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Course title microbial Ecology

Assignment:As a microbial ecologist discuss the variety of diverse analytical techniques you will employ to understand the critical role of microbes in specific ecosystems and in maintaining life on earth.

ANSWER

MICROBIAL ECOLOGY

MICROBIAL METAGENOMICS,METATRANSCRIPTOMICS,AND METAPROTEOMICS

Microbial [ecology](/topics/agricultural-and-biological-sciences/ecology%22%20%5Co%20%22Learn%20more%20about%20Ecology%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) explores the diversity, distribution, and abundance of [microorganisms](/topics/earth-and-planetary-sciences/micro-organism%22%20%5Co%20%22Learn%20more%20about%20Micro-Organism%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages), their specific interactions, and the effect that they have on ecosystems. Although not traditionally thought of as a central discipline within ecology, [microbial ecology](/topics/earth-and-planetary-sciences/microbial-ecology%22%20%5Co%20%22Learn%20more%20about%20Microbial%20Ecology%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) is of critical importance because microorganisms represent the vast majority of the genetic and metabolic diversity on the planet and drive most of the critical ecosystem processes which recycle matter and energy. [Microorganisms](/topics/earth-and-planetary-sciences/micro-organism%22%20%5Co%20%22Learn%20more%20about%20Micro-Organism%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) have evolved to occupy almost every conceivable [ecological niche](/topics/earth-and-planetary-sciences/ecological-niche%22%20%5Co%20%22Learn%20more%20about%20Ecological%20Niche%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) and energy-generating mechanism. In so doing microorganisms engage in a wide range of ecological interactions with each other and with higher forms of life. Laboratory culture and culture-independent molecular approaches are typically used to identify microbial species, their evolutionary relationships, and the environmental variables that dictate abundance, distribution, and specific activity. Although these approaches are often complementary, they each study microbial ecology from a different perspective. Culture-independent approaches allow the in situ study of microbial interactions and dynamics in complex natural communities. Laboratory cultures have determined the wide metabolic diversity of microorganisms and due to their simplicity and ease of manipulation are invaluable for testing fundamental ecological theories relating to evolutionary adaptation, competition, and demographic.

IMPACT OF UNIT OPERATIONS FROM FARM TO FORK ON MICROBIAL SAFETY AND QUALITY OF FOODS

The microbial ecology of foods is dependent not only upon their composition, packaging systems, the origin of raw materials, but also on the unit operations employed during food processing, their intensity, and combinations. Unit operations modify material properties aiming to produce uniform and high-quality food products both with greater acceptance by the consumers and with longer shelf life. Microorganisms, including bacteria, yeasts, molds, viruses, and parasites may have different susceptibilities to unit operations employed during food processing. While viruses and parasites are not able to grow but to survive in foods, bacteria, molds, and yeasts can survive, grow as well as be inactivated, inhibited, or removed from foods. Several technologies can be applied to achieve such objectives, like on-farm (cleaning, selection and classification, cooling, storage, and transport) or on-factory unit operations (heating, refrigeration/freezing, dehydration, modification of atmosphere, irradiation and physical, chemical, and microbial-based operations).

The microbial ecology of foods can be studied by conventional culturing microbiological methods, which are time-consuming and laborious. However, a variety of novel methodologies with more sensitive, precise, and reproducible results can be employed to get insights into the microbial ecology of foods. Even with the effort of industries and government regulatory agencies to guarantee good quality food products, [foodborne diseases](/topics/food-science/food-borne-disease%22%20%5Co%20%22Learn%20more%20about%20Food-Borne%20Disease%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) remain occurring. This chapter discusses the impact of unit operations from farm to fork on microbial ecology (safety and quality) of foods.

STRATEGIES FOR MICROBIAL SURVIVAL IN THE CLEAN ROOM ENVIRONMENT.

