PREFACE

Salmonella are ubiquitous enteric bacteria, responsible for thousands of deaths worldwide. In this book, the authors present current research in the study of the classification, genetics and disease outbreak cases relating to salmonella. Topics include the pre- and post-harvest intervention strategies for controlling salmonella contamination in broiler production; *Salmonella enterica* survival to biocides and antibiotics; salmonella newport contamination in produce; genome comparisons of salmonella; salmonella in sub-Antarctica and Antarctica; and hazard of salmonella in the intact shell egg.

Chapter 1 - Contamination of poultry with *Salmonella* occurs prior to entry into the processing facility and by cross-contamination in the processing plant. Breeder flocks, hatcheries, contaminated feed and water, and environmental sources/vectors such as litter, humans, and insects are potential pre-harvest sources of *Salmonella* contamination in poultry. Knowledge of key pre-harvest factors responsible for *Salmonella* colonization would help integrators and poultry producers in defining critical, pre-harvest control points on the farm. Intervention at these points could reduce or eliminate horizontal transmission and *Salmonella* positive birds arriving at the processing plants. It is difficult to improve the microbiological quality of chickens once they have reached the processing plant. Several physical and chemical decontamination procedures have been applied to poultry carcasses and have been useful in reducing the level and frequency of *Salmonella* on poultry carcasses at the processing plants. A combination of pre- and post-harvest intervention strategies can reduce or eliminate *Salmonella* on fresh poultry delivered to consumers, thereby significantly reducing the medical and productivity costs associated with salmonellosis.

Chapter 2 - Many strains of *Salmonella enterica* represent zoonotic pathogens which are capable of causing disease in humans. One major source of food poisoning is *Salmonella enterica* serovar Enteritidis which is often found associated with chicken meat, meat products and eggs. A number of virulence factors enable this bacterium to colonize and systemically infect poultry. In this review the authors will concentrate on the *S.* Enteritidis Type III Secretion System (T3SS) located on *Salmonella* Pathogenicity Island-1 and highlight its role in invasion of chickens. The structural components of the complex secretion apparatus and the secreted effector proteins will be introduced. Aspects of T3SS assembly, its expression regulation under different conditions as well as its involvement in colonization and invasion of poultry will be discussed in the context of the recent findings.

Chapter 3 - It is well known that the main microbiological hazard derived from egg consumption is the presence of *Salmonellae*, being the serovars *S.* Enteritidis and *S.*

Typhimurium the most frequently implicated in the outbreaks. Although bacteria can reach the inside of the eggs by transovarian or through the shell pores, the main way of contamination probably occurs from organisms located in the shell, which can attain the inner part of the egg when it is broken for cooking other foods, e.g. custard, salads, desserts. In developed countries, the outbreaks of salmonellosis increased until 2004, observing a clear decline afterward, which is attributed to the success of implementing programs to control *Salmonella* in poultry populations. In spite of this, salmonellosis is still the main cause of foodborne illness. Therefore, the decontamination of shell eggs remains of public health concern yet. Many chemical (e.g. washing, hydrogen peroxide, ozone and electrolyzed water) and physical (e.g. water boiling, pasteurization, ionizing and ultraviolet radiation, pulsed light, high pressure,) methods for shell eggs sanitation have been envisaged, being the pasteurization that of the most concern. Many heat treatment conditions have been proposed to remove *Salmonellae* from eggshells, but none have been completely satisfactory due to the adverse effects of the heat on the functional properties of the white and yolk. An attempt to develop a procedure to reach this goal has been recently performed by the authors' group. The ability of thermoultrasonication (ultrasounds and heat simultaneously applied) to eliminate *Salmonella* from eggshell was studied, using *S.* Enteritidis and *S.* Senftenberg as models. The thermoultrasonication dramatically reduced the heat resistant of *Salmonella*. E.g., the D-value of *S.* Enteritidis at 55 ºC (2.89 minutes for the heat treatment alone) was reduced a 77.16 %, i.e. it passed to 0.66 minutes when the thermoultrasonication was applied. In the case of *S.* Senftenberg the reduction was lower. The same figures at 57 ºC were 10.54 and 3.92 minutes, respectively. No effects of the thermoultrasonication on several attributes (shelf-life, loss of freshness, eggshell breaking strength, emulsifying and foaming abilities, etc.) were observed. The fundamentals of the new process for shell egg sanitation have been established at a laboratory scale. As an ultrasonic treatment added to an existing heat process would increase its bactericidal efficiency, the process might have important practical implications for the egg industry. It is now necessary to validate the process at a higher scale to confirm the results.

