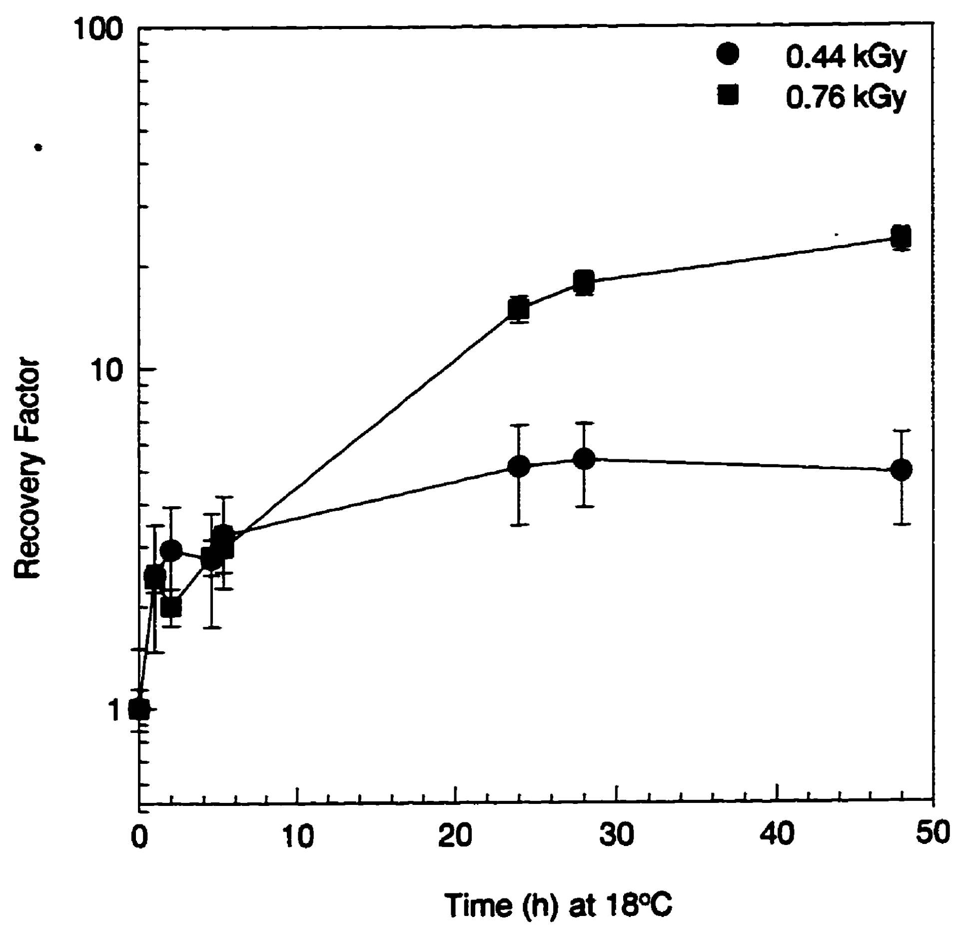

100
10
1
Recovery Factor
0.44 kGy
0.76 kGy
0
10
20
30
40
50
Time (h) at 18°C

Figure 2. Kinetics of fixation of potentially lethal radiation damage in *E. coli*. All points represent the means of two determinations, each performed in duplicate ± SD.
Appendix table 33.

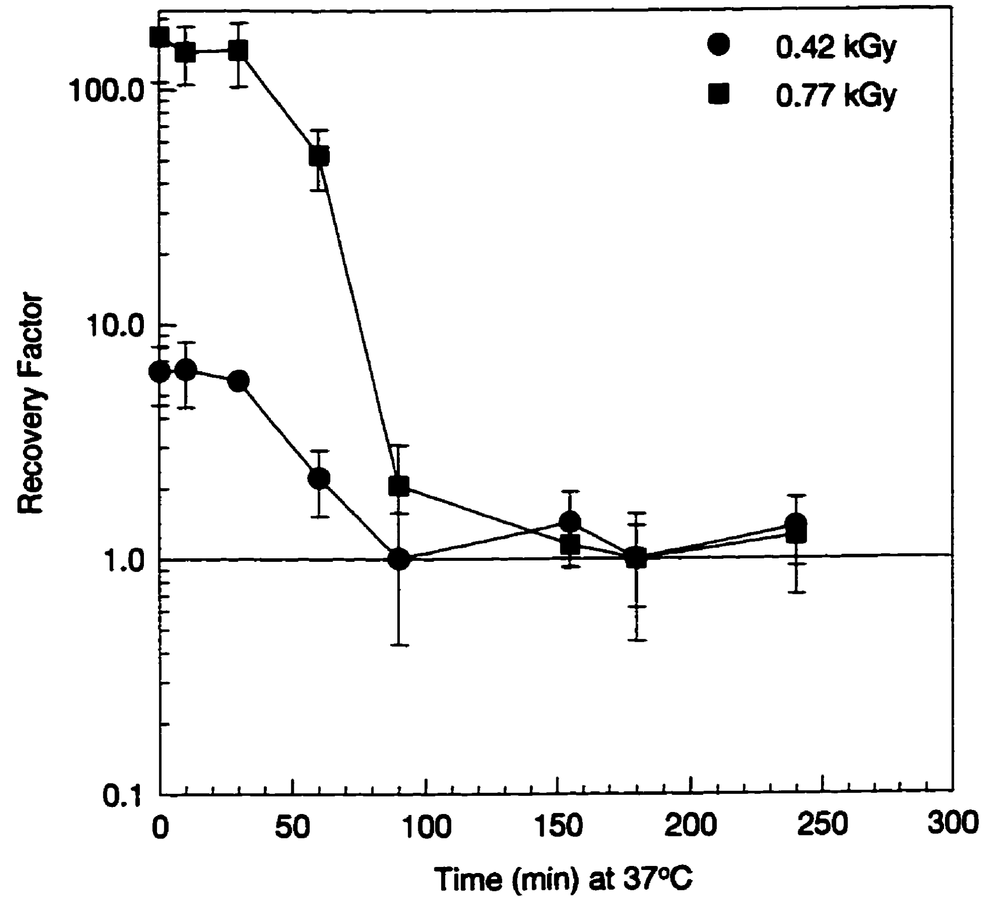
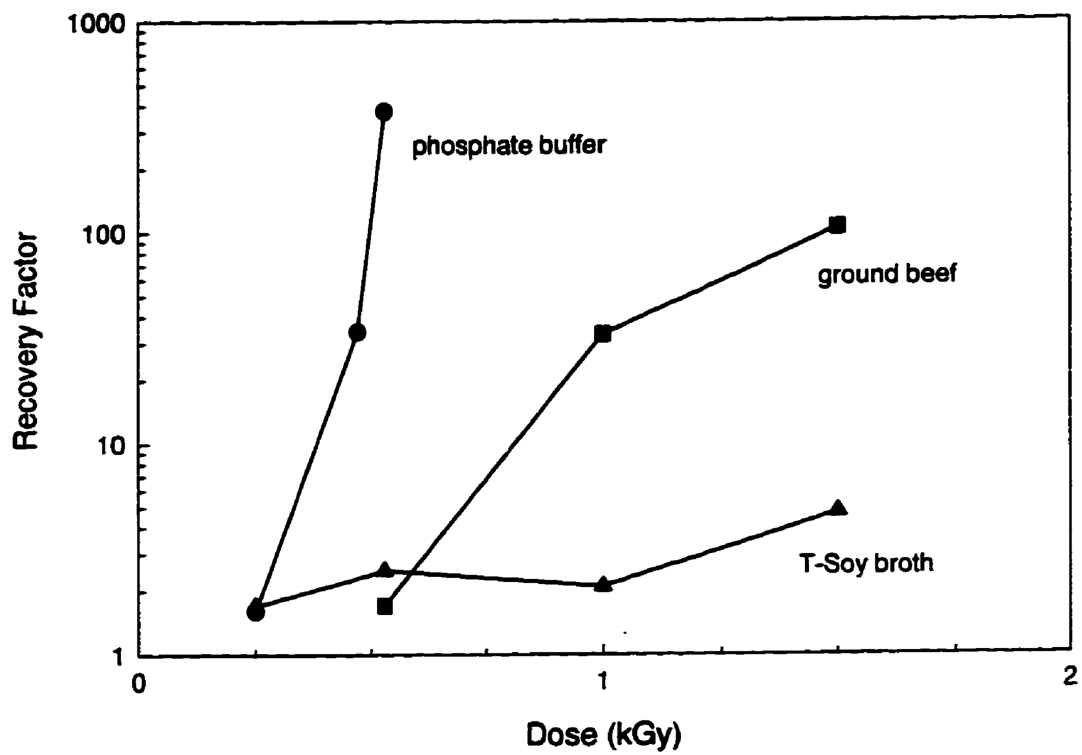

0.42 kGy
0.77 kGy
100.0
10.0
1.0
0.1
Recovery Factor
0
50
100
150
200
250
300
Time (min) at 37°C

Figure 3. Effect of different suspending matrices during irradiation on the recovery phenomenon in *E. coli*. All points represent the means of two determinations, each performed in duplicate.
Appendix table 40.



1000
100
10
1
0
1
2
Recovery Factor
Dose (kGy)
phosphate buffer
ground beef
T-Soy broth

Figure 4. Comparison of recovery from potentially lethal radiation damage for *E. coli* irradiated with either gamma rays or high energy electrons. All points represent the means of two determinations, each performed in duplicate ± SD. Appendix table 38.

