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Bacteria: Pathogenicity and virulence-factors

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ABSTRACT

This chapter provides guidance on topics and issues relevant to the risk/safety assessment of commercial environmental applications involving genetically engineered micro-organisms, especially bacteria. It explores the important aspects in bacteria for causing adverse human health effects, and how this knowledge can be used in biosafety regulatory assessment. It contains information on bacterial pathogenicity (general considerations, factors and determinants, genetics and molecular biology), and also elements on assessing potential for bacteria-mediated adverse human health effects.

General considerations for bacterial pathogenicity

This chapter provides guidance on the concept of bacterial pathogenicity in the context of risk/safety assessment of deliberate release of "genetically engineered", or "genetically modified",1 micro-organisms intended for commercial environmental applications (e.g. bioremediation, biosensors, biofertilisers, biopesticides, biomining, biomass conversion or oil recovery). It is limited in scope to bacteria that may exhibit properties pathogenic to human beings. Not included in the scope are environmental releases of known (potential) pathogens, e.g. vaccine strains.

The chapter explores the factors that are important in bacteria for causing adverse human health effects and assesses how this knowledge can be used in risk/safety assessment of environmental applications of bacteria. Where appropriate, the chapter also refers to certain aspects of mamalian bacterial pathogens. For specific aspects of plant and/or other animal (e.g. fish, insects and other invertebrates) pathogens, separate documents on these issues would be needed. Genetically engineered bacteria applied for environmental purposes, including field trials, should be evaluated to determine whether they may pose hazards to human health, which this chapter addresses. The analysis from the OECD "Blue Book" on recombinant DNA safety (OECD, 1986) appears to be still valid: Agricultural applications may result in release of large quantities of modified [micro]-organisms into terrestrial or aquatic ecosystems.

Recombinant DNA-derived vaccines for animals and humans, as well as certain plant-associated micro-organisms, may in some cases have a limited pattern of environmental exposure because of biological specificity to the host, but incidental release to the environment certainly occurs in sewage and feed-lot or run-off waters, and may be significant. Environmental applications (e.g.

metal extraction, pollutant and toxic waste degradation) may be confined initially to a specific location or may result in broad ecosystem exposure. The scientific considerations for assessing risk/safety will vary with each particular environmental application, depending on the organism, the physical and biological proximity to man and/or other significant biota. Local quarantine regulations, confinement measures and monitoring methodologies utilised during research and development will also be relevant. In general, prior to their release, bacterial strains should be submitted to an assessment of their potential health effects, including their pathogenicity. As "virulence" is the quantitative measure of the pathogenicity of a micro-organism, the virulence factors of a bacterial strain are its traits that will be taken into account in the risk/safety assessment. For the special case of genetically engineered micro-organisms, the risk/safety assessment should take into account any characteristics of the engineered micro-organism related to pathogenicity, and whether any introduced traits are associated with pathogenicity. When performing a regulatory review of the role of a donor gene as a virulence factor in the recipient micro-organism, regulators need a good understanding of the significance of a given virulence gene in the physiological background of the donor organism, as well as of the constitution of the recipient micro-organism. A large number of interacting factors affect the ability of a micro-organism to become pathogenic, and acquisition of a single gene in the absence of other genes necessary for pathogenicity will not likely convert a non-pathogen to a pathogen.

Only if the newly acquired gene can have a role in the pathogenicity of the recipient micro-organism can an interaction be expected between the newly acquired gene and the resident genes contributing to a pathogenic lifestyle. Pathogenicity is a multifactorial process which depends on the immune status of the host, the nature of the bacterial species or strain, and the number of organisms in the exposure. Therefore, the risk/safety assessment for human health can only be done on a case-by-case basis, taking into account the activity(s) of the introduced gene(s), the (potential) health hazards of the bacterial strain depending on the route of exposure (e.g. ingestion, inhalation, dermal contact) and the actual way that exposure to the strain is expected to occur under the conditions of the release. Exposure can depend on a number of factors, including the pattern of release (e.g. aerial spray, ground application, deep well injection, application into water bodies or effluent streams, shedding from inoculated humans or animals) and the scale of use (e.g. pilot, field trial, commercial use). Because this chapter is intended as an aid to general risk/safety assessment tool, its nature is generic, i.e. not organism specific, and refers to specific bacteria and characteristics only to illustrate specific concepts. In addition to describing potential adverse health effects, and the bacterial factors that can contribute to these effects, the chapter describes general considerations in assessing the potential hazard of unmodified bacteria, e.g. a description of some tools available for predicting pathogenicity. Lastly, the chapter addresses considerations for the potential to introduce or alter pathogenicity as a result of genetic modifications to the micro-organism.

