Molecular mechanisms of pathogenicity: how do pathogenic microorganisms develop cross-kingdom host jumps?

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Received 3 July 2006; revised 22 December 2006; accepted 23 December 2006.
First published online 26 February 2007.

DOI:10.1111/j.1574-6976.2007.00065.x

Editor: Ramón Díaz Orejas

Keywords
pathogen; evolution; adaptation; disease; infection; pathogenicity.

Abstract

It is common knowledge that pathogenic viruses can change hosts, with avian influenza, the HIV, and the causal agent of variant Creutzfeldt–Jacob encephalitis as well-known examples. Less well known, however, is that host jumps also occur with more complex pathogenic microorganisms such as bacteria and fungi. In extreme cases, these host jumps even cross kingdom of life barriers. A number of requirements need to be met to enable a microorganism to cross such kingdom barriers. Potential cross-kingdom pathogenic microorganisms must be able to come into close and frequent contact with potential hosts, and must be able to overcome or evade host defences. Reproduction on, in, or near the new host will ensure the transmission or release of successful genotypes. An unexpectedly high number of cross-kingdom host shifts of bacterial and fungal pathogens are described in the literature. Interestingly, the molecular mechanisms underlying these shifts show commonalities. The evolution of pathogenicity towards novel hosts may be based on traits that were originally developed to ensure survival in the microorganism's original habitat, including former hosts.

Introduction

Life on Earth is diverse and interactive: not one ecosystem harbouring a single species has yet been described. Interactions between organisms can be very diverse in nature, ranging from beneficial through neutral to detrimental. Infectious disease is usually defined as the result of a detrimental (set of) interaction(s), and is usually restricted to a given combination of host (the diseased) and pathogen (the disease-causing agent). From the anthropocentric perspective, infectious disease may be defined as a biological process leading to the disruption of the normal physiology of a multicellular organism in reaction to the presence of a pathogenic microorganism on or in the body. Diseases may differ in their presentation but are always preceded by infection and colonization. Interestingly, several pathogenic microorganisms are capable of infecting a variety of organisms, and in some cases even members of different biological kingdoms of life may be susceptible. Such cross-kingdom host jumps are defined when a microorganism normally colonizing a species from one taxonomic kingdom becomes able repeatedly to infect a species belonging to another kingdom. Microbial pathogen host jumps occurring at a cross-kingdom level are not as well known as those occurring within the animal kingdom, such as those associated with the human diseases avian influenza, acquired immune deficiency syndrome (AIDS) after infection with the HIV and variant Creutzfeldt–Jakob disease, which are thought to originate from birds, primates and cattle, respectively, but several cross-kingdom host jumps have been described. An example of a well-established microbial cross-kingdom pathogen is *Agrobacterium tumefaciens* (syn. *A. radiobacter*), a globally dispersed soil-borne alphaproteobacterium. *Agrobacterium tumefaciens* was initially characterized as a pathogen of rosaceous plants, in which it induces crown gall or tumour formation (hence the name, *tumefaciens*) in susceptible genotypes (Hayward & Waterston, 1965). *Agrobacterium tumefaciens* induces these tumours by transfer of a segment of a tumour-inducing (Ti) plasmid DNA to the genome of plant cells (Zambryski *et al.*, 1980). Because of these DNA transmission abilities, *Agrobacterium tumefaciens* has been exploited as a laboratory tool to deliver DNA into plant genomes. However, *Agrobacterium tumefaciens* can transfer parts of its DNA not only into plant genomes, but also into fungal and human genomes (de Groot *et al.*, 1998; Kunik *et al.*, 2001; Wood *et al.*, 2001; Lacroix *et al.*, 2006). Moreover, *Agrobacterium tumefaciens* does not only cause plant diseases, but has also been recorded as a human pathogen (Paphitou &

Rolston, 2003). This leads to the question of how *Agrobacterium tumefaciens* evolved into a pathogen that is able to infect such different organisms as plants, fungi and humans. In this review we address questions of what is needed for a microbial pathogen, in particular for a living microorganism such as a bacterium or a fungus, to extend its host range to include species that belong to a different kingdom of life. The environmental conditions, population biology traits and molecular processes that enable microorganisms to infect hosts belonging to different kingdoms are discussed.