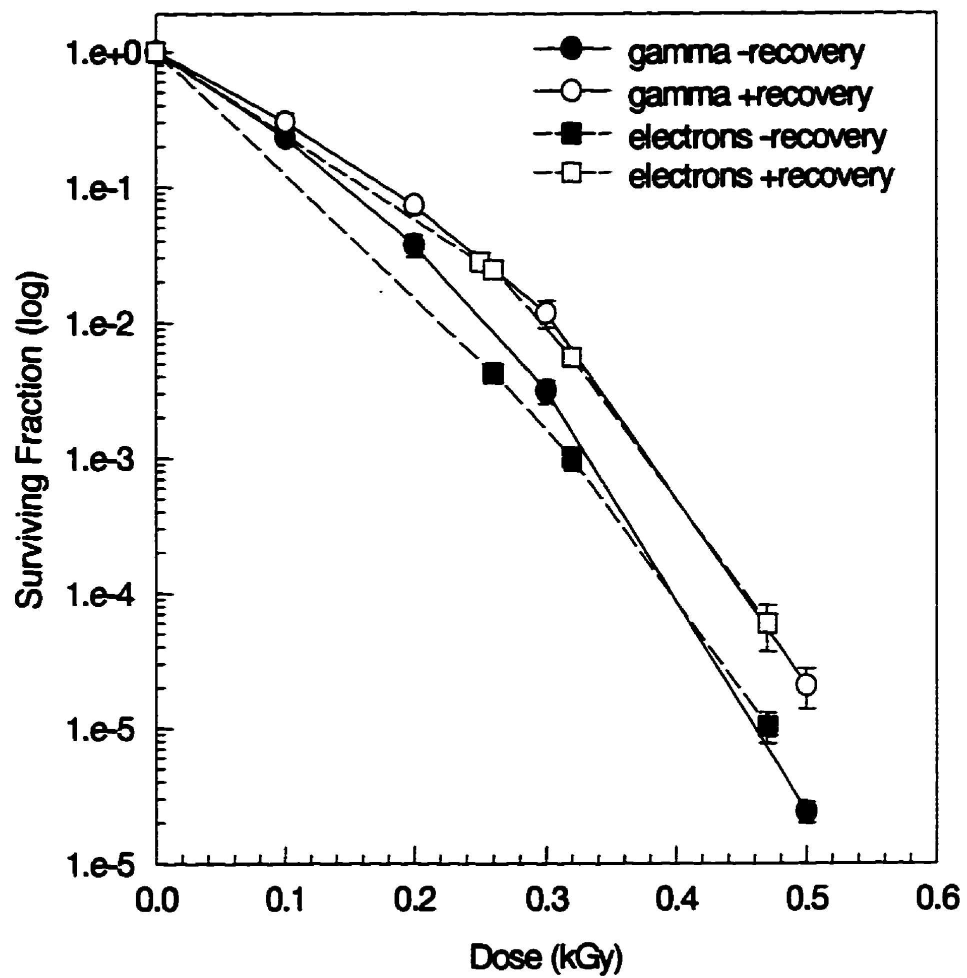

gamma -recovery
gamma +recovery
electrons -recovery
electrons +recovery
1.e+0
1.e-1
1.e-2
1.e-3
1.e-4
1.e-5
1.e-5
Surviving Fraction (log)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Dose (kGy)

Figure 5. Detection of potentially lethal heat or radiation damage in *L. monocytogenes* using selective media. All points represent the means of two determinations, each performed in duplicate ± SD.
Appendix table 34.

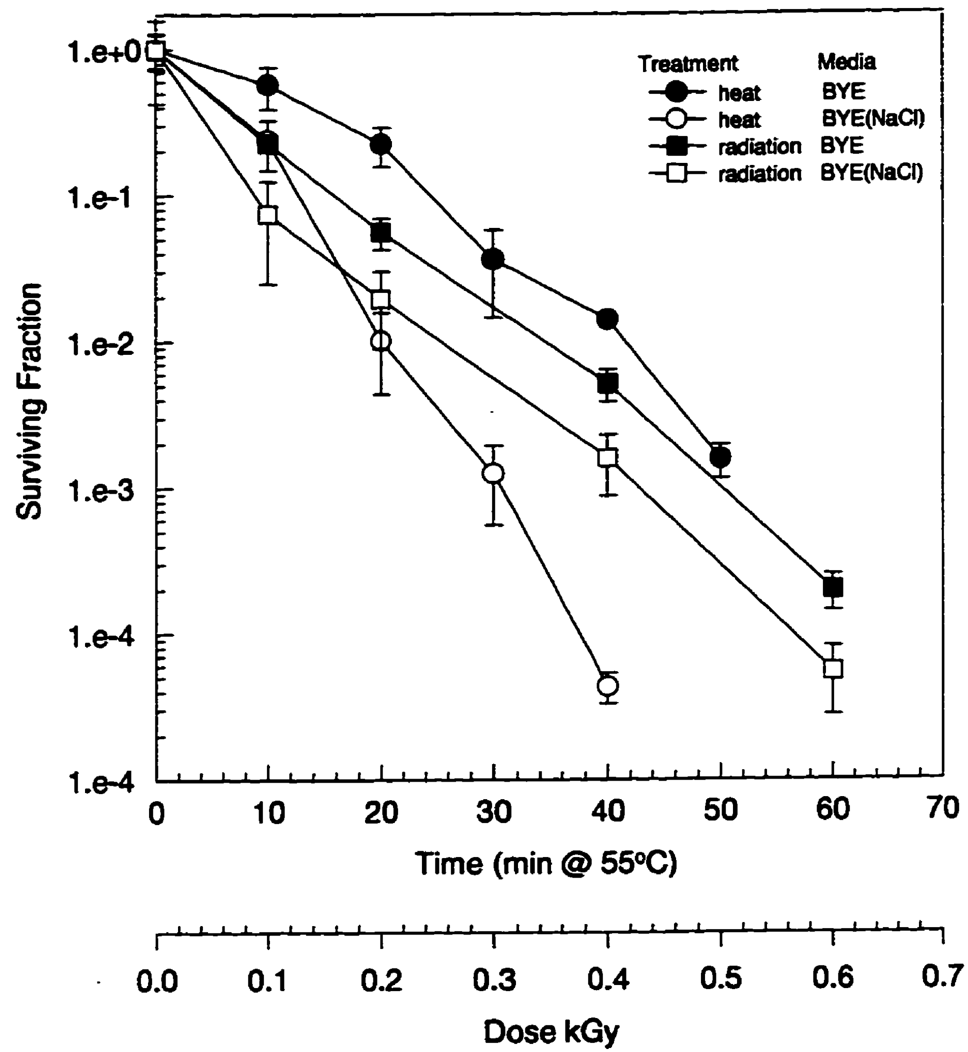
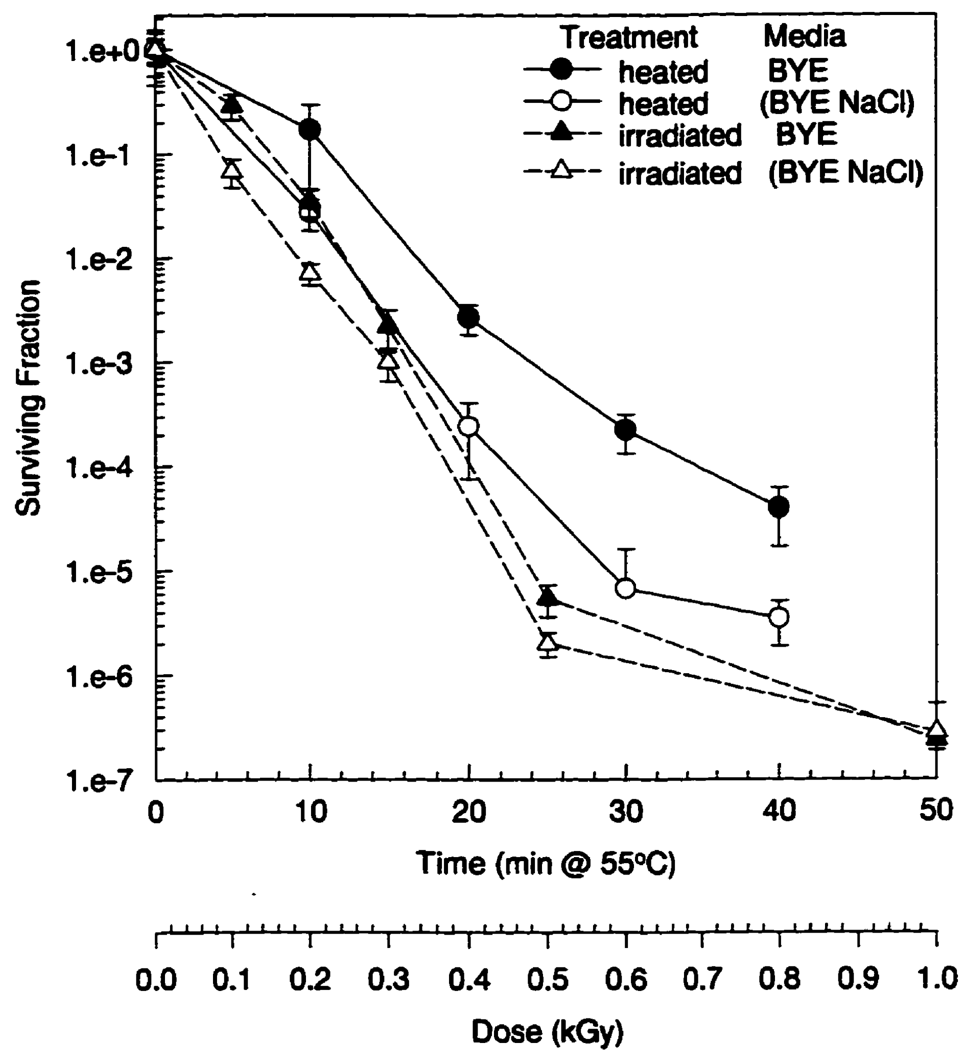
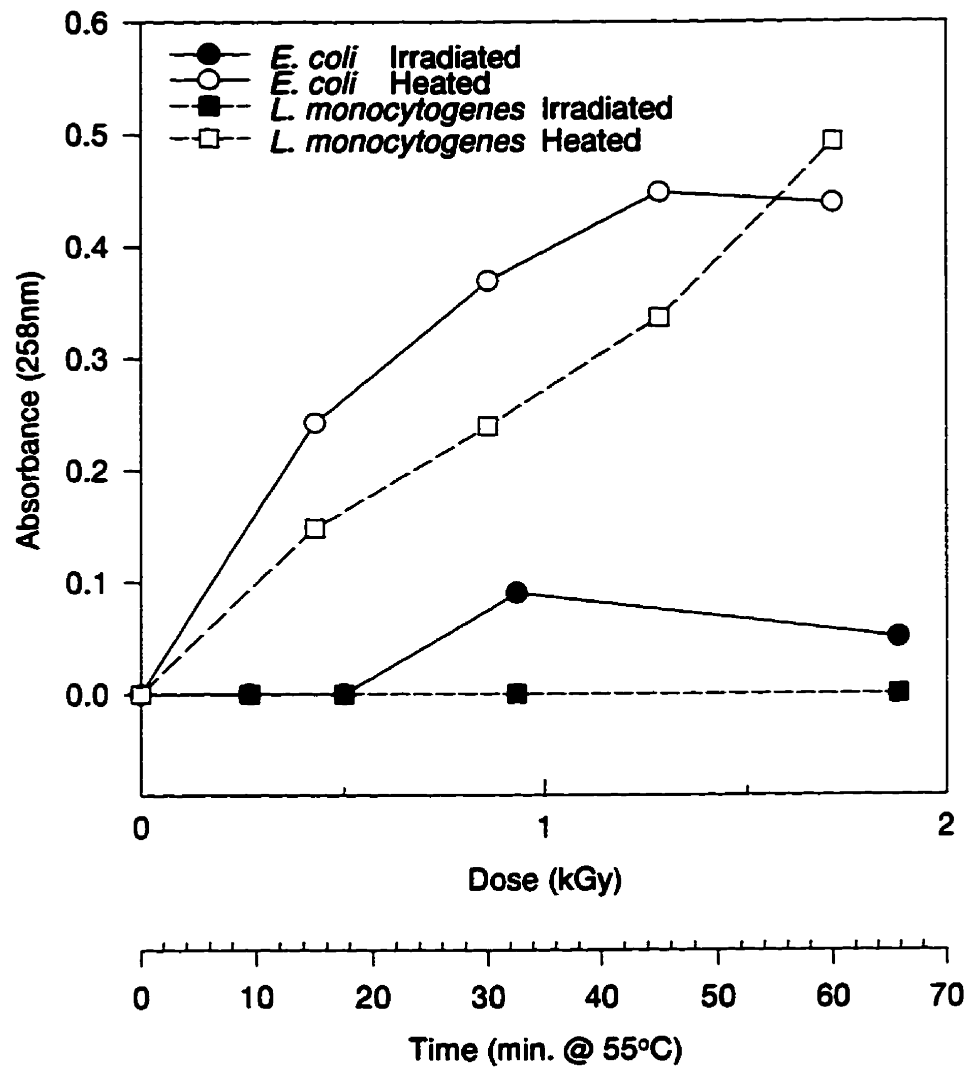