Microbial ecology in pharmaceutical environments is controlled by various environmental factors such as temperature, pH, nutrient availability, pressure, and water availability. Microbial flora in cleanroom environments can be effectively controlled by adjusting different parameters (Hyde, 1998). Unlike microorganisms growing in culture, microorganisms within the pharmaceutical environment—the cleanroom—behave in different ways according to different environmental conditions.

How do microorganisms respond to different environmental fluctuations in the environment? Pharmaceutical manufacturing comprises physical processes such as blending, compression, filtration, heating, encapsulation, shearing, tableting, granulation, coating, and drying. These processes expose microbial cells to extensive environmental stresses. Microorganisms respond to the lack of nutrients and other environmental fluctuations by undertaking different survival strategies (Roszak & Colwell, 1987). Thus microorganisms recovered from production environments are stressed due to the fluctuations of parameters during manufacturing processes, lack of nutrients, low water activity, contact with chemicals, and temperature changes. With these strategies, microorganisms are not always metabolically active and reproducing.

Under conditions of limited supply of nutrition vegetative forms of certain bacteria, notably Gram-positive rods and Actinomycetes, form highly resistant and dehydrated forms which are called as endospores. Bacterial endospores can resist extreme conditions and survive for years in the absence of nutrients. However, when nutrients return, these spores can germinate and become growing cells (Setlow & Johnson, 2007). Bacterial spores are more resistant than fungal spores and yeasts, and considerably more resistant than vegetative bacteria to the actions of antiseptics and disinfectants (Russell & Furr, 1996).

Biofilm formation is another important survival strategy against environmental stress. A biofilm is a complex aggregation of microorganisms growing on solid substrate. A biofilm contains about 15% of microbial cells and 85% extra polymeric substance (EPS). EPS is composed of polysaccharides, proteins, other polymers, and water. Biofilms are characterized by structural heterogeneity, genetic diversity, and complex community interactions. A biofilm formation is often initiated by micro colonies from one type of organism. However, biofilms quickly become heterogeneous as mixed cultures of bacteria, as well as fungi, algae, and protozoa join the established structure and become intermixed. In fact, within a biofilm different types of microorganisms can coexist and form stable communities. It is resistant to phagocytic amoebae, and much more resistant than planktonic cells to antimicrobial agents. For example, chlorination of a biofilm is usually unsuccessful because the biocide only kills the bacteria in the outer layers of biofilm. The bacteria within the biofilm remain healthy and the biofilm can regrow. Repeated use of antimicrobial agents on biofilms can cause bacteria within the biofilm to develop an increased resistance to biocides. In the pharmaceutical environments, biofilms can develop on the product contact surfaces of equipment and interiors of water purification and distribution system, which leads to clogs, corrosion, and biological contamination of the system (Reidewald, 1997).

ADAPTATION POTENTIAL OF MICROBES TREATED BY PULSED ELECTRIC FIELDS.

The microbial ecology of foods is influenced dramatically by food processing and preservation techniques. Some of the processes used are traditional and are known to be effective in controlling any foodborne pathogens present. Control may be achieved by the effects of the product formulation, including the incorporation of preservatives, use of a decontamination treatment and other hygiene measures. Although vegetative bacteria may sometimes survive such conditions, they are often sub-lethally injured. In fact, almost all microorganisms of concern in the food industry are susceptible to stress and injury by chemical or physical treatments involved in food production. Sub-lethal injury was first demonstrated with [lactic acid bacteria](/topics/agricultural-and-biological-sciences/lactic-acid-bacteria%22%20%5Co%20%22Learn%20more%20about%20Lactic%20Acid%20Bacteria%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)used as starter cultures in dairy fermentations. In this case, injury arose from exposure to chill conditions and the organisms were found to require additional nutrients to restore growth at the normal rate (IFT, 2002). The manifestations of injury can include more exacting growth requirements, an increased time lag prior to exponential growth, and greater sensitivity to inhibitory agents. On the other hand, there may be subsequent resistance to inactivation by agents that are normally inhibitory, a change in the virulence of a pathogen or acquisition of new characteristics (IFT, 2002). When only mild antimicrobial treatments are used in food processing, as in some newer techniques, the number of surviving organisms will be greater and sub-lethal injury will be less.