Pathogenicity

A pathogen or pathogenic microorganism is usually defined as a biological agent that can cause damage to its host during, or as a consequence of, the host–microorganism interaction. Damage may be inflicted directly by the microorganism (e.g. by toxins or other so-called virulence factors) or indirectly through the activity of the host immune responses (Casadevall & Pirofski, 1999). The ability of the pathogen to infect is called its pathogenicity. Microorganisms express their pathogenicity by means of their virulence, a term that refers to the relative, quantitative degree of pathogenicity (Casadevall & Pirofski, 1999). Moreover, pathogens are distinguished by their virulence from non-pathogens, which are considered to be avirulent (Casadevall & Pirofski, 1999).

Pathogenic microorganisms causing disease (or, more generally, physiological damage) have traditionally been divided into opportunistic, facultative and obligate pathogens. Obligate pathogens are capable of infecting only within a narrow host range, but can infect healthy, immunocompetent individuals of susceptible host species. *Mycobacterium tuberculosis* is a key example of an obligate pathogen to humans. It causes tuberculosis (TBC) and is one of the major infectious threats that includes rising multidrug resistance (Cohen & Murray, 2004). It requires living hosts for replication and survival in the same way as obligate symbionts do. Facultative pathogens similarly infect within a narrow host range, but are also capable of surviving outside the host in the (inanimate) environment. Opportunistic pathogens thrive on a wide range of organic substrates, and generally exhibit low virulence towards a broad array of living hosts. However, if potential host species become injured or become compromised in their immune responses, opportunistic pathogens may be able to attack aggressively, or in a manner that is indolent but progressive. It has been speculated that obligate and facultative pathogens have evolved from microbial lineages that originally caused only opportunistic infections (Scheffer, 1991). According to the damage-response concept (Casadevall & Pirofski, 2003), the division into opportunistic, facultative and obligate pathogens may also be accommodated on the basis of host damage predominantly being induced by the microorganism, by the host's innate and acquired immune responses, or by a combination of these processes established during the interaction, respectively. The evolution of pathogenicity cannot be considered independently from the host (e.g. Casadevall & Pirofski 1999, 2003; Brown *et al.*, 2006), so, whenever evolving pathogenicity is discussed throughout this review, evolution of host susceptibility is implied as well. The interaction between hosts and pathogens may depend on adequate 'key-and-lock' situations. In order to render a host susceptible to colonization and/or infection, the genetic make-up of both parties needs to facilitate molecular interactions that form a solid basis for a mutually agreeable or detrimental interaction. Genetic variation, selection of the best-fitting mutants, and retention (or progression into disease) are the fundamental processes for tweaking the interactions (Lynch & Conery, 2003).

The kingdoms of life

Originally, Charles Darwin described the evolution of species as 'the tree of life', an expression implying that all life originates from a single common root (Darwin, 1859). Until the 1960s, only three kingdoms of life were recognized: the single-celled protista, and the multi-cellular plantae and animalia. In 1959, Whittaker defined a five-domain system distinguishing prokaryotic and eukaryotic single-celled organisms and, in addition, adding fungi as a recognized kingdom (Whittaker, 1959). The resulting five-kingdom system became a widely accepted standard. Since then, additional kingdoms have been proposed, of which only two are more or less commonly accepted. Based on rRNA gene differences, a division of prokaryotes into eubacteria and archaea was proposed (Woese & Fox, 1977; Woese *et al.*, 1990). In addition, the kingdom Chromista, containing many algal groups as well as most water moulds, was put forward (Cavalier-Smith, 1981). As a consequence, the generally recognized seven kingdoms of life (see Fig. 1) are as follows.

(1) Kingdom Eubacteria (Woese & Fox, 1977). These unicellular organisms are prokaryotic and lack a nucleus and other membrane-bounded organelles. The cell wall is composed partially of peptidoglycan, a complex structural molecule that is not found in eukaryotic cells.