Treatment
Media
heat
BYE
heat
BYE(NaCl)
radiation
BYE
radiation
BYE(NaCl)
Surviving Fraction
1.e+0
1.e-1
1.e-2
1.e-3
1.e-4
1.e-4
0
10
20
30
40
50
60
70
Time (min @ 55°C)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Dose kGy

Figure 6. Detection of potentially lethal heat or radiation damage in *E. coli* using selective media. All points represent the means of two determinations, each performed in duplicate ± SD.
Appendix table 35.



Treatment Media
heated BYE
heated (BYE NaCl)
irradiated BYE
irradiated (BYE NaCl)
Surviving Fraction
1.e+0
1.e-1
1.e-2
1.e-3
1.e-4
1.e-5
1.e-6
1.e-7
0
10
20
30
40
50
Time (min @ 55°C)
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Dose (kGy)

Figure 7. Detection of leakage through the cellular membrane using UV absorbance after heat or radiation treatment in *E. coli* and *L. monocytogenes*. Appendix table 36 and 37.


E. coli Irradiated
E. coli Heated
L. monocytogenes Irradiated
L. monocytogenes Heated
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Absorbance (258nm)
0
1
2
Dose (kGy)
0
10
20
30
40
50
60
70
Time (min. @ 55°C)

UV absorbing material (Figure 7). In contrast absorbance levels of 0.44 to 0.49 were obtained from supernatants of these microorganisms following heating at 55°C for 60 min.

DISCUSSION

The rate and total amount of repair of PLD in injured microorganisms are dependent on various environmental factors including the post-treatment incubation temperature (17). In general, *E. coli* repair optimally at a temperature range from 30 to 37°C (17,18). Although the time of maximum repair differs considerably with the nature of sublethal injury, *E. coli* subjected to either freezing, drying, acid or salt treatment have been shown to repair within 30 min. In contrast heat-injured cells have been reported to take 3 to 4h (24).

Results of the present study indicated that, at 37°C which is also the optimal temperature of growth for *E. coli*, the fixation of potentially lethal damage to permanent damage is essentially complete within ca. 90 min. Holding irradiated *E. coli* at 18°C prior to their incubation at 37°C has been reported to optimally facilitate repair and thereby increase survivor numbers (22). In this study results agreed with those reported by Stapleton (22) and indicated that at 18°C, the repair process continued for as long as 48h. In both repair and fixation studies, the data suggested a logarithmic relationship between treatment dose and the amount of PLD.

The observation that optimal repair of *E. coli* following irradiation occurred when initially maintained at a suboptimal growth temperature is perhaps a phenomenon based on the physiological nature of the stress. Mackey and Derrick (12) for example, reported that after equivalent stress treatments (freezing, drying, heating, acidification and gamma radiation) the repair times were shorter for cells injured by radiation. It is well recognised that the major critical target in irradiated treated bacteria is their DNA (2,7).

It is possible therefore that radiation injured bacteria may differ (either site of injury and/or severity) from those cells injured by other stress agents such as heat. Results in this study, based on survivor numbers obtained on selective (BYEA-NACL) and nonselective media (BYEA) and UV absorption profiles, do indicate that such differences exist between heat and radiation stressed cells. In this respect various authors (5,17,18,20) have reported that the major site of injury caused by heating was located at the cell membrane and in the case of Gram negative bacteria, could include lipopolysaccharide molecules in the outer membrane. Lesions in the cell membrane allow NaCl molecules to pass through the membrane at which point they may interfere with metabolic processes. However, membrane damage has not been reported to be a major consequence as a result of irradiation treatment (14).

It is plausible that a variety of repair mechanisms could operate concurrently within a cell each addressing different sites of injury; individual mechanisms would be expected to operate under their own defined optimal conditions. Stapleton *et al.* (22) for example, suggested that the ability of irradiated *E. coli* to survive treatment was dependent on two counter-current metabolic processes. One process, involved in synthesis, was thought to promote repair and operated at temperatures up to 18°C. The other process, promoted damage and/or permanent injury leading to death and was favoured at temperatures higher than 18°C. Although the exact nature of this destructive process is unknown it is thought to promote increased thermolability of various cellular enzyme-mediated physiological reaction(s) and/or structural components. In support of this explanation is the observation that many microorganisms and spores show enhanced thermal sensitization if pre-irradiated (13).

Holding injured cells at 18°C following either electron or gamma radiation appeared to result in similar levels of repair. Although one major difference between these treatment methods is their dose rate, (with electron beam irradiation, the instantaneous dose rate can be several orders of magnitude higher than with gamma rays) (11,12 21) they have both been reported to affect similar target sites (6,7). Overall the efficacy of the treatments was reduced ca. 10-15% as a result of the repair process.

Recovery of *E. coli* following resuscitation at 18°C was particularly effective for cells irradiated in the least complex matrix, namely phosphate buffer. The reason(s) for this are speculative but the results are consistent with the suggestion that in complex media, a significant fraction of the initial damage is chemically repaired very rapidly (14,17,18). Such repair could result from the presence of naturally occurring repair agents in complex media. The action of these agents is believed to involve oxygen removal, perhaps via oxidation and/or protection of radio-sensitive sites (6)

In phosphate buffer the absence or low concentration of repair agents would limit the recovery process. Only when subsequently cultured on complex medium would increased survivor levels then be expected. Since the lowest RFs were obtained with TSB, it could suggest high intrinsic levels of repair agents. Alternatively, since the log reduction, for a given dose was lower in TSB and ground beef compared to buffer, the results could suggest protective effects. In this respect various studies have demonstrated an increase in radiation resistance in complex matrices (8,15,16).

In conclusion, for *E. coli*, potentially lethal radiation damage is fixed into an irreversible form within ca. 90 minutes if incubation is carried out at its optimal growth temperature. Under conditions optimal for resuscitation, the recovery process appears to be biphasic as evidenced by an initial rapid phase (complete

within ca. two hours) followed by a slower phase which appears to continue for at least 24 hours. Recovery from potentially lethal damage takes place irrespective of the type of menstruum, although the extent of recovery appeared enhanced when cells were irradiated in simple buffer. In addition, the magnitude of the recovery factor following irradiation appeared to be exponentially related to dose. Finally it appeared that the resuscitation protocol was effective for cells treated by either gamma or electron beam irradiation.