Whatever the proportion of injured cells, these can usually revert to the normal state by repairing the cellular damage under suitable conditions. Thus, the original capabilities of the cell will be regained following a period of recovery in the absence of inhibitory agents. In practice, however, the effectiveness of a particular treatment for inactivating micro-organisms is often measured by enumerating any survivors on a selective isolation medium. On such a medium, injured organisms would appear to be dead, because viability is generally based on the ability of an organism to multiply to a measurable extent and death has been defined as an irreversible loss of this capability (Mackey, 2000).

Microbial response to stress in a food system may play a major role in the behaviour of pathogens (Sheridan and McDowell, 1998). Not only can the stress response increase resistance to inactivation treatments, but there may be an enhanced ability to cause human illness if the organism is ingested. For these and other reasons, there is a need for scientific study of multiple stress treatments in order to develop new and improved control systems for particular purposes, not least in relation to the food-processing environment. While new processing techniques can provide opportunities for better control of [microbial contamination](/topics/agricultural-and-biological-sciences/microbial-contamination%22%20%5Co%20%22Learn%20more%20about%20Microbial%20Contamination%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages), their impact on overall food safety and the possible emergence of unexpected food safety problems should not be ignored. The same concerns apply to combined treatments that are intended to offer additive or even synergistic effects.

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LABORATORY TRAINING AND WRITING COMPREHENSION FOR FOOD SAFETY EDUCATION

Understanding the gastrointestinal microbial ecology of a variety of food animals has always been a very important component of nutritional research studies. Traditionally, much of the early research focused on ruminant animals and rumen microbiology, in part because of the interest in understanding how ruminants were able to subsist on cellulose. Although it was known for some time that ruminant animals could derive [nutritional value](/topics/food-science/nutritive-value%22%20%5Co%20%22Learn%20more%20about%20Nutritive%20Value%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) from forages, it was unclear how that was accomplished. Research efforts beginning in the mid-twentieth century led to the isolation of many of the anaerobic microorganisms that were prominent in the rumen, including the primary cellulolytic bacteria and anaerobic protozoa (Bryant, 1959; Hungate, 1950, 1960, 1966, 1979; Schaechter, 2013). Once pure culture collections of the primary rumen bacterial isolates were established, this opened the door for numerous groups of researchers to enter the field of rumen microbiology and initiate studies to develop an in-depth understanding of individual organisms as well as metabolic interactions among groups of organisms. Consequently, rumen ecological concepts were identified and characterized such as the cross-feeding among cellulolytics and noncellulolytics during fiber degradation in the rumen, identifying organisms with multiple fermentation pathways to generate ATP for growth and metabolism, and discovering the ability of some organisms to both produce certain metabolites, such as lactic acid, and subsequently utilize these same metabolites as growth substrates under specific growth conditions (Hungate, 1966; Russell and Hespell, 1981; Hobson and Wallace, 1982a,b; Wolin and Miller, 1982, 1983; Ricke et al., 1996; Mackie, 2002; Weimer, 1992; Weimer et al., 2009). A key development was the identification of methanogens responsible for production of methane in the rumen, but perhaps more importantly from a rumen functionality standpoint, their ability to consume hydrogen generated by the saccharolytic population and drive overall fermentation to a more oxidized series of end products via a process referred to as interspecies hydrogen transfer (Hungate, 1966; Wolin and Miller, 1982, 1983; Wolin, 1979; Saengkerdsub and Ricke, 2014). As environmental interest in reducing rumen methane production became more important, competitors for hydrogen as a substrate such as acetogens were identified that had the capability of using hydrogen and carbon dioxide to form acetate (Sharak Genther and Bryant, 1987; Le Van et al., 1998; Boccazzi and Patterson, 2011, 2013, 2014; Jiang et al., 2012a,b; Pinder and Patterson, 2012, 2013).