(2) Kingdom Archaea (Woese & Fox, 1977; Woese *et al.*, 1990). Many archaeans are anaerobic and thrive under extreme conditions. Together with the eubacteria they compose the prokaryotes. With respect to cellular structure and metabolism, they resemble eubacteria, while with respect to transcription and translation, they are similar to eukaryotes. In contrast to eubacteria, archaea lack peptidoglycans in their cell wall. This kingdom is not

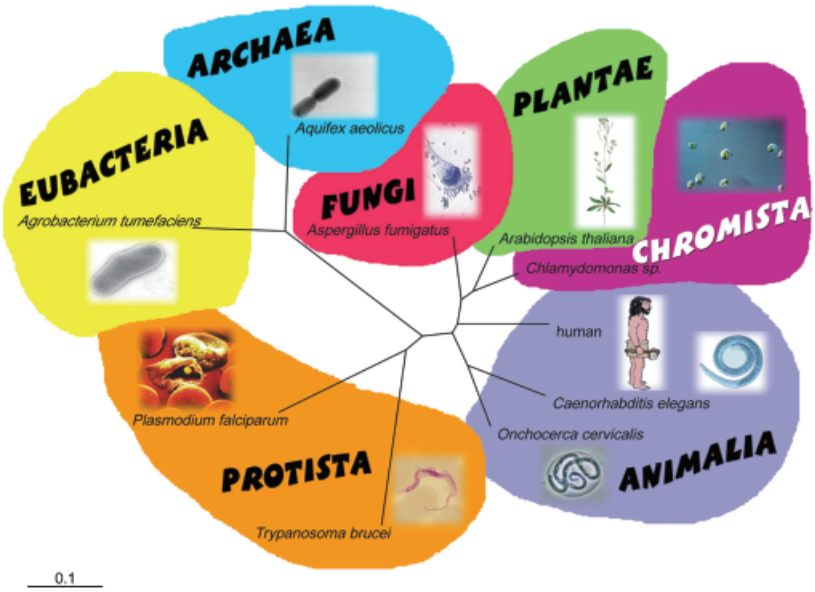

Fig. 1. The seven kingdoms of life as represented by examples of pathogens and hosts that are discussed in the text. *Plasmodium falciparum* (Protista) is visible as yellow substructures inside the red blood cells of its human host. The unrooted tree illustrating the phylogenetic distances between the groups representing the kingdoms is based on 16S and 18S rRNA gene sequences (retrieved from GenBank, June 2006) and is constructed using neighbour-joining (Saitou & Nei, 1987). The sizes of the areas representing the kingdoms and the illustrations of the respective organisms are not drawn to scale.

universally recognized, and some systematicians classify archaea as an infrakingdom of the bacteria (Cavalier-Smith, 2004).

(3) Kingdom Protista (Whittaker, 1959). This kingdom contains single-celled eukaryotes. Protista are unicellular or colonial homokaryotic organisms that can be either autotrophic or heterotrophic. Locomotion occurs by means of flagella or pseudopodia.

(4) Kingdom Chromista (Cavalier-Smith, 1981). Almost all of the species of the Chromista are photosynthetic, except for the water moulds, and most are also aquatic. Almost all fall into the traditional category of 'algae'. The photosynthetic members possess chlorophyll c, which does not occur in plants or the related 'green algae' (*Chlorophyta*, *Charophyta*, etc.). The best-known colourless members of the Chromista are a group with fungus-like morphology, the oomycetes.

(5) Kingdom Fungi (Whittaker, 1959). These are heterotrophic eukaryotes relatively closely related to animalia but distinguished in part by the possession of cells with a carbohydrate cell wall. Reproduction is usually by means of nonmotile spores or, in one phylum, flagellated zoospores. Somatic structures often appear filamentous and branched, growing only at the apex. Unicellular organisms reproducing by budding are also characteristic of certain groups. Many but not all fungi reproduce sexually as well as asexually. They include moulds, mushrooms, yeasts, mildews, smuts, and rumen symbionts, as well as the conspicuous component of lichens (a symbiosis between fungi and algae or cyanobacteria).