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SUMMARY

The recovery of injured microorganisms has been reported to vary with post treatment incubation temperature. The current study found that the ability of radiation injured microorganisms to recover under a low temperature incubation resuscitation protocol varied greatly between species. Results showed the patterns of recovery to be determined at the genus level. *Escherichia coli*, *Salmonella typhimurium* and *Brocothrix thermosphacta* showed an enhanced survival attributable to the repair of potentially lethal radiation damage during the recovery step. The RF appeared to be dose dependent, with larger doses giving rise to larger RFs in a logarithmic relationship. *S. typhimurium* irradiated to a dose of 1.5 kGy, and allowed to recover at 22°C exhibited one of the highest RFs of 161. *E. coli* had an RF of 375 following irradiation to a dose of 0.53 kGy in phosphate buffer and an RF of 105 following irradiation to a dose of 1.50 kGy in ground beef. Other organisms including listeriae, *Staphylococcus aureus*, *Aeromonas hydrophila* and *Saccharomyces cerevisiae* exhibited an RF of less than 2, regardless of the incubation temperature during the recovery step. The viability of all the listeriae species tested appeared to show a dose dependent enhanced mortality under the same resuscitation protocol which enhanced survival in *E. coli*, *Salmonella* and *Brocothrix*. The rate and total amount of repair of PLD varied depending on environmental conditions. This study found that at 18°C the repair

process for irradiated *E. coli* appeared biphasic, with an initial rapid phase (about 2h) and a slower phase which continued for at least 48h. For *E. coli*, PLD was fixed to a permanent form within 90 min if incubation was carried out at 37°C. Again, there was a logarithmic relationship between dose and the magnitude of repair or fixation. *E. coli* showed the ability to repair PLD, using the resuscitation protocol, following irradiation in both simple and complex media. The highest RF values followed irradiation in the simplest media (phosphate buffer), and the lowest in T-Soy broth. Ostensibly, this was attributed to the protective effect offered by a more complex media and the greater ability to repair under more nutritionally favorable conditions. Following the resuscitation protocol *E. coli* appeared able to repair PLD following gamma ray or electron beam irradiation equally well. As a means of identifying a possible site of radiation injury, PLD was assessed using selective and non-selective media and the presence of UV absorbing materials in supernatants of treated cells. These results indicated that differences exist between heat and radiation injured cells. Membrane damage does not appear to be a major consequence of irradiation treatment.

In conclusion, these studies indicated that radiation-induced PLD must be considered when evaluating the efficacy of irradiation as a lethal treatment. This resuscitation protocol may be useful in regards to the detection of salmonellae on irradiated poultry. This type of information may be useful when applying "hurdle"

technology to processed foods as a means of achieving maximum benefits with minimal processing. The results of these studies underscore the importance of the effects of environmental conditions on the recovery of irradiated microorganisms. Comparatively few studies have examined irradiation as a stress agent in regards to the recovery of sublethally injured microorganisms, despite its increasing use as a decontamination process in the food industry. Future investigations might expand on this work by examining other conditions during and post-irradiation treatment. These might include irradiation in other food matrices, examining the effect of the growth phase of the treated microorganism, and examining the ability to recover on other media types particularly traditional isolation and enumeration media.

APPENDIX

Appendix table 1. Survivor profile of *E. coli* (ATCC 11775) following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.86 (± 0.29) 10^8
0.26	8.23 (± 0.46) 10^6
0.52	5.25 (± 3.30) 10^3
0.66	< 10
0.92	< 10

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 2. Survivor profile of *E. coli* (O157:H7) following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	2.77 (± 0.34) 10^9
0.10	4.41 (± 0.48) 10^8
0.20	9.87 (± 2.06) 10^7
0.40	3.41 (± 0.30) 10^6
0.50	1.37 (± 0.26) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 3. Survivor profile of *S. typhimurium* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	2.58 (± 0.34) 10^8
0.10	1.61 (± 0.23) 10^8
0.20	8.10 (± 1.70) 10^7
0.40	5.11 (± 0.36) 10^6
0.60	5.25 (± 0.35) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 4. Survivor profile of *A. hydrophila* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	6.90 (± 2.68) 10^7
0.05	1.56 (± 0.02) 10^6
0.10	3.19 (± 1.44) 10^5
0.15	5.18 (± 3.03) 10^4
0.20	5.07 (± 2.56) 10^4

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 5. Survivor profile of *P. fluorescens* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.35 (± 0.18) 10^9
0.05	1.01 (± 0.20) 10^8
0.10	4.28 (± 0.22) 10^6
0.15	1.03 (± 0.22) 10^5
0.20	1.86 (± 0.11) 10^4

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 6. Survivor profile of *Y. enterocolitica* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.04 (± 0.14) 10^8
0.05	8.98 (± 0.77) 10^6
0.10	4.85 (± 1.47) 10^5
0.15	4.00 (± 0.20) 10^4
0.20	2.28 (± 0.10) 10^3

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 7. Survivor profile of *B. thermosphacta* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.09 (± 0.11) 10^9
0.10	1.25 (± 0.06) 10^8
0.20	8.53 (± 1.58) 10^7
0.40	3.65 (± 0.24) 10^6
0.60	3.05 (± 0.24) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 8. Survivor profile of *L. monocytogenes* (81-861) following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	3.65 (± 0.93) 10^7
0.10	1.20 (± 0. 84) 10^7
0.20	4.73 (± 1.46) 10^6
0.40	1.18 (± 0.24) 10^6
0.60	2.31 (± 0.29) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 9. Survivor profile of *L. innocua* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	2.93 (± 0.93) 10^8
0.10	1.33 (± 0. 12) 10^8
0.20	5.18 (± 0.95) 10^7
0.40	5.36 (± 0.31) 10^6
0.60	9.94 (± 1.53) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 10. Survivor profile of *L. ivanovii* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.00 (± 0.11) 10^8
0.10	6.50 (± 0. 46) 10^7
0.20	1.06 (± 0.08) 10^7
0.40	1.18 (± 0.17) 10^6
0.60	4.55 (± 1.33) 10^4

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 11. Survivor profile of *L. monocytogenes* (Scott A) following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	5.63 (± 0.40) 10^8
0.11	1.11 (± 0. 09) 10^8
0.23	1.92 (± 0.60) 10^7
0.41	1.36 (± 0.33) 10^6
0.64	1.07 (± 0.26) 10^5

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 12. Survivor profile of *B. subtilis* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	1.93 (± 0.26) 10^7
0.26	8.83 (± 2.52) 10^5
0.56	3.00 (± 1.41) 10^3
1.16	1.65 (± 0.26) 10^2
1.82	6.5 (± 2.1) 10^1

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 13. Survivor profile of *S. aureus* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	2.35 (± 0.22) 10^9
0.22	2.22 (± 0. 12) 10^8
0.40	1.20 (± 0.29) 10^7
0.60	7.08 (± 1.50) 10^5
0.73	2.42 (± 0.36) 10^4

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 14. Survivor profile of *S. cerevisiae* following irradiation.

Dose (kGy)	[1]Survivors (CFU/mL)
0	2.61 (± 0.30) 10^7
0.50	2.75 (± 0. 74) 10^6
1.00	7.43 (± 2.30) 10^5
1.50	8.00 (± 3.00) 10^4
2.00	1.72 (± 0.74) 10^4

[1] Values represent the means of two trials each performed in duplicate ± SD.

Appendix table 15. Evaluation of recovery media and temperature protocol for *S. typhimurium* following 1.0 kGy irradiation.

Recovery medium	Sample	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)		
			Trial 1	Trial 2	[2]Mean ± SD
BYEA	non irradiated	37	(1.26) 10^9	(1.14) 10^9	1.2 (± 0.09) 10^9
	irradiated but not resuscitated	37	(2.15) 10^4	(2.05) 10^4	2.10 (± 0.21) 10^4
	irradiated and resuscitated	8	(1.54) 10^4	(1.98) 10^4	1.76 (± 0.27) 10^4
		14	(9.95) 10^4	(9.80) 10^4	9.88 (± 1.00) 10^4
		18	(2.79) 10^4	(2.47) 10^4	2.63 (± 0.19) 10^5
		22	(2.54) 10^4	(2.67) 10^4	2.60 (± 0.15) 10^5
		30	(5.70) 10^4	(7.05) 10^4	6.38 (± 0.84) 10^4
TSA	non irradiated	37	(1.65) 10^9	(1.45) 10^9	1.55 (± 0.14) 10^9
	irradiated but not resuscitated	37	(3.60) 10^3	(4.60) 10^3	4.10 (± 0.70) 10^3
	irradiated and resuscitated	8	(3.70) 10^3	(2.60) 10^3	3.15 (± 0.78) 10^3
		14	(4.50) 10^4	(4.01) 10^4	4.26 (± 0.30) 10^4
		18	(1.23) 10^5	(1.22) 10^5	1.23 (± 0.07) 10^5
		22	(9.20) 10^4	(9.40) 10^4	9.30 (± 0.14) 10^4
		30	(2.60) 10^4	(2.27) 10^4	2.43 (± 0.26) 10^4

[1] Followed by additional incubation (24 h, 37°C)

[2] Two trials each performed in duplicate ± SD

Appendix table 16. Evaluation of recovery media for irradiated *E. coli* (11775).

Recovery Medium	Irradiation Dose (kGy)	[1]Survivors (CFU/mL)		
		Trial 1	Trial 2	[2]Mean ± SD
BYEA	0	(1.35) 10^9	(7.15) 10^8	1.03 (± 0.65) 10^9
	0.1	(4.35) 10^8	(4.98) 10^8	4.66 (± 0.65) 10^8
	0.2	(9.45) 10^7	(1.04) 10^8	9.90 (± 1.38) 10^7
	0.3	(3.50) 10^6	(8.35) 10^6	5.93 (± 2.94) 10^6
	0.4	(6.75) 10^5	(1.14) 10^6	9.08 (± 2.97) 10^5
	0.5	(2.25) 10^4	(2.60) 10^3	2.43 (± 0.39) 10^4
TSA	0	(6.00) 10^8	(1.13) 10^9	8.63 (± 3.04) 10^8
	0.1	(2.76) 10^8	(3.40) 10^8	3.08 (± 0.44) 10^8
	0.2	(3.65) 10^7	(4.05) 10^7	3.85 (± 0.82) 10^7
	0.3	(1.45) 10^6	(1.25) 10^6	1.35 (± 0.14) 10^6
	0.4	(1.30) 10^5	(1.35) 10^5	1.33 (± 0.32) 10^5
	0.5	(3.00) 10^3	(1.50) 10^3	2.00 (± 1.73) 10^3

[1] Incubation was carried out at 18°C for 20 h followed by 24 h at 37°C.