Chapter 4 - Nowadays, *Salmonella* spp. is still considered one of the major foodborne pathogens, being responsible for thousands of deaths worldwide and widespread economic losses every year. *Salmonella enterica* strains have proven to be highly adaptable and able to survive in the external environment, and their ability to respond effectively to environmental changes is important in their survival in the food chain. Moreover, like many other microorganisms, *S. enterica* is able to adhere and form biofilms on several different surfaces, which increases its inherent resistance and, thus, significantly affects plant sanitation and product safety. Bacterial resistance to antimicrobial agents in general, as well as the underlying mechanisms, have been widely studied by several authors. Additionally, a number of scientists have recently expressed concern that the common and indiscriminate use of biocides may be a contributing factor to the development and selection of antibiotic-resistant strains, giving rise to a phenomenon known as "cross-resistance". In this context, the present chapter intends to review the scientific results concerning the mechanisms by which *S. enterica* planktonic and biofilm cells may become resistant to biocides action, as well as the relation between biocide and antibiotic resistance by this foodborne pathogen. Moreover, genomic aspects involved in such adaptive and resistant behaviours will also be considered.

Chapter 5 - Culture-based methods for detection of *Salmonella* spp. in food demand the performance of several steps in stages (preenrichment, enrichment, selective plating, identification, and confirmation). This results in significant time-to-detection, media and

reagents and labor needed. As a consequence of the need for rapid and specific detection techniques, molecular-based rapid detection methods such as real time polymerase chain reaction (RT-PCR), nucleic acid sequence based amplification (NASBA) or immunoassays have been developed. These methods, however, may still be negatively affected by interference from inhibitors, may be qualitative in nature or require extraction of target molecules (DNA, rRNA, mRNA) prior to analysis allowing for possible contamination or amplification of target sequences from non-viable cells. Alternative rapid detection techniques for *Salmonella* spp. in food have been developed which accomplish identification and enumeration of whole-cell targets. Fluorescence *in situ* hybridization (FISH) employs sequence-specific rRNA-targeted fluorescently-labeled oligonucleotide probes to specifically hybridize whole permeabilized bacterial cells. FISH can be coupled with fluorescence microscopy, flow cytometry or capillary electrophoresis to achieve detection and enumeration. Bacteriophage-based detection techniques for *Salmonella* spp. which make use of the inherent specificity of phages for their target bacterial host have also been described. In addition, biosensor-based detection may offer simplicity and high throughput capability for whole-cell detection of *Salmonella* spp. in food. These alternative methods for whole-cell detection of *Salmonella* spp. have shown potential as sensitive, specific, and quantitative methods for detection and identification of this pathogen in foods. The advantages and disadvantages of these methods as well as the current status of development and future directions are discussed here.

Chapter 6 - *Salmonella enterica* is the most frequent source of bacterial foodborne illness in the U.S., causing an estimated 1.4 million cases of salmonellosis annually. Historically, infection by *Salmonella* occurs after eating undercooked meats, poultry and eggs. However, over the past decade, the consumption of raw ready-to-eat produce has been implicated in several severe and widespread outbreaks within the U. S. Most notably, an *S. enterica* Saintpaul outbreak associated with jalapeño and Serrano peppers sickened over 1400 individuals during the summer of 2008. One *Salmonella* serovar in particular, *Salmonella enterica* Newport, has been associated perennially with contamination events involving fresh tomatoes and prepared fresh foods containing tomatoes. *S.* Newport is a non-typhoidal salmonellae classified taxonomically as subspecies (i.e. "group") I *Salmonella enterica enterica*. Prior to its emergence in produce and in produce growing environments, *S.* Newport was found predominantly in swine, cattle and other livestock commodities. *S.* Newport association with tomatoes retains a geographically skewed distribution. Metagenomic analyses of tomato phyllopsheres, based on 16S rDNA sequences and deep genome sequencing of tomato microbial communities, point to resident colonization on tomatoes from the Mid-Atlantic growing regions but not other predominant fresh tomato growing regions such as Florida or California, signaling niche adaptation of *S.* Newport in some growing environments but not others. Genetic analysis of *S.* Newport has revealed specific differences among strains from disparate locales. Pulsed-field gel electrophoresis, for example, has yielded several pulsotypes of environmental *S.* Newport primarily associated with the Virginia eastern shore, a prominent fresh tomato production region of the county. Whole-genome sequencing affirmed these phylo-geographic differences within the serovar. Surprisingly, certain genotypes of *S.* Newport have been isolated from this region that genetically match previous outbreaks where tomatoes were a suspected vehicle. *S.* Newport can be readily detected in the environment of the Virginian eastern shore growing region using both molecular (i.e. PCR) and microbiological (i.e. FDA-BAM) screening methods.