General considerations in assessing the hazardous potential of bacteria:

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The concept of bacterial pathogenicity This section and the following two sections deal with the concept of bacterial pathogenicity in general, as it is discussed for unmodified bacteria; the concept also applies to genetically modified bacteria. Pathogenic bacteria have the ability to invade their hosts and produce disease. In this chapter, "pathogenicity" is referred to as the property of a micro-organism to cause disease. The great majority of bacteria that are encountered in the environment usually do not present problems to human health, in the sense that no record exists of them behaving as pathogens. Many bacteria are even beneficial, e.g. because of their role in essential processes in the environment such as mineralization, or their function as human symbionts.

There are many bacteria that may act as opportunistic pathogens, i.e. organisms that are normally present in the environment or as part of the commensal bacterial population of a host, but that may cause disease when defense systems of the host become debilitated, or when the equilibrium within the existing bacterial population is disrupted. In general, given the interplay between members of microbial communities and the interplay between microorganisms and potential hosts, it is unrealistic to say that a bacterium can never be a pathogen, and probably "non-pathogenic" bacteria can best be seen as bacteria that have not yet proven to have pathogenic potential. Although "pathogenicity" can be defined in terms of properties of a micro-organism, it is important to keep in mind that the concept of pathogenicity is highly anthropomorphic, as it implies that a micro-organism would cause disease "on purpose". A more realistic view is that the body is a habitat for micro-organisms to adapt to and use as a favourable environment for survival and growth. Some bacteria have developed a "lifestyle" that enables them to colonise this niche in symbiotic as well as in pathogenic ways (Wassenaar and Gaastra, 2001). Each body surface – skin, conjunctiva, mucous membranes of the upper and lower respiratory tract, intestinal tract, genital tract and so forth – harbors a characteristic commensal bacterial population which differs qualitatively from the population of other areas of the body. Bacteria with pathogenic behaviour may establish a foothold in this microbial ecosystem. Once established, other pathogenic properties allow the pathogen to penetrate into deeper tissues, to avoid or counteract host defense mechanisms, and to multiply. As they pursue this strategy, pathogenic bacteria produce damage to the host. Virulence-associated factors may be defined as all factors that are essential for expressing pathogenicity. Whether a host will develop disease is, however, not just determined by the pathogenic potential of the bacterium, but also by host factors. There is a formidable array of specific and non-specific host factors that affect the outcome of an encounter between a host and a pathogenic bacterium. For example, the normal commensal population plays an important role in protecting the host from invasion by pathogenic organisms.

They do this by mechanisms such as:

1) competition for the same nutrients;

2) competition for the same receptors on the host cells (tropism);

3) production of bacteriocins or other antimicrobial agents (interference); and

4) stimulation of cross-protective immune factors. The commensal population of the host may be affected by a number of activities (e.g. use of antibiotics).

Additional host factors that can affect pathogenicity include the production of antimicrobial substances (e.g. lysozyme in bronchial secretions; or the pancreatic enzymes, bile or intestinal secretions; or secretion of acid [HCl] for low pH of the stomach). Also, humans have an innate immune system that protects against invasion. When this system breaks down, e.g. in advanced stages of acquired immunodeficiency syndrome (AIDS) (Gradon, Timpone and Schnittman, 1992), bacteria that are normally not able to cause disease in humans may become opportunistic pathogens that cause conditions that clinically mimic the more commonly encountered "frank" pathogens. The potential of bacteria that normally occur in the environment to cause opportunistic infections in hosts with debilitated defense systems is recognised as an important human health hazard. The case of the Burkholderia cepacia complex (Bcc) is an example (Mahenthiralingham, Urban and Goldberg, 2005). Bacteria of the Bcc are found throughout the environment, some as plant pathogens.

General considerations in assessing the hazardous potential of bacteria:

Classification of risk groups of bacteria Pathogenic bacteria are commonly classified in risk groups, according to their pathogenic potential. The classification of the World Health Organization (WHO), as found in its Laboratory Biosafety Manual (WHO, 2004), is generally accepted. It should be noted, though, that these risk groups are primarily concerned with laboratory applications, where exposure may be high. They are valid for persons that are not immune compromised. According to this classification,

risk group 1 ("no or low individual or community risk") comprises micro-organisms that are unlikely to cause human or animal disease.