[2] Mean of two trials each performed in duplicate ± SD.

Appendix table 17. Survivors and recovery factor for *E. coli* (11775) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[4]Recovery Factor
		Trial 1	Trial 2	[3]Mean ± SD	(± SD)
0.20	2	(1.16) 10^8	(1.53) 10^8	1.34 (± 0.25) 10^8	0.87 (± 0.26)
	5	(1.34) 10^8	(1.36) 10^8	1.35 (± 0.15) 10^8	0.88 (± 0.19)
	8	(1.51) 10^8	(1.62) 10^8	1.56 (± 0.14) 10^8	1.01 (± 0.18)
	14	(1.46) 10^8	(1.53) 10^8	1.50 (± 0.21) 10^8	0.97 (± 0.23)
	18	(2.46) 10^8	(2.48) 10^8	2.47 (± 0.07) 10^8	1.60 (± 0.17)
	22	(2.01) 10^8	(2.07) 10^8	2.04 (± 0.11) 10^8	1.32 (± 0.17)
	26	(1.14) 10^8	(1.77) 10^8	1.52 (± 0.35) 10^8	0.99 (± 0.32)
	30	(1.83) 10^8	(1.26) 10^8	1.54 (± 0.36) 10^8	1.00 (± 0.32)
	[2]37	(1.43) 10^8	(2.37) 10^8	1.54 (± 0.14) 10^8	1.00 (± 0.18)
0.32	2	(3.44) 10^6	(3.42) 10^6	3.43 (± 0.58) 10^6	1.02 (± 0.33)
	5	(4.50) 10^6	(3.54) 10^6	4.02 (± 1.00) 10^6	1.20 (± 0.44)
	8	(4.30) 10^6	(4.22) 10^6	4.26 (± 0.29) 10^6	1.27 (± 0.27)
	14	(1.02) 10^7	(7.84) 10^6	9.10 (± 1.58) 10^6	2.72 (± 0.61)
	18	(1.00) 10^7	(9.36) 10^6	9.68 (± 0.74) 10^6	2.89 (± 0.54)
	22	(1.59) 10^7	(1.50) 10^7	1.54 (± 0.17) 10^7	4.60 (± 0.85)
	30	(8.67) 10^6	(9.11) 10^6	8.89 (± 0.64) 10^6	2.65 (± 0.50)
	[2]37	(3.46) 10^6	(3.25) 10^6	3.35 (± 0.55) 10^6	1.00 (± 0.32)
0.44	5	(1.65) 10^5	(1.27) 10^5	1.46 (± 0.23) 10^5	1.87 (± 0.59)
	8	(1.39) 10^5	(1.84) 10^5	1.39 (± 0.09) 10^5	1.78 (± 0.48)
	14	(2.07) 10^5	(1.93) 10^5	2.00 (± 0.28) 10^5	2.56 (± 0.73)
	18	(5.40) 10^5	(4.25) 10^5	5.25 (± 0.94) 10^5	6.73 (± 1.73)
	24	(2.66) 10^5	(3.11) 10^5	2.88 (± 0.52) 10^5	3.69 (± 1.03)
	30	(1.52) 10^5	(1.55) 10^5	1.54 (± 0.05) 10^5	1.97 (± 0.49)
	[2]37	(8.05) 10^4	(7.55) 10^4	7.80 (± 1.80) 10^4	1.00 (± 0.46)
0.45	2	(2.14) 10^5	(1.96) 10^5	2.05 (± 0.16) 10^5	0.68 (± 0.18)
	5	(3.95) 10^5	(5.20) 10^5	4.58 (± 0.85) 10^5	1.53 (± 0.40)
	8	(9.35) 10^5	(1.03) 10^5	9.83 (± 1.61) 10^5	3.28 (± 0.63)
	15	(2.49) 10^6	(2.21) 10^6	2.35(± 0.24) 10^6	7.83 (± 1.21)
	18	(2.51) 10^6	(1.97) 10^6	2.33 (± 0.32) 10^6	7.77 (± 1.24)
	22	(1.43) 10^6	(1.43) 10^6	1.43 (± 0.11) 10^6	4.77 (± 0.75)
	30	(7.50) 10^5	(7.25) 10^5	7.38 (± 0.85) 10^5	2.46 (± 0.46)
	[2]37	(7.50) 10^5	(7.25) 10^5	3.00 (± 0.42) 10^5	1.00 (± 0.28)
0.60	14	(4.01) 10^4	(5.50) 10^4	4.76(± 0.88) 10^4	20.88 (± 4.78)
	18	(1.22) 10^5	(1.34) 10^5	1.28 (± 0.09) 10^5	56.14 (± 12.42)
	22	(3.61) 10^4	(3.40) 10^4	3.54 (± 0.54) 10^4	15.53 (± 3.57)
	26	(8.10) 10^3	(8.35) 10^3	8.28 (± 1.19) 10^3	3.76 (± 0.94)
	30	(6.10) 10^3	(3.70) 10^3	3.38 (± 0.76) 10^3	1.54 (± 0.55)
	[2]37	(1.85) 10^3	(2.70) 10^4	2.28 (± 0.51) 10^3	1.00 (± 0.44)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.

[2] Served as control; incubated at 37°C for 44 h.

[3] Mean of two trials, each performed in duplicate ± SD

[4] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 18. Survivors and recovery factor for *E. coli* (OH157:H7) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[4]Recovery Factor
		Trial 1	Trial 2	[3]Mean ± SD	(± SD)
0.20	2	(3.65) 10^7	(1.90) 10^7	2.78 (± 1.13) 10^7	1.54 (± 0.79)
	5	(4.80) 10^7	(1.55) 10^7	3.18 (± 1.89) 10^7	1.77 (± 1.04)
	8	(3.70) 10^7	(3.10) 10^7	3.30 (± 0.78) 10^7	1.83 (± 0.69)
	14	(1.08) 10^7	(5.90) 10^7	8.35 (± 2.80) 10^7	4.64 (± 1.50
	18	(9.60) 10^7	(7.10) 10^7	8.35 (± 1.75) 10^7	4.64 (± 1.37)
	22	(3.50) 10^7	(2.55) 10^7	3.03 (± 0.79) 10^7	1.68 (± 0.68)
	26	(4.15) 10^7	(2.90) 10^7	3.53 (± 0.81) 10^7	1.96 (± 0.72)
	30	(2.55) 10^7	(2.75) 10^7	2.65 (± 0.51) 10^7	1.47 (± 0.56)
	[2]37	(1.65) 10^7	1.95) 10^7	1.80 (± 0.45) 10^7	1.00 (± 0.50)
0.50	2	(9.20) 10^5	(1.12) 10^5	1.02 (± 0.14) 10^5	1.16 (± 0.16)
	5	(2.87) 10^5	(2.62) 10^5	2.74 (± 0.48) 10^5	3.12 (± 0.24)
	8	(2.56) 10^5	(2.82) 10^5	2.69 (± 0.26) 10^5	3.06 (± 0.16)
	14	(7.85) 10^4	(6.95) 10^4	7.40 (± 0.63) 10^4	0.84 (± 0.10)
	18	(8.75) 10^4	(8.75) 10^4	8.75 (± 0.53) 10^4	1.00 (± 0.08)
	22	(7.80) 10^4	(6.35) 10^4	7.08 (± 1.34) 10^4	0.81 (± 0.21)
	30	(9.80) 10^4	(8.15) 10^4	8.98 (± 1.28) 10^4	1.02 (± 0.16)
	[2]37	(7.55) 10^4	(1.00) 10^4	8.78 (± 0.15) 10^4	1.00 (± 0.04)
0.60	2	(6.50) 10^3	(9.40) 10^3	7.95 (± 2.05) 10^3	2.09 (± 0.40)
	5	(8.90) 10^3	(1.01) 10^3	9.50 (± 0.85) 10^3	2.50 (± 0.26)
	8	(1.69) 10^4	(2.54) 10^4	2.12 (± 0.60) 10^4	5.58 (± 0.67)
	14	(2.34) 10^4	(2.30) 10^4	2.32 (± 0.28) 10^4	6.11 (± 0.55)
	18	(1.29) 10^4	(1.30) 10^4	1.30 (± 0.07) 10^4	3.42 (± 0.78)
	22	(3.50) 10^3	(4.10) 10^3	3.80 (± 0.42) 10^3	1.00 (± 0.18)
	26	(4.00) 10^3	(5.20) 10^3	4.60 (± 0.85) 10^3	1.21 (± 0.27)
	30	(3.10) 10^3	(4.40) 10^3	3.75 (± 0.92) 10^3	0.99 (± 0.31)
	[2]37	(4.00) 10^3	(3.60) 10^3	3.80 (± 0.28) 10^3	1.00 (± 0.14)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.

[2] Served as control; incubated at 37°C for 44 h.

[3] Mean of two trials, each performed in duplicate

[4] RF calculated as no. of survivors (CFU/mL) enumeratedwith resuscitation/control (no resuscitation protocol).