Taken together, these issues highlight the important intersection of microbiological, genetic, and ecological investigation for fully understanding the complex life cycle of this dangerous foodborne pathogen.

Chapter 7 - *Salmonella* was identified as the first cause of food-borne diseases in the last decade in Brazil. Currently, Brazil is an important producer of pork and is the major poultry and beef exporter in the world. Brazilian animal products have been recognized worldwide, and to attend international standards the Brazilian food industries have been putting considerable effort in order to prevent food contamination, especially by *Salmonella*. A representative part of the Brazilian eggs, beef, poultry, and pork meats is produced in the State of Rio Grande do Sul (RS), in southern Brazil, where *Salmonella* was pointed out as the first cause of food-borne diseases for almost two decades. In this region, homemade mayonnaise was identified as the food preparation mostly involved with salmonellosis, and both meat and meat products were also identified as frequent vehicles for salmonellosis. A specific strain of *Salmonella* Enteritidis (named SE86) was identified as the responsible agent in more than 95 % of investigated food-borne diseases throughout the last decade. SE86 strains isolated from several outbreaks demonstrated the same DNA banding patterns when typed by PCR-methods and PFGE, suggesting that it is spread out through RS. SE86 demonstrated high percentages of sensitivity to many antibiotics; however, an increasing resistance to nalidixic acid and ampicillin has been identified. After exposure to sub-letal pHs, SE86 demonstrated a greater acid adaptation and also to be more acid-and-thermal-resistant than *S.* Typhimurium and *S.* Bredeney also isolated in RS State. Acid-adapted SE86 presents higher virulence than non-acid-adapted SE86 and acid-adapted *S.* Typhimurium in germ-free mice. This strain has shown to be able to form biofilms on stainless steel and polypropylene, and was more resistant to 200 and 400 ppm sodium hypochlorite than others *Salmonella* serovars, but was not more resistant than other *S.* Enteritidis isolated in countries such as Albania, Morocco, Pakistan and Zimbabwe. SE86 mutants (*dps, rpoS*) were constructed in order to investigate the involvement of these genes with oxidative stress caused by sodium hypochlorite exposure. The results demonstrated that both genes are expressed during such exposure; however, *dps* was expressed in much higher amounts, increasing survival rates of SE86. Poultry meat produced in RS State demonstrated *Salmonella* prevalence of 4.4 % to 15 %, whilst expressively higher percentages of *Salmonella* were found in pork meat samples. Strains isolated from poultry and pork belonged to diverse serovars and, in general, demonstrated different patterns and higher antimicrobial resistance than SE86 isolated from salmonellosis.

Chapter 8 - *Salmonella spp.* are ubiquitous enteric bacteria. These gram-negative rods are the etiologic agents of food-borne salmonellosis and other food borne diseases **(1, 2)**. It is also the agents that cause typhoid and paratyphoid fevers. An estimated 2 to 4 million cases of salmonellosis occur annually in the United States **(3).** In Tunisia, bacteriological results showed that the rate of *salmonella* contamination remains quite high. In fact, the infection progresses, mostly under sporadic and rarely in an epidemic mode. *Salmonella spp.* can survive for long periods in natural waters, and the persistence of specific and epidemic strains is of great concern in public health. However, information on the diversity and occurrence of *Salmonella* strains is very scarce (4, 5), and as a consequence, the ecology of these species remains unknown. *Salmonella* bacteria cause, also, much of the food poisoning in the world, including an estimated 1,400,000 cases of salmonellosis in the United States each year. Although foods of animal origin (eggs, poultry, beef, dairy products…) are the vehicles of