Risk group 2 ("moderate individual risk, low community risk") comprises pathogens that can cause human or animal disease but that are unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment; laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of the spread of infection is limited.

Risk group 3 ("high individual risk, low community risk") comprises pathogens that usually cause serious human or animal disease but do not ordinarily spread from one infected individual to another; effective treatment and preventive measures are available.

Risk group 4 ("high individual and community risk") comprises pathogens that usually cause serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly; effective treatment and preventive measures are not usually available For practical reasons, also in regulatory practice, a distinction is drawn between bacteria that are pathogenic to humans and bacteria that are pathogenic to other animals.

Host specificity of bacteria is the result of differences between the environment that bacteria encounter in different hosts, i.e. in the human body and the bodies of other animals. If there are similarities between these environments, it may be expected that pathogenic organisms frequently "jump the species barrier". Indeed, there are a number of bacteria that are primarily pathogenic to other vertebrates that are also pathogenic to humans, e.g. Bacillus anthracis, Brucella abortus, Yersinia pestis, Leptospira spp. and a number of Salmonella species. Human diseases caused by these bacteria are called zoonoses (see also Blancou et al., 2005, for a review). In some cases insect vectors play a specific role in passing the pathogenic bacteria from the animal to the human host. Zoonotic diseases are "animal borne": animals, or animal products, act as a source of the disease. Consequently, exposure to the disease may change with changing social, behavioral and consumer practices. The risk class of a zoonotic bacterial species may differ depending on the host. For environmental risk/safety evaluations of activities with these bacterial species, the highest risk class has to be taken into consideration. As pointed out previously, it is difficult to definitively state that a bacterial strain is non-pathogenic. The evidence given for non-pathogenicity can only be tentative. The determination of whether a bacterial strain may be considered non-pathogenic is usually made in a stepwise fashion. The strain may be considered non-pathogenic if it belongs to a species or taxonomic group for which no pathogenic strains are known. If it has direct relatives that are pathogenic, or if it is derived as an attenuated pathogenic strain, it should be shown that the strain effectively lacks the virulence determinants of its pathogenic relatives. If this fails, evidence for non-pathogenicity can be obtained through appropriate animal testing. This requires, however, a validated animal model. If none of this evidence is available or can be obtained, the strain may be considered non-pathogenic because it has a long history of safe use under conditions where no specific physical containment, like a closed fermentor system, has been applied to reduce worker exposure. Although there is a clear value in using risk groups in practice (e.g. refer to WHO, 2004, Chapters 1 and 2), the concept of "opportunistic pathogenicity" implies that there is a continuum from non-pathogens to full frank pathogens. Some bacteria complete their life cycle independent of a human or animal host. Others that lack the ability to cause disease may still be able to recognise, adhere to and multiply in or on the host, as commensals. Opportunistic pathogens have some limited ability to cause disease, but are normally kept under control by the host immune response and defense systems and the competitive, harmless micro-organisms with which they compete in the host's habitat. However, they may acquire a toehold, with adverse consequences for the host, generally under circumstances where the host's defense mechanisms are compromised (e.g. weakening of the immune system through age or HIV infection) or

destroyed (e.g. through skin lesions or burns). Some opportunistic pathogens are acquired from the environment while others may constitute part of the host's normal bacterial population. Some bacterial species causing infections at hospitals are used in bioremediation and/or bioaugmentation processes that may involve inoculation of soil with large amounts of bacteria. For instance, Pseudomonas aeruginosa and Stenotrophomonas maltophilia are organisms used industrially that cause nosocomial infections in cystic fibrosis and burn patients. Serratia marcescens, a common soil bacterium, causes pneumonia, urinary tract infections and bacteremia in compromised human hosts and is lethal to certain insect species with commercial use as a biopesiticde while commensal on the rhizoplane of many plant species. Other bacteria, such as Lactobacillus acidophilus, may be considered to be non-pathogens, because they rarely or never cause human disease. However, it should be noted that categorisation as non-pathogens may change due to the inherent variability and adaptability of bacteria and the potential for detrimental effects on host defense systems caused, for example, by radiation therapy, chemotherapy and immunotherapy; genetic defects (cystic fibrosis); or immunosuppressive infection (HIV). General considerations in assessing the hazardous potential of bacteria: Approaches to bacterial virulence In 1890 Robert Koch established his "postulates", a standard for the evidence of causation in infectious disease.