Appendix table 19. Survivors and recovery factor for *S. typhimurium* (13311) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[4]Recovery Factor (± SD)
		Trial 1	Trial 2	[3]Mean ± SD	
0.50	2	(1.24) 10^8	(8.85) 10^7	1.06 (±0.25) 10^8	2.38 (±0.66)
	5	(4.55) 10^7	(5.10) 10^7	4.83 (±0.83) 10^7	1.09 (±0.37)
	8	(6.35) 10^7	(6.10) 10^7	6.23 (±0.43) 10^7	1.40 (±0.32)
	14	(1.86) 10^8	(1.29) 10^8	1.57 (±0.39) 10^8	3.53 (±0.88)
	18	(2.15) 10^8	(1.59) 10^8	1.87 (±0.34) 10^8	4.20 (±0.94)
	22	(2.18) 10^8	(2.10) 10^8	2.14 (±0.14) 10^8	4.81 (±0.93)
	30	(1.72) 10^8	(1.76) 10^8	1.74 (±0.12) 10^8	3.91 (±0.77)
	[2]37	(4.45) 10^7	(4.45) 10^7	4.45 (±0.81) 10^7	1.00 (±0.36)
0.97	2	(5.95) 10^4	(6.65) 10^4	6.30 (±0.15) 10^4	0.56 (±0.09)
	5	(4.90) 10^4	(6.95) 10^4	4.70 (±0.77) 10^4	0.42 (±0.22)
	8	(4.65) 10^4	(5.05) 10^4	4.85 (±0.57) 10^4	0.43 (±0.17)
	14	(1.87) 10^5	(1.71) 10^5	1.79 (±0.19) 10^5	1.60 (±0.31)
	18	(4.70) 10^5	(7.25) 10^5	6.00 (±0.17) 10^5	5.36 (±0.95)
	22	(8.65) 10^5	(1.06) 10^6	9.60 (±1.23) 10^5	8.57 (±1.20)
	30	(3.50) 10^5	(3.75) 10^5	3.63 (±0.35) 10^5	3.24 (±0.50)
	[2]37	(1.18) 10^5	(1.06) 10^5	1.12 (±0.14) 10^5	1.00 (±0.25)
1.50	2	(1.30) 10^2	(3.00) 10^1	8.00 (±6.00) 10^1	0.52 (±0.27)
	5	(6.00) 10^1	(2.00) 10^1	4.00 (±3.00) 10^1	0.26 (±0.85)
	8	(4.00) 10^1	(4.00) 10^1	4.00 (±3.00) 10^1	0.26 (±0.85)
	14	(1.90) 10^2	(3.10) 10^2	2.50 (±0.90) 10^2	1.61 (±0.97)
	18	(2.50)10^2	(2.20) 10^2	2.35 (±0.77) 10^2	1.52 (±0.90)
	22	(2.08) 10^4	(2.93) 10^4	2.50 (±0.50) 10^4	161.3 (±61.5)
	30	(9.80) 10^2	(1.01) 10^3	9.95 (±0.55) 10^2	6.42 (±2.49)
	[2]37	(1.50)10^2	(1.60) 10^2	1.55 (±0.59) 10^2	1.00 (±0.76)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] Mean of two trials, each performed in duplicate
[4] RF calculated as no. of survivors (CFU/mL) enumeratedwith resuscitation/control (no resuscitation protocol).

Appendix table 20. Survivors and recovery factor for *P. fluorescens* (13325) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.15	2	(8.20) 10^4	(9.70) 10^4	8.95 (± 1.06) 10^4	1.09 (±0.32)
	5	(1.07) 10^5	(1.23) 10^5	1.15 (±0.11) 10^5	1.41 (±0.36)
	8	(1.19) 10^5	(1.04) 10^5	1.11 (±0.11) 10^5	1.36 (±0.35)
	14	(1.18) 10^5	(9.60) 10^5	1.08 (±0.14) 10^5	1.32 (±0.38)
	18	(1.32) 10^5	(8.85) 10^4	1.10 (±0.26) 10^5	1.34 (±0.49)
	22	(1.46) 10^5	(1.04) 10^5	1.25 (±0.24) 10^5	1.53 (±0.48)
	[2]26	(9.45) 10^4	(6.90) 10^4	8.18 (± 1.52) 10^4	1.00 (±0.37)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 21. Survivors and recovery factor for *Y. enterocolitica* (23715) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.20	2	(3.10) 10^4	(2.65) 10^4	2.88 (±0.56) 10^4	0.81 (±0.35)
	5	(4.65) 10^4	(3.00) 10^4	3.83 (± 1.02) 10^4	1.07 (±0.47)
	8	(3.15) 10^4	(2.50) 10^4	3.23 (±0.15) 10^4	0.90 (±0.22)
	14	(3.80) 10^4	(4.10) 10^4	3.95 (±0.25) 10^4	1.11 (±0.27)
	18	(2.75) 10^4	(4.10) 10^4	3.43 (±0.84) 10^4	0.96 (±0.43)
	22	(2.00) 10^4	(3.35) 10^4	2.68 (±0.75) 10^4	0.75 (±0.42)
	[2]30	(4.30) 10^4	(3.20) 10^4	3.57 (±0.66) 10^4	1.00 (±0.37)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 22. Survivors and recovery factor for *A. hydrophila* (7965) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.18	2	(4.70) 10^4	(4.30) 10^4	4.50 (± 0.28) 10^4	0.43 (± 0.09)
	5	(8.10) 10^4	(6.50) 10^4	7.30 (± 1.13) 10^4	0.70 (± 0.20)
	8	(4.55) 10^4	(7.70) 10^4	6.13 (± 1.87) 10^4	0.58 (± 0.34)
	14	(1.15) 10^5	(8.65) 10^4	1.01 (± 0.17) 10^5	0.96 (± 0.23)
	18	(8.85) 10^5	(1.12) 10^5	1.00 (± 0.14) 10^5	0.95 (± 0.20)
	22	(1.42) 10^5	(1.20) 10^5	1.31 (± 0.15) 10^5	1.25 (± 0.19)
	[2]26	(1.01) 10^5	(1.09) 10^5	1.05 (± 0.06) 10^5	1.00 (± 0.12)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.

[2] Served as control; incubated at 37°C for 44 h.