To accomplish the tremendous amount of information on the rumen microbiota required the adaptation of traditional microbiology culture methods to accommodate strict anaerobes that were incapable of tolerating oxygen (Bryant, 1959, 1972; Hungate, 1960). However, routine anaerobic microbiology required a number of technological laboratory challenges to be overcome. Certainly first and foremost was developing media-handling techniques that limited initial oxygen exposure, removed residual oxygen, and retained oxygen-free liquid and headspace conditions during growth of strict anaerobes. The use of combinations of heating of media solutions and addition of chemical reductants allowed for poising of culture media at low oxidation-reduction levels for optimal growth (Bryant, 1972; Zehnder and Wuhrmann, 1976; Brock and O’Dea, 1977; Carlsson et al., 1979; Jones and Pickard, 1980; Moench and Zeikus, 1983; Wachenheim and Hespell, 1984; Ricke and Schaefer, 1990). Development of a roll tube technique with an inner layer of agar media that could be properly prepared anaerobically, oxygen-free gasses added, and sealed with oxygen-impermeable butyl rubber stoppers opened the door for isolation of individual colonies of strict anaerobic bacteria for further characterization (Hungate et al., 1966; Hungate, 1969; Holdeman and Moore, 1972). Utilization of serum bottles that could be sealed with stoppers via aluminum crimp sealing and inoculated by syringes allowed for pressurized anaerobic headspaces that revolutionized the cultivation and characterization of methanogens (Macy et al., 1972; Miller and Wolin, 1974; Balch and Wolfe, 1976; Balch et al., 1979). Characterizing strict anaerobic bacteria became more routine with the advent of anaerobic glove boxes that enabled manipulation of cultures using conventional plating techniques and subsequent enumeration in a carefully controlled anaerobic atmosphere and with some modifications that could also be used for growth of methanogens (Aranki et al., 1969; Aranki and Freter, 1972; Edwards and McBride, 1975; Cox and Herbert, 1978; Gill et al., 1978).

Once anaerobic methodology became well established, inevitably efforts to train the next generation of students became important. Consequently, rumen microbiology course work, particularly at the graduate level, was offered in several dairy and animal science departments of land grant institutions from the 1960s onward. One of the primary sources of material was the comprehensive monograph written by R.E. Hungate in 1966, which was still considered the seminal treatise on rumen microbiology 25 years later (Ling, 1991). Since the publication of Hungate’s book, several monographs and edited books emerged to provide supplemental material on rumen theoretical aspects as well as protocols and descriptive assessments of the rumen microbial species and rumen function (Holdeman et al., 1977; Ogimoto and Imai, 1981; Van Soest, 1982; Hobson, 1988; Dehority, 1993; Russell, 2002). When laboratory principles developed for rumen microorganisms were applied to other animal gastrointestinal intestinal systems a series of book publications were generated that covered most of the major animal species as well as humans (Clark and Bauchop, 1977; Holdeman et al., 1977; Hentges, 1983; Drasar and Barrow, 1985; Woolcock, 1991; Ewing and Cole, 1994; Mackie and White, 1997a,b). Publications that focused on methodology protocols also emerged that proved useful (Holdeman et al., 1977; Levett, 1991). Along with gastrointestinal microbiology oriented material, complementary textbooks on fermentation biochemistry and microbial physiology could also be included for some of the fundamental tenets of anaerobic microbiology (Gottschalk, 1979, 1986; Ingraham et al., 1983; Gerhardt et al., 1994; Moat et al., 2002).

For the mid-twentieth century, anaerobic microbiology training and course work were considered the domain of food animal-oriented academic departments and, for the most part, primarily focused on rumen microbiology with some attempts to expand to other gastrointestinal systems. Consequently, the audience for this type of material was fairly limited and training remained confined to those dedicated to this academic discipline. However, this was soon to change dramatically and with these changes the target audiences for this type of training. While the fundamentals and principles would not necessarily be expected to change, the manner in which they might be taught would potentially have to be modified. To some extent, this also required a change in instructional philosophy as well. Some approaches and the underlying philosophies on how to develop and repackage effective delivery of this type of training are discussed in the following sections.