transmission in most *Salmonella* outbreaks, contaminated fresh fruits and vegetables are now recognized as the main cause of salmonellosis (1, 2). The purpose of the present review was to provide information on the diversity of *Salmonella* strains isolated from environmental, animal, food and human samples which allows to follow trends in *Salmonella enterica* seotypes that provide information about source of infection and the efficacy of prevention and control measures. *Salmonella* is an important source of food-borne disease in humans throughout the world. An important change in the epidemiology of *Salmonella* occurred since the mid 1980s, when *Salmonella Enteritidis* (*S. Enteritidis*) became a major contaminant of eggs and egg products, hereby causing a worldwide pandemic. In France, salmonellosis is also the largest documented cause of foodborne infections (6). A total of 21 *S. Muenster* isolates had been received by the the French Food Safety Agency (Afssa) between January 2006 and February 2008. Among them, four were food isolates (poultry); the other 17 strains were from different origins (meat and bone meal, environmental isolates). In Canada, in 1982, a documented food poisoning outbreak caused by *S. Muenster* occurred and implicated cheddar cheese made from unpasteurised milk as the source of infection (7). On March 2008, the National Reference Centre (NRC) for *Salmonella* reported three laboratory-confirmed cases of *S. Muenster* to the (Institut de veille sanitaire, InVS). In Tunisia, during the study period (1994-2004), Nazek and coll showed that the top three *Salmonella* serotypes frequentely isolated with interchangeable ranking were: *Anatum, Enteritidis* and *Corvallis* (8). Aknown food origine was reported for all *Salmonella* isolates: red meat for *S.enterica* and *S.Anatum*. Whereas, *S.Enteritidis*, *Corvallis* and *Anatum* were most commonly isolated from poultry. The highest serotype commonly isolated from vegetables and fruits were *S.enterica* serotypes: *Anatum, Enteritidis* and *Corvallis*. Serotypes most frequentely isolated from milk and dairy products was *S.enterica* serotype *Anatum*. Finally, on November 2007, the Singapore Ministry of Health was notified of an outbreak of food poisoning. This outbreak was the largest common source outbreak of gastroenteritis caused by *Salmonella enterica* subspecies *enterica* serotype *Enteritidis* in Singapore. The epidemiological evidence implicates cream cake as the vehicle of transmission (9). In addition to food contamination, *Salmonella* species are pathogenic bacteria often detected in wastewater, freshwater, marine coastal water, and groundwater. Municipal sewage and storm water runoff become the conduits for the passage of pathogens into surface waters (10, 11). Serotype *Typhimurium* was dominant in all river samples and in marine and freshwater sediments. A high occurrence of this serotype has already been reported for polluted freshwaters (12, 13). In contrast, serotype *Newport* was the dominant serotype in wastewater, and consequently, it was also found in river samples. In Tunisia, Nazek and coll. reported that *S.enterica* serotype *Corvallis* was the most frequentely reported *Salmonella* serotype, accounting for 17.3% of all environmental isolates. Whereas, *S.enterica* serotype *Enteritidis* and *S.enterica* serotype *Anatum* came in second and third position, respectively, with non consistent trends during the study period (8). The most frequent source of isolation was tap water samples (8). Another study analyzing 60 wastewater samples showed that in entrance point 66.6% of samples were contaminated with *Salmonella* whereas, in exit points, a percentage of 20% were found. In entrance points, three serotypes of *Salmonella spp*. were detected: *Typhimurium*, *Enteritidis* and *Montevideo*; however, in exit points only the *Typhimurim* serotype was isolated (14). In 1999 and 2000, in Spain, the most frequent serotypes isolated from wastewater were *S. Enteritidis* and *S. Anatum* (15). Regarding *Salmonella* strains isolated from animal, the most frequently isolated *Salmonella* serotype during the study period was *S.enterica* serotype