[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 23. Survivors and recovery factor for *L. monocytogenes* (Scott A) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor
		Trial 1	Trial 2	Mean ± SD	(± SD)
0.32	2	(3.67) 10^7	(3.82) 10^7	3.74 (±0.16) 10^7	0.99 (±0.18)
	5	(3.57) 10^7	(2.84) 10^7	3.22 (±0.95) 10^7	1.21 (±0.12)
	8	(3.60) 10^7	(2.52) 10^7	3.05 (±0.93) 10^7	1.04 (±0.36)
	14	(2.89) 10^7	(2.13) 10^7	2.51 (±0.45) 10^7	0.99 (±0.36)
	18	(2.69) 10^7	(2.54) 10^7	2.62 (±0.14) 10^7	0.85 (±0.23)
	22	(2.85) 10^7	(3.03) 10^7	2.94 (±0.27) 10^7	0.93 (±0.16)
	30	(3.15) 10^7	(3.03) 10^7	3.09 (±0.20) 10^7	0.95 (±0.15)
	[2]37	(3.33) 10^7	(2.82) 10^7	3.07 (±0.37) 10^7	1.00 (±0.12)
0.50	2	(1.29) 10^6	(1.52) 10^6	1.41 (±0.26) 10^6	1.13 (±0.29)
	5	(1.81) 10^6	(1.96) 10^6	1.88 (±0.13) 10^6	1.50 (±0.21)
	8	(1.41) 10^6	(1.56) 10^6	1.48 (±0.14) 10^6	1.18 (±0.21)
	14	(5.05) 10^5	(7.60) 10^5	6.30 (±0.18) 10^5	0.50 (±0.33)
	18	(1.06) 10^6	(7.70) 10^5	9.13 (± 1.25) 10^5	0.73 (±0.26)
	22	(6.85) 10^6	(7.50) 10^5	7.18 (± 1.12) 10^5	0.57 (±0.21)
	30	(1.11) 10^6	(9.35) 10^5	1.02 (±0.12) 10^6	0.82 (±0.20)
	[2]37	(1.21) 10^6	(1.30) 10^6	1.25 (±0.12) 10^6	1.00 (±0.19)
0.85	2	(6.70) 10^3	(6.50) 10^3	6.60 (± 1.14) 10^3	0.70 (±0.18)
	5	(5.55) 10^3	(4.25) 10^3	4.90 (±0.81) 10^3	0.52 (±0.13)
	8	(5.35) 10^3	(4.90) 10^3	5.13 (± 1.21) 10^3	0.54 (±0.18)
	14	(1.75) 10^3	(2.50) 10^3	2.13 (±0.49) 10^3	0.23 (±0.07)
	18	(1.50) 10^3	(1.85) 10^3	1.68 (±0.21) 10^3	0.18 (±0.04)
	22	(2.20) 10^3	(2.10) 10^3	2.15 (±0.64) 10^3	0.23 (±0.09)
	30	(4.75) 10^3	(6.00) 10^3	5.38 (±0.11) 10^3	0.57 (±0.17)
	[2]37	(9.65) 10^3	(9.25) 10^3	9.45 (±0.84) 10^3	1.00 (±0.18)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 24. Survivors and recovery factor for *L. monocytogenes* (81-861) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor
		Trial 1	Trial 2	Mean ± SD	(± SD)
0.60	2	(1.25) 10^4	(1.24) 10^5	1.24 (±0.14) 10^5	0.92 (±0.25)
	5	(1.18) 10^5	(1.20) 10^5	1.19 (±0.11) 10^5	0.88 (±0.22)
	8	(1.10) 10^5	(1.13) 10^5	1.12 (±0.12) 10^5	0.83 (±0.23)
	14	(1.03) 10^5	(1.03) 10^5	1.03 (±0.11) 10^5	0.76 (±0.22)
	18	(1.04) 10^5	(9.10) 10^4	9.75 (±0.13) 10^4	0.72 (±0.24)
	22	(1.18) 10^5	(1.14) 10^5	1.17 (±0.40) 10^5	0.87 (±0.16)
	30	(1.30) 10^5	(1.38) 10^5	1.34 (±0.08) 10^5	0.99 (±0.21)
	[2]37	(1.25) 10^5	(1.46) 10^5	1.35 (±0.20) 10^5	1.00 (±0.30)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 25. Survivors and recovery factor for *L. innocua* (CFPDC) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor
		Trial 1	Trial 2	Mean ± SD	(± SD)
0.55	2	(6.44) 10^6	(6.09) 10^6	6.26 (±0.65) 10^6	0.93 (±0.16)
	5	(5.25) 10^6	(4.55) 10^6	4.90 (±0.52) 10^6	0.73 (±0.15)
	8	(4.90) 10^6	(5.18) 10^6	5.24 (±0.51) 10^6	0.78 (±0.14)
	14	(3.65) 10^6	(3.33) 10^6	3.49 (±0.23) 10^6	0.52 (±0.10)
	18	(4.11) 10^6	(3.92) 10^6	4.01 (±0.32) 10^6	0.60 (±0.12)
	22	(5.23) 10^6	(4.42) 10^6	4.83 (±0.47) 10^6	0.72 (±0.14)
	30	(4.76) 10^6	(4.76) 10^6	4.76 (±0.26) 10^6	0.71 (±0.10)
	[2]37	(6.74) 10^6	(6.72) 10^6	6.73 (±0.40) 10^6	1.00 (±0.12)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 26. Survivors and recovery factor for *L. ivanovii* (CFPDC) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor
		Trial 1	Trial 2	Mean ± SD	(± SD)
0.55	2	(1.10) 10^6	(8.05) 10^5	9.50 (± 1.90) 10^5	0.98 (±0.29)
	5	(8.85) 10^5	(8.15) 10^5	8.50 (± 1.42) 10^5	0.88 (±0.25)
	8	(1.08) 10^6	(1.09) 10^6	1.08 (±0.09) 10^6	1.11 (±0.19)
	14	(6.95) 10^5	(5.95) 10^5	6.45 (±0.78) 10^5	0.66 (±0.18
	18	(6.10) 10^5	(6.90) 10^5	6.50 (±0.53) 10^5	0.67 (±0.14)
	22	(9.80) 10^5	(9.30) 10^5	9.55 (± 1.19) 10^5	0.98 (±0.21)
	30	(1.16) 10^6	(1.17) 10^6	1.16 (±0.04) 10^6	1.20 (±0.14)
	[2]37	(8.95) 10^5	(1.05) 10^6	9.70 (±0.89) 10^5	1.00 (±0.18)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 27. Survivors and recovery factor for *B. thermosphacta* (11509) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor
		Trial 1	Trial 2	Mean ± SD	(± SD)
0.48	2	(5.85) 10^4	(1.53) 10^4	1.05 (±0.57) 10^4	0.45 (±0.62)
	5	(1.21) 10^4	(5.30) 10^3	8.70 (± 4.00) 10^3	0.37 (±0.53)
	8	(1.68) 10^4	(1.46) 10^4	1.57 (±0.17) 10^4	0.67 (±0.23)
	14	(3.50) 10^4	(4.60) 10^4	4.10 (±0.88) 10^4	1.76 (±0.53)
	18	(7.50) 10^4	(4.95) 10^4	6.23 (± 1.52) 10^4	2.67 (±0.73)
	22	(2.55) 10^4	(1.65) 10^4	2.10 (±0.87) 10^4	0.90 (±0.58)
	[2]26	(2.65) 10^4	(2.00) 10^4	2.33 (±0.41) 10^4	1.00 (±0.36)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 28. Survivors and recovery factor for *B. subtilis* using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.48	2	(7.60) 10^4	(6.20) 10^4	6.90 (± 1.32) 10^4	1.43 (± 0.61)
	5	(7.30) 10^4	(6.25) 10^4	6.78 (± 0.66) 10^4	1.40 (± 0.50)
	8	(6.20) 10^4	(8.00) 10^4	7.10 (± 1.18) 10^4	1.47 (± 0.59)
	14	(6.10) 10^4	(8.75) 10^4	7.43 (± 1.85) 10^4	1.54 (± 0.70)
	18	(7.45) 10^4	(8.00) 10^4	7.73 (± 1.36) 10^4	1.60 (± 0.64)
	22	(5.50) 10^4	(8.55) 10^4	7.03 (± 1.99) 10^4	1.46 (± 0.71)
	26	(5.80) 10^4	(7.20) 10^4	6.73 (± 0.90) 10^4	1.39 (± 0.54)
	30	(5.45) 10^4	(5.05) 10^4	5.25 (± 0.61) 10^4	1.09 (± 0.43)
	[2]37	(5.20) 10^4	(4.45) 10^4	4.83 (± 1.42) 10^4	1.00 (± 0.58)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 29. Survivors and recovery factor for *S. aureus* using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.60	2	(9.85) 10^5	(8.60) 10^5	9.23 (± 1.07) 10^5	1.30 (± 0.39)
	5	(5.55) 10^5	(6.70) 10^5	6.13 (± 0.69) 10^5	0.87 (± 0.29)
	8	(5.55) 10^5	(3.90) 10^5	4.73 (± 1.30) 10^5	0.67 (± 0.42)
	14	(3.70) 10^5	(4.40) 10^5	4.05 (± 5.30) 10^5	0.57 (± 0.25)
	18	(4.40) 10^5	(6.40) 10^5	5.40 (± 1.40) 10^5	0.76 (± 0.42)
	22	(6.65) 10^5	(8.60) 10^5	7.30 (± 1.35) 10^5	1.03 (± 0.40)
	30	(7.55) 10^5	(8.55) 10^5	8.05 (± 0.78) 10^5	1.14 (± 0.34)
	[2]37	(5.80) 10^5	(8.35) 10^5	7.08 (± 1.50) 10^5	1.00 (± 0.42)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.
[2] Served as control; incubated at 37°C for 44 h.
[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 30. Survivors and recovery factor for *S. cerevisiae* (2360) using resuscitation following irradiation treatment.

Irradiation dose (kGy)	[1]Resuscitation temp. (°C) for 20 h	Survivors (CFU/mL)			[3]Recovery Factor (± SD)
		Trial 1	Trial 2	Mean ± SD	
0.50	2	(5.20) 10^5	(4.60) 10^5	4.90 (± 0.61) 10^5	0.56 (± 0.25)
	5	(5.50) 10^5	(5.00) 10^5	5.25 (± 0.35) 10^5	0.60 (± 0.20)
	8	(6.25) 10^5	(8.65) 10^5	7.45 (± 1.49) 10^5	0.86 (± 0.39)
	14	(6.20) 10^5	(6.40) 10^5	6.30 (± 1.41) 10^5	0.73 (± 0.38)
	18	(7.50) 10^5	(7.25) 10^5	7.34 (± 0.95) 10^5	0.85 (± 0.32)
	22	(1.11) 10^6	(9.25) 10^6	1.02 (± 0.20) 10^6	1.18 (± 0.45)
	[2]30	(9.25) 10^5	(8.10) 10^5	8.68 (± 1.90) 10^5	1.00 (± 0.44)

[1] Following resuscitation protocol samples were further incubated at 37°C for 24 h.

[2] Served as control; incubated at 37°C for 44 h.

[3] RF calculated as no. of survivors (CFU/mL) enumerated with resuscitation/control (no resuscitation protocol).

Appendix table 31. Maximum recovery factor for irradiated microorganism and corresponding resuscitation temperature.

Organism	Irradiation dose (kGy)	Maximum RF(± SD)	Resuscitation temperature (°C)
E. coli (11775)	0.20	1.60 ± 0.17	18
	0.32	4.60 ± 0.85	22
	0.44	6.73 ± 1.73	18
	0.45	7.83 ± 1.21	15
	0.60	56.14 ± 12.42	18
E. coli (OH157:H7)	0.20	4.64 ± 1.50	14
	0.20	4.64 ± 1.37	18
	0.50	3.12 ± 0.24	5
	0.60	6.11 ± 0.55	14
S. typhimurium (13311)	0.50	4.81 ± 0.93	22
	0.97	8.57 ± 1.20	22
	1.50	161.29 ± 61.5	22
A. hydrophila (7965)	0.18	1.25 ± 0.19	22
Y. enterocolitica (23715)	0.60	1.11 ± 0.27	14
P. fluorescens (13325)	0.15	1.53 ± 0.48	22
B. thermosphacta (11509)	0.48	2.67 ± 0.73	18
L. monocytogenes (Scott A)	0.32	1.21 ± 0.12	5
	0.50	1.50 ± 0.21	5
	0.85	1.00 ± 0.18	37
L. monocytogenes (81-861)	0.60	1.00 ± 0.30	37
L. innocua (CFPDC)	0.55	1.00 ± 0.12	37
L. ivanovii (CFPDC)	0.55	1.11 ± 0.19	37
B. subtilis	0.48	1.60 ± 0.64	18
S. aureus (25923)	0.60	1.30 ± 0.39	2
S. cerevisiae (2360)	0.50	1.18 ± 0.45	22

Appendix table 32. Kinetics of repair of potentially lethal radiation damage in *E. coli* (11775)

Irradiation Dose (kGy)	Resuscitation time at 18°C (h)	CFU/mL ± (SD)	Recovery Factor ± (SD)
0.44	0	[a]$6.73 \pm (1.33)\ 10^6$	1.00 ± (0.50)
	1	$1.66 \pm (0.61)\ 10^7$	2.47 ± (1.00)
	2	$1.97 \pm (0.50)\ 10^7$	2.93 ± (1.00)
	4.5	$1.85 \pm (0.59)\ 10^7$	2.75 ± (1.01)
	5.25	$2.18 \pm (0.38)\ 10^7$	3.24 ± (0.98)
	24	$3.43 \pm (1.32)\ 10^7$	5.10 ± (1.66)
	28	$3.61 \pm (0.47)\ 10^7$	5.36 ± (1.47)
	48	$3.29 \pm (0.93)\ 10^7$	4.89 ± (1.50)
0.76	0	[a]$5.70 \pm (0.42)\ 10^3$	1.00 (± 0.14)
	1	$1.38 \pm (0.07)\ 10^4$	2.42 (± 0.22)
	2	$1.14 \pm (0.13)\ 10^4$	2.00 (± 0.25)
	4.5	$1.60 \pm (0.23)\ 10^4$	2.81 (± 0.34)
	5.25	$1.70 \pm (0.42)\ 10^4$	2.98 (± 0.46)
	24	$8.45 \pm (2.12)\ 10^4$	14.82 (± 1.29)
	28	$1.01 \pm (0.16)\ 10^5$	17.72 (± 1.40)
	48	$1.34 \pm (0.25)\ 10^5$	23.51 (± 1.83)

[a] CFU determined following incubation (resuscitation) time at 18°C plus 24 h at 37°C. All values represent the means of two trials each performed in duplicate.