MICROBIAL ECOLOGY IN EXTREME ACIDIC ENVIRONMENTS.

A major issue in microbial ecology is to identify the limits of life for growth and survival and to understand the molecular mechanisms that define these limits. Thus, interest in the biodiversity and ecology of extreme environments has grown in recent years for several reasons. Some are basic and revolve around the idea that extreme environments are believed to reflect early Earth conditions. Other issues are related to the biotechnological potential of [extremophiles](/topics/biochemistry-genetics-and-molecular-biology/extremophile%22%20%5Co%20%22Learn%20more%20about%20Extremophile%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages), such as the use of the metabolic properties of some [microorganisms](/topics/biochemistry-genetics-and-molecular-biology/microorganism%22%20%5Co%20%22Learn%20more%20about%20Microorganism%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)for metal extraction. In this regard, the study of extremely acidic environments (pH<3) has become increasingly important since environmental acidity is often caused by [microbial activity](/topics/biochemistry-genetics-and-molecular-biology/microbial-activity%22%20%5Co%20%22Learn%20more%20about%20Microbial%20Activity%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages). The advent of [high-throughput sequencing](/topics/biochemistry-genetics-and-molecular-biology/high-throughput-sequencing%22%20%5Co%20%22Learn%20more%20about%20High%20Throughput%20Sequencing%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) technologies has allowed sampling [microbial diversity](/topics/biochemistry-genetics-and-molecular-biology/microbial-diversity%22%20%5Co%20%22Learn%20more%20about%20Microbial%20Diversity%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) more deeply and widely than ever before and thus affords new opportunities for comprehensively examining broader trends of microbial distribution with larger numbers of ecological samples. Acidic extreme environments are unique ecological niche for acid- and toxic-metal-adapted microorganisms. These low-complexity systems offer a special opportunity for the ecological and evolutionary analyses of natural microbial assemblages. The last decade has witnessed an unprecedented interest in the study of acidophilic communities using 16S rRNA high-throughput sequencing and community genomic and postgenomic methodologies, significantly advancing our understanding of microbial diversity, community function, and evolution in acidic environments. This chapter summarizes the current status of our knowledge of extreme acidic environments microbial ecology through the use of meta-“omic” molecular techniques.

MICROBIOLOGY OF A TYPICAL ENVIRONMENT.

### Metagenomics-derived fungal diversity and functional characterization

Substantial advances in studying microbial ecology were made following the implementation of [metagenomic](/topics/immunology-and-microbiology/metagenomics%22%20%5Co%20%22Learn%20more%20about%20Metagenomics%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) techniques. Metagenomics unveils the functional gene composition of environmental communities, allowing broader characterization than based on the diversity of one gene, including [16S rRNA](/topics/medicine-and-dentistry/rna-16s%22%20%5Co%20%22Learn%20more%20about%20RNA%2016S%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) and [internal transcribed spacer](/topics/medicine-and-dentistry/internal-transcribed-spacer%22%20%5Co%20%22Learn%20more%20about%20Internal%20Transcribed%20Spacer%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) used in bacterial and fungal [phylogenetic](/topics/immunology-and-microbiology/phylogeny%22%20%5Co%20%22Learn%20more%20about%20Phylogeny%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) studies, respectively. This novel approach allows linking function and phylogeny of yet-to-be-cultivable organisms (Thomas, Gilbert, & Meyer, 2012). When metagenomic analysis of samples collected from a JPL cleanroom and its adjacent gowning area was performed, a remarkably complex ecosystem was observed. Viable bacteria, fungi and viruses were detected, with fungal sequences predominantly observed in samples collected from the gowning area. Interestingly, the abundance of fungal sequences in samples treated with PMA prior to [DNA extraction](/topics/immunology-and-microbiology/dna-extraction%22%20%5Co%20%22Learn%20more%20about%20DNA%20Extraction%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) was higher than in untreated samples. Additionally, PMA treatment enabled the detection of viral sequences that were undetectable in untreated samples due to their low abundance relative to nonviable primal and eukaryotic DNA. Similar to previous reports, PMA treatment led to increased resolution in detecting microbial species and additionally revealed viral presence in the cleanroom environment (Weinmaier et al., 2015).