Enteritidis followed by *S.enterica* serotype *Corvallis*. For *Enteritidis* serotypes, the source of isolation was poultry (70%), cattle (69%) and fish and shellfish (22%). While, *S.enterica* serotype *Amesterdam* and *S. enterica* serotype *Corvallis* were in a lead for cattles (8). *Salmonella* strains present a great impact on human health. In Tunisia, during 11 year surveillance periode (1994-2004), the top three reported *Salmonella* serotypes (*Enteritidis, Corvallis and Linvingstone*) accounted for 48.4% of all human isolates (8). *S.enterica* serotype *Enteritidis* came in first position during the study period, except for the years 2001, 2002, and 2003 where it came in the second position, prececed by *S.enterica* serotype *Livingstone* (8). The next not frequently reported serotype was *Corvallis*. Data from 2000 to 2004 suggest that *Salmonella Corvallis* infections are decreasing. *S. enterica* serotype *Linvingstone* was the third most common serotype (8). The most frequent source of isolation was stool samples (95% of *Salmonella* serotypes that were most commonly isolated) (8).Human infection due to *Salmonella enterica* serovar *Senftenberg* is infrequent in the United States. This bacterium colonizes the gastrointestinal tract of birds and mammals, and has been isolated from sewage systems, poultry processing plants, and different animal feeds (16, 17). *S. Senftenberg* infections in humans have been documented, mainly in Africa (18, 19) and India (20, 21), as well as in 1 US outbreak of gastroenteritis (22). Nonintestinal infectious syndromes due to *S. Senftenberg* were also reported in the Indian study (23, 24). At the end of February 2005, the National Institute for Health Surveillance was informed by the National Reference Centre for *Salmonella* of an increase of *Salmonella enterica* serotype *Agona* isolated in infants in January-February 2005. This is the first documented outbreak of *Salmonella Agona* in France. This investigation shows that regular microbiological examinations of formula were insufficient to detect low-grade or inhomogeneous contamination (http://www.invs.sante.fr). The authors' study highlighted the role of *Salmonella* as a pathogen widely dispersed in nature. These organisms can cause disease states that range from self-limited diarrhea to bacteremia considered as a public health problem. Thus, routine controls of all food, animal and human samples must be done in order to prevent community wide outbreaks of *salmonellosis.* The availability of routine molecular typing techniques in outbreak settings would facilitate tracing the source of infection and confirming epidemiological linkages of the *Salmonella* strains isolated from humans, food, animals and the environment.

Chapter 9 - Salmonellosis is of public health concern, accounting for about a quarter of all bacterial foodborne infections. According to the CDC, Salmonella enterica serovar Typhimurium is the most common Salmonella serotype reported. Of great concern is the possession and dissemination of antimicrobial resistance among Salmonella strains. Many antimicrobial resistance genes associated with Salmonella are found on conjugative plasmids. The misuse of antimicrobials in medical and animal-production settings may provide selective pressures that contribute to the proliferation of antimicrobial resistance, primarily by plasmid transfer. This study aims to present a selective pressure (i.e., varying antibiotic concentrations of four antibiotics) on the plasmid transfer rates of Salmonella via conjugation in-vitro. The four antimicrobials were selected for their plasmid-encoded resistance genes, activity against gram negative bacteria, and FDA approval for use in animal production. Successful conjugation pairs of S. Typhimurium isolates were mated under varying pressure conditions, plated on double antibiotic plates to select for transconjugants, and then conjugation rates were calculated. Preliminary results indicate a difference in conjugation rates across the four antimicrobials and within antimicrobials of varying concentration. Final

results of the study may be useful in identifying antibiotics that may be more or less likely to initiate plasmid transfer between S. Typhimurium isolates in-vitro.

Chapter 10 - Human salmonellosis infections are usually acquired via the food chain as a result of the ability of *Salmonella* serovars to colonize and persist within the gastrointestinal tract of food-producing animals and the avian reproductive tract, being able to contaminate, survive and grow inside eggs. This review gives an overview of the cellular and molecular processes involved in the colonization of hen's eggs and in *Salmonella* persistence in the harsh conditions of the egg albumen with special focus on serovar Enteritidis, which shows a particular tropism and ability to persist in chicken oviducts and ovaries and to contaminate, survive and grow inside eggs. The trans-ovarian (also referred to as vertical transmission) and the trans-shell (referred to as horizontal transmission) routes of colonization of the egg are briefly described. In addition, several gene candidates involved in bacterial survival in the egg albumen are identified as potential biomarkers, appropriate for the design of ambitious strategies for the prevention of salmonellosis.