The evidence should show that:

1) The micro-organism occurs in every case of the disease in question and under circumstances which can account for the pathological changes and clinical course of the disease;

2) After being isolated from the body and grown in pure culture,

3) The micro-organism can be inoculated into a healthy host and induce the disease anew; and

4) The micro-organism can be re-isolated after this experimental infection.

Virulence factors can be defined in terms of Koch's postulates as phenotypic properties of a micro-organism that are present in pathogenic strains that fulfill Koch's postulates but that are not observed in related strains that are not pathogenic. Although the postulates have been generally accepted for over 100 years (Fredricks and Relman, 1996), Koch himself already recognised the limitations of these guidelines. For instance, the ability to cause disease as an invariant virulence trait has been challenged. In recent years, a more integrated view of microbial pathogenesis has been developed which recognises that the contributions of both the pathogen and its host are required. The lack of experimental models for human-specific pathogens limits testing of the third postulate, and consequently also the rigorous testing of the role of a human-specific virulence factor. Still, based on the notions of Koch's postulates, a number of virulence factors have been identified because of their clear role in the pathogenesis or their clear-cut

coincidence with pathogenic strains, (e.g adhesins, invasins, haemolysins or, in general, cytolysins). With the development of molecular biological techniques, it became possible to identify the genes encoding these known virulence factors and to identify genes of unknown function for which a possible role in virulence could be determined. This resulted in a new approach of research on bacterial pathogenicity, in which the role of specific genes in bacterial virulence was the key point. Virulence of a micro-organism is usually considered as the "degree" of pathogenicity of the micro-organism in a susceptible host. Finlay and Falkow (1997) discussed the various definitions of microbial pathogenicity, and the idea that pathogens can be distinguished from their non-virulent counterparts by the presence of such virulence genes. A virulence factor is a phenotypic trait associated with the virulence level of a micro-organism. The term is also used for a gene product (or group of gene products) that is responsible for the phenotypic trait.

Virulence factors add to the pathogenicity, by enhancing one or more of the processes involved in the stages of pathogenicity:

1) The ability of the bacterial pathogen to gain access to the individual by surviving on or penetrating skin and mucous membranes;

2) The in vivo multiplication of the pathogen;

3) The inhibition or avoidance of host protective mechanisms; and

4) The production of disease or damage to the host. In this chapter microbial toxins are regarded as virulence factors even though these toxins are defined as gene products to produced by a bacterium that can cause harmful effects in the absence of the active living bacterium because most cases the bacterium producing the toxin has to be established within the host in order to deliver the toxin most effectively. Therefore, the phenotypic trait of toxin production may be seen as increasing the pathogenic potential of a bacterium, while the full-blown effects of a toxin may be dependent on other virulence factors of the producing micro-organism, (e.g. the ability to colonise the host). It should, however, be noted that some bacteria that are not regarded as pathogenic (e.g. neurotoxin producing cyanobacteria) may also produce toxins, and that some bacteria producing toxins that can act at a distance (e.g. Clostridium botulinum causing foodborne disease) are characterised as pathogens. Bacterial factors and determinants for pathogenicity "Virulence" is a quantitative measure of the pathogenicity of a micro-organism that may be expressed by the ratio of the number of individuals developing clinical illness to the number of individuals exposed to the micro-organism, or in a comparative manner, by the number of individuals that develop clinical illness if the same dose of different microorganisms is applied to each of them. Pathogenic bacteria have evolved a number of different mechanisms, which result in disease in the host. The virulence factors and determinants used by bacteria to