Appendix table 33. Kinetics of fixation of potentially lethal radiation damage in *E. coli* (11775).

Irradiation Dose (kGy)	Fixation time at 37°C(min)	CFU/mL ± (SD)	Recovery Factor ± (SD)
0.42	0	[a]3.60 ± (0.51) 10^7	6.32 ± (1.78)
	10	3.66 ± (0.63) 10^7	6.42 ± (1.99)
	30	3.27 ± (0.33) 10^7	5.73 ± (0.25)
	60	1.26 ± (0.21) 10^7	2.21 ± (0.69)
	90	5.70 ± (2.30) 10^6	1.00 ± (0.57)
	155	8.10 ± (1.70) 10^6	1.42 ± (0.51)
	180	5.70 ± (1.50) 10^6	1.00 ± (0.38)
	240	7.80 ± (1.50) 10^6	1.36 ± (0.44)
0.77	0	[a]2.79 ± (0.50) 10^5	168.07 ± (60.37)
	10	2.41 ± (0.24) 10^5	145.18 ± (40.33)
	30	2.45± (0.26) 10^5	147.59 ± (44.40)
	60	8.73 ± (0.93) 10^4	52.59 ± (15.12)
	90	3.40 ± (1.10) 10^3	2.05 ± (1.00)
	155	1.90± (0.71) 10^3	1.14 ± (0.22)
	180	1.66 ± (0.61) 10^3	0.10 ± (0.55)
	240	2.08 ± (0.54) 10^3	1.25 ± (0.55)

[a] CFU determined following fixation incubation at 18°C, 20 h plus incubation at 37°C for 24 h. All values are means of two trials, each performed in duplicate.

Appendix table 34. Assay for potentially lethal damage caused by either heat or radiation in *L. monocytogenes* (Scott A) using BYE agar with and without 4 % NaCl.

Treatment	[a]Survivors BYE agar (± SD)	[a]Survivors BYE (4 % NaCl) agar (± SD)	Ratio
Heating minutes at 55°C			
0	7.15 (± 0.99) 10^7	7.43 (± 0.97) 10^7	0.96
10	4.10 (± 0.75) 10^7	9.45 (± 2.30) 10^6	4.34
20	1.60 (± 0.25) 10^7	2.25 (± 0.98) 10^5	71.11
30	2.58 (± 1.18) 10^5	2.85 (± 1.21) 10^4	9.05
40	1.00 (± 0.24) 10^5	4.03 (± 0.42) 10^3	24.81
50	1.11 (± 0.13) 10^5	<10	> 1.11 x 10^4
Irradiation Dose (kGy)			
0	2.61 (± 0.18) 10^8	2.07 (± 0.59) 10^8	1.26
0.10	5.90 (± 0.10) 10^7	1.54 (± 0.58) 10^7	3.83
0.20	1.46 (± 0.25) 10^7	4.04 (± 1.03) 10^6	3.61
0.40	1.34 (± 0.24) 10^6	3.28 (± 5.10)10^5	4.08
0.60	5.18 (± 1.10) 10^4	1.13 (± 0.23)10^4	4.58

[a] means of two trials, each performed in duplicate ± SD

Appendix table 35. Assay for potentially lethal damage caused by either heat or radiation in *E. coli* using BYE agar with and without 2 % NaCl.

Treatment	[a]Survivors BYE agar (± SD)	[a]Survivors BYE (2 % NaCl) agar (± SD)	Ratio
Heating minutes at 55°C			
0	2.27 (± 0.62) 10^9	2.91 (± 0.65) 10^9	0.78
10	3.88 (± 0.18) 10^8	7.98 (± 0.87) 10^7	4.86
20	6.05 (± 0.28) 10^6	6.95 (± 3.25) 10^5	8.70
30	5.05 (± 3.62)10^5	1.95 (± 2.29) 10^4	25.90
40	8.89 (± 2.67) 10^4	1.01 (± 0.24) 10^3	8.80
Irradiation Dose (kGy)			
0	1.89 (± 0.27) 10^9	1.53 (± 0.19) 10^9	1.24
0.10	5.48 (± 0.71) 10^8	1.04 (± 0.19) 10^8	5.27
0.20	6.57 (± 1.04) 10^7	1.09 (± 0.12) 10^7	6.03
0.30	4.15 (± 0.12) 10^6	1.52 (± 0.34) 10^6	2.73
0.50	1.03 (± 0.20) 10^4	3.07 (± 0.42) 10^3	3.36
1.00	4.48 (± 0.28) 10^2	4.30 (± 3.30) 10^2	1.04

[a] means of two trials, each performed in duplicate ± SD

Appendix table 36. UV absorbance (258 nm) in supernatant after heat or radiation treatment of *E. coli*.

Treatment	Absorbance 0 h	Absorbance 48 h
Heating minutes at 55°C		
0	0	0
15	0.24	0.20
30	0.37	0.25
45	0.45	0.47
60	0.44	0.89
Irradiation Dose (kGy)		
0	0	0
0.27	0	0.04
0.50	0	0.03
0.93	0.09	0.06
1.88	0.05	0.06

Appendix table 37. UV absorbance (258 nm) in supernatant after heat or radiation treatment of *L. monocytogenes* (Scott A).

Treatment	Absorbance 0 h	Absorbance 48 h
Heating minutes at 55°C		
0	0	0
15	0.15	0.12
30	0.24	0.21
45	0.34	0.35
60	0.49	0.38
Irradiation Dose (kGy)		
0	0	0
0.27	0	0
0.50	0	0
0.93	0	0
1.88	0	0

Appendix table 38. Comparison of recovery in gamma and electron irradiated *E. coli.*

Treatment	Survivors [a](± SD) [b]without resuscitation	Survivors (± SD) [c]with resuscitation	RF
gamma Irradiation Dose (kGy)			
0	4.41 (±0.41) 10^9	3.40 (±0.18) 10^9	0.77
0.10	1.02 (±0.06) 10^9	1.02 (±0.12) 10^9	0.10
0.20	1.64 (±0.25) 10^8	2.49 (±0.21) 10^8	0.51
0.30	1.38 (±0.22) 10^7	3.99 (±0.69) 10^7	2.89
0.50	1.06 (±0.15) 10^4	7.00 (± 1.98) 10^4	6.60
Electron Irradiation Dose (kGy)			
0	4.41 (±0.41) 10^9	3.40 (±0.18) 10^9	0.77
0.25	3.36 (±0.64) 10^8	9.50 (±0.71) 10^7	2.83
0.26	1.87 (±0.12) 10^7	8.35 (±0.49) 10^7	4.47
0.32	4.40 (±0.71) 10^6	1.88 (±0.11) 10^7	4.27
0.47	4.48 (±0.99) 10^4	2.00 (±0.65) 10^5	4.46

[a]mean of two trials each performed in duplicate ± SD
[b]Incubation at 37°C for 44 h
[c]Incubation at 18 °C for 20 h, followed by 24 h at 37°C

Appendix table 39. Numbers of *E. coli* (11775) counted at the specified times following irradiation

Dose (kGy)	Time (Hours at 37°C)	Survivors [a]cfu/mL ± SD
0	24	3.64 ± (0.53) 10^9
	25	3.62 ± (0.52) 10^9
	26	3.73 ± (0.46) 10^9
	28.5	3.59 ± (0.47) 10^9
	29.25	3.72 ± (0.44) 10^9
	48	3.64 ± (0.51) 10^9
	52	3.64 ± (0.55) 10^9
0.42	24	5.70 ± (2.49) 10^6
	25	6.45 ± (3.22) 10^6
	26	6.53 ± (2.63) 10^6
	28.5	6.80 ± (2.83) 10^6
	29.25	6.53 ± (2.63) 10^6
	48	5.80 ± (2.45) 10^6
	52	6.28 ± (2.91) 10^6
0.78	24	3.00 ± (2.45) 10^2
	25	2.50 ± (2.50) 10^2
	26	3.00 ± (0.82) 10^2
	28.5	2.00 ± (3.37) 10^2
	29.25	3.00 ± (0.82) 10^2
	48	3.00 ± (1.41) 10^2
	52	3.00 ± (0.82) 10^2
	72	3.50 ± (1.29) 10^2

[a] Mean of two trials each performed in duplicate

Appendix table 40. Comparative effectiveness of resuscitation protocol on *E. coli* based on treatment medium

Treatment medium	Irradiation dose (kGy)	[a]Survivors (CFU/mL ± SD) [a]Resuscitation	[b]No Resuscitation	[c]RF
BPB	0.25	5.68 (± 0.61) 10^4	3.43 (± 0.40) 10^4	1.61
	0.47	2.13 (± 0.26) 10^3	6.25 (± 0.28) 10^1	34.08
	0.53	2.42 (± 0.37) 10^4	6.45 (± 2.01) 10^1	375.19
TSB	0.25	7.10 (± 0.86) 10^4	4.08 (± 0.90) 10^4	1.74
	0.53	4.23 (± 0.46) 10^6	1.68 (± 0.25) 10^6	2.52
	1.00	7.29 (± 2.67) 10^6	3.46 (± 1.69) 10^6	2.11
	1.50	3.78 (± 0.54) 10^4	7.98 (± 2.06) 10^3	4.74
Ground beef	0.53	6.42 (± 0.70) 10^6	3.89 (± 0.72) 10^6	1.65
	1.00	5.38 (± 1.17) 10^4	1.63 (± 0.71) 10^3	33.01
	1.50	1.05 (± 0.97) 10^3	1.00 (± 1.40) 10^1	105.00

[a] Means of two trials, each performed in duplicate. Culture plates incubated at 18°C for 20 h followed by incubation at 37°C for 24 h.
[b] Culture plates incubated at 37°C for 44 h.
[c] RF = ratio of survivors obtained (a/b).