Chapter 11 - Members of *Salmonella* are able to infect a wide range of hosts with some subspecies and serovars being host-specific while others are wide host range organisms. Salmonella is most commonly associated with humans, food animals (bovine, swine and poultry) and reptiles such as turtles, lizards, and iguanas. The exact nature of this diversity towards host susceptibility is still unclear. In the present study, whole genomes and subsequent protein homologues from Salmonella Pathogenicity Islands 1 and 2 of several *Salmonella* serovars were analyzed for their structure-function constraints to identify evolutionary pressures. Whole genome alignment showed no explicit pattern of rearrangement, phylogenetic trees of pathogenicity islands genes revealed that the branch lengths were very small, and functional constraint analysis revealed that most of these genes were operating under negative selection. The results indicate that genes of Salmonella Pathogenicity Island 1 and 2 are functionally conserved across serovars, regardless of host range. Thus, the present result suggests that genes located in the pathogenic islands may not be sufficient solely for host specificity and pathogenesis, and other accessory proteins from different strains as well as host factors may play an important role in influencing the severity of the outcome of the diseases.

Chapter 12 - The *Salmonella*/microsome assay is a technique for detection of mutagens based on the principle that mutagens can cause the the reversion of genetic engineered mutations that suppress biosynthesis of histidine in strains of *Salmonella typhimurium*. It started and has been employed since the beggining of the 70's to investigate chemical and biological samples (urine, feces and plasma) and is currently the most used to evaluate the presence of mutagenic compounds in environmental samples (soil, water, atmospheric material and sediment). Due to its importance, it is nowadays included in environmental legislation in several countries. Several improvements were introduced to enhance the sensibility of the test and broaden its applicability and reliability. Even about to complete its forty-year birthday, the assay keeps receiving improvements, including the development of new sensitive strains for detection of groups of compounds and new ways of evaluation and interpretation of the results. This chapter brings a brief review covering the forty years of the *Salmonella*/microsome assay studies and future perspectives and trends of this important mutagenicity assay.

Chapter 13 - *Salmonella enterica* subspecies *enterica* has been isolated from every region around the world and is considered as a potential pathogen for many animal species. The

epidemiology, reservoirs and transmission of *Salmonella* is very complex owing to humans, animals and environment are involved. The purpose of this work was to review the current knowledge of *Salmonella* in the wildlife of sub-Antarctic and Antarctic region. For a long time Antarctic was a region that remained isolated from human contact, but in recent years the human presence has notably increased and as consequence, the danger of introduction of microorganisms to the Antarctic fauna. One of the most extensively studied bacterial species in the region is *Salmonella*, because of its characteristics mentioned above. *Salmonella* infections in Antarctic wildlife were first reported in 1970. Since then, several studies have been conducted, which showed that *Salmonella enterica* subspecies *enterica* isolated from sub-Antarctic and Antarctic fauna belonged to the most prevalent serovars in humans and domesticated animals, with the exception of few rare or exotic serovars. Some of *Salmonella* serovars found in Antarctic wildlife were: Enteritidis, Typhimurium, Newport, Infantis which are among the main serovars isolated from salmonellosis cases in humans and animals in the world. *Salmonella* infections in sub-Antarctic and Antarctic wildlife were reported in the following animals: *Adelie* penguins (*Pygoscelis adelie*), south polar skuas (*Catharacta maccormicki*), gentoo penguins (*Pygoscelis papua*), fur seal (*Arctocephalus gazelle*), black-browed albatross (*Diomedea melanophrys*), sea lions (*Phocarctos hookeri*), southern petrels (*Macronectes giganteus*), kelp gulls (*Larus dominicanus*), Weddell seals (*Leptonychotes weddelli*), emperor penguin (*Aptenodytes forsteri*). All of these animal species were *Salmonella* carriers and none of them had clinical signs of salmonellosis. Different routes of introduction of *Salmonella* to Antarctic region have been proposed: by human carriers, by contaminated foods, by infected birds, sewage from some land-based operations or from discharge of this material from ships sailing in waters of the region. One of the most possible routes of introduction is by birds, because many Antarctic birds have long routes of migration. Studies on *Salmonella* carried out in Antarctic region allow the scientific community to know the sanitary status of Antarctic fauna and also, they can be used as an indicator to know the local ecosystem status. Up to now, in spite of the different studies that have been performed in sub-Antarctic and Antarctic, it was impossible to determine if *Salmonella* was introduced or was and indigenous bacterium in the Antarctic ecosystem.