interact with the host can be unique to specific pathogens or conserved across several different species or even genera. For instance, common mechanisms for adherence, invasion, evasion of host defenses and damage to host cells are shared by profoundly different microbial pathogens. However, a virulence factor can only contribute to the pathogenic potential of a bacterium in and as far as the micro-organism possesses the constellation of traits conducive to pathogenicity. This section examines bacterial factors/determinants that contribute to pathogenicity in bacteria. While these are the determinants that would generally be considered in a risk/safety assessment, it should be noted that the same factor/determinant will not necessarily have a similar effect on the virulence of two different bacteria, and thus simple possession of a trait is not an indicator that the micro-organism is pathogenic. The concept of the "pathogenicity" of bacteria is further discussed in the next section. Host recognition/adherence Bacterial adherence to host surfaces is an essential first step in colonisation, infection and disease production. Colonisation establishes the organism at the portal of entry. Whereas intact outer skin is generally impervious to invasion by organisms, surface penetration of the urogenital, digestive and respiratory tracts as well as the mucosal barrier is more easily accomplished. Much of the body that is usually regarded as internal is topologically connected to the exterior. For example, the surfaces of the intestinal lumen, the lung alveoli, the bile cannaliculi and the kidney tubules are continuous with the outside skin. Organisms infecting these regions usually have elaborate adherence mechanisms and some ability to overcome or withstand the constant pressure of the host defenses on the surface. Bacterial adherence to host cells is usually a prerequisite to invasion. Consequently, a great deal of research has focused on elucidating bacterial mechanisms of adherence to host cells (adhesin biosynthesis, regulation of adhesins, identification of host receptors).

Adhesion can be defined as the coupling of a bacterium with a substratum. For molecules on the surface of the bacterium to interact with molecules on the surface of a host cell or the extracellular matrix, the two molecules must come into contact, an action that leads to the creation of intermolecular bonds requiring a certain amount of energy or effort to break. Bacterial adherence to a eukaryotic cell or tissue surface requires the participation of two factors: a receptor and an adhesin. The receptors so far defined are usually specific carbohydrate or peptide residues on the eukaryotic cell surface. Many bacterial adhesins are a macromolecular component of the bacterial cell surface which interacts with the host cell receptor. This interaction is usually complementary and specific, although most receptors can bind several ligands. It is this specificity which determines the tropism of the bacteria for a particular tissue (or a specific animal). Bacterial adherence to cells or tissue surfaces may be specific or non-specific. Non-specific adherence or "docking" involves attractive forces and allows for the approach and reversible attachment of the bacterium to the eukaryotic surface (Kachlany et al., 2000). Possible interactions and forces involved include: hydrophobic interactions, electrostatic attractions, Brownian movement, recruitment and trapping by biofilm polymers interacting with the bacterial glycocalyx or capsule (Gilbert, Das and Foley, 1997; An, Dickinson and Doyle, 2000; Ukuku and Fett, 2002; Foong and Dickson, 2004). Specific adherence occurs when the

bacterium forms a more permanent, yet still reversible, attachment with the eukaryotic surface and may proceed as one or more steps. Many specific lock-and-key bonds between complementary molecules on each cell surface are formed. Complementary receptor and adhesin molecules must be accessible and arranged in such a way that many bonds form over the area of contact between the two cells. Once the bonds are formed, separation under physiological conditions requires significant energy input.

Some Gram positive bacteria with microbial surface components recognising adhesive matrix molecules (MSCRAMMs) employ a dock, lock and latch mode of ligand binding (Ponnuraj et al., 2003). Generally, reversible attachment precedes irreversible attachment, but in some cases specific adherence is not observed. Mammalian cells communicate with each other through cell surface receptors. Once a receptor is bound with its ligand, a cellular response is triggered. Bacterial recognition of and interaction with host cell ligands facilitates the initial adherence to, and subsequent invasion of, host cells Through host receptor binding, bacteria exploit normal cellular processes to invade host cells. Many micro-organisms have elaborate properties that can be used for industrial purposes in extensive biotechnological applications. For example, Rhodococcus spp. have elaborated adhesive properties for attachment to environmental surfaces or for biofilm formation that are particularly useful for adherence to heavy metals and hydrocarbons (Shabtai and Fleminger, 1994; Stratton et al., 2002). Although Rhodococcus spp. are not generally considered to be human pathogens, some species have emerged as rare opportunistic human pathogens. Rhodococcus equi infection is characterised by bronchiopneumonia following adherence and entry into alveolar macrophages. Garton et al. (2002) postulated that a novel lipoarbinomannan (LAM) variant may contribute to pathogenesis of disease caused by R. equi., similar to Manosylated LAM of Mycobacterium tuberculosis which facilitates adherence to alveolar macrophages via mannose receptors. Evaluators must always be cognisant that those factors which have extensive industrial applications (for instance, adhesive properties) may also confer one of the properties that allow a micro-organism to cause disease in susceptible individuals.

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