A follow-up study used metagenomics to detect [virulence factors](/topics/immunology-and-microbiology/virulence-factors%22%20%5Co%20%22Learn%20more%20about%20Virulence%20Factors%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) associated with [human pathogens](/topics/immunology-and-microbiology/human-pathogen%22%20%5Co%20%22Learn%20more%20about%20Human%20Pathogen%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) in environmental samples collected from various cleanrooms at Kennedy Space Center (KSC) and JPL at several time points. It was the first study that targeted functional genes associated with potentially health hazardous [microorganisms](/topics/immunology-and-microbiology/microorganism%22%20%5Co%20%22Learn%20more%20about%20Microorganism%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) to determine if strict maintenance procedures favoured natural selection of [pathogenic microbes](/topics/immunology-and-microbiology/pathogenic-microbes%22%20%5Co%20%22Learn%20more%20about%20Pathogenic%20Microbes%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages). Analysis of samples collected during assembly of the Phoenix spacecraft, when rigid cleaning procedures were applied, revealed a decrease in pathogen abundance and overall [microbial diversity](/topics/immunology-and-microbiology/microbial-diversity%22%20%5Co%20%22Learn%20more%20about%20Microbial%20Diversity%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages). However, during this time the abundance of potential virulence factors increased when compared to samples collected prior or postspacecraft assembly, suggesting that survival advantage was provided by virulence factors. Although the major focus of the study were bacterial pathogens, pathogenic genes associated with [Candida parapsilosis](/topics/immunology-and-microbiology/candida-parapsilosis%22%20%5Co%20%22Learn%20more%20about%20Candida%20Parapsilosis%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) were also detected, implying that fungi present in “clean” environments may also pose a threat to human health (Bashir et al., 2016).

The previously characterized HEPA and vacuum debris collected from the ISS were later subjected to functional metagenomic analysis. In contrast to pyrosequenced and Illumina-sequenced samples (Checinska et al., 2015), metagenomic analysis of the HEPA filter revealed the presence of viable fungal sequences belonging to genus [Aspergillus](/topics/immunology-and-microbiology/aspergillus%22%20%5Co%20%22Learn%20more%20about%20Aspergillus%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages), while dust samples contained [Penicillium](/topics/immunology-and-microbiology/penicillium%22%20%5Co%20%22Learn%20more%20about%20Penicillium%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) and [Malassezia](/topics/immunology-and-microbiology/malassezia%22%20%5Co%20%22Learn%20more%20about%20Malassezia%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) genera. The predominant fungal species in samples collected from JPL cleanrooms were [Aureobasidium pullulans](/topics/immunology-and-microbiology/aureobasidium-pullulans%22%20%5Co%20%22Learn%20more%20about%20Aureobasidium%20pullulans%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages), [Alternaria](/topics/immunology-and-microbiology/alternaria%22%20%5Co%20%22Learn%20more%20about%20Alternaria%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages) arborescens and Coniosporium apollinis. Functional pathway analysis revealed higher abundance of [antimicrobial resistance](/topics/immunology-and-microbiology/antimicrobial-resistance%22%20%5Co%20%22Learn%20more%20about%20Antimicrobial%20Resistance%20from%20ScienceDirect%27s%20AI-generated%20Topic%20Pages)(AMR) and virulence genes in ISS debris than HEPA samples. However both ISS-derived samples had a higher abundance of sequences associated with virulence or AMR than cleanroom samples, implying survival advantage (Be et al., 2017). This is in agreement with a previous observation of samples from KSC and JPL cleanrooms, suggesting that harsh environments promote increased abundance of virulence factors (Bashir et al., 2016).

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