(6) Kingdom Plantae (Whittaker, 1959). These are autotrophic, mostly multi-cellular organisms, usually with haplo-diploid life cycles. They typically develop from embryos and use chlorophyll to convert CO_2 with the aid of sunlight into complex carbohydrates.

(7) Kingdom Animalia (Whittaker, 1959). This kingdom encompasses heterotrophic multi-cellular organisms whose cells do not synthesise cell walls or photosynthetic pigments. They develop from a diploid blastula.

Within these seven kingdoms of life, five major groups of microbial pathogens are currently recognized: bacteria, fungi, protozoa, helminths and oomycetes (Fig. 1). Protozoa (kingdom Protista) are single-celled eukaryotes that include amoeba (for instance the *Entamoeba* species that cause gastrointestinal disease) and a large diversity of other microorganisms (for example the malaria parasites *Plasmodium* spp. and the flagellated *Trypanosoma* spp.). Helminths (kingdom Animalia) are multicellular invertebrates that, unlike protozoa, have differentiated tissues. Various types of vermiform animals, such as the nematodes that cause elephantiasis and river blindness, are included in this category. Finally, oomycetes (kingdom Chromista) are filamentous aquatic organisms that have cell walls composed of cellulose and a predominantly diploid lifecycle. Asexual reproduction is by biflagellated zoospores. The most well-known oomycete pathogen is *Phytophthora infestans*, causal agent of potato late blight that caused the Great Irish Famine (1845–1847), when up to one million people died and a similar number emigrated, many to the USA. So far, archaea are not identified as direct causal agents of infectious diseases. However, recent studies show a correlation between infections and the presence of archaea (Vianna *et al.*, 2006). Therefore it is expected that archaea will be identified as causes of infectious diseases.

General blueprint of a microbial infection

Although various pathogenic microorganisms can be responsible for many different disease syndromes on hosts of all kinds, several factors are common to the establishment of an infection (Fig. 2). The first step is primarily oriented towards outer surfaces of the host and involves the adhesion of the microorganism to host cells or tissues. This can be achieved through biophysical means (electrostatic or Van der Waals interactions) or through more sophisticated tools. Despite the major structural difference between plant and animal cells, namely the rigid and porous cellulose-containing cell wall surrounding the plant cell membrane that provides stability and protection, the eukaryotic plant and animal host cells share many similarities. Many pathogenic microorganisms have been shown to utilize specialized surface-associated adherence factors or adhesins to attach themselves firmly to their hosts (Hahn, 1997). The bacterial plant pathogen *Erwinia chrysanthemi* was found to express an adhesin that shares immunological identity with intimins, which is conserved in mammalian bacterial pathogens, such as the intestinal bacterium *Escherichia coli* (Higgins *et al.*, 1999; Duarté *et al.*, 2000). It has been shown that *Erwinia chrysanthemi* is able to attach not only to plant cells, but also to human cells, leading to an oxidative burst that results in the death of these cells. Furthermore, a type III secretion mutant of *Erwinia chrysanthemi* is compromised in the ability to kill human cells (Duarté *et al.*, 2000). Thus, *Erwinia chrysanthemi* possesses the basic tools to adhere to, penetrate, and kill human cells, although no human infection has yet been documented. However, *Dickeya dadantii*, a synonym of *Erwinia chrysanthemi*, has recently been shown to be highly virulent towards the pea aphid *Acyrthosiphon pisum* (Grenier *et al.*, 2006). Several human pathogenic microorganisms also contain members of a protein family called Microbial Surface Components Recognizing Adhesive Matrix Molecules (or MSCRAMMs), which interact in a ligand-receptor fashion with common host surface molecules including collagen, fibronectin, vitronectin, cytokeratin and others (Kreikemeyer *et al.*, 2004; Rivas *et al.*, 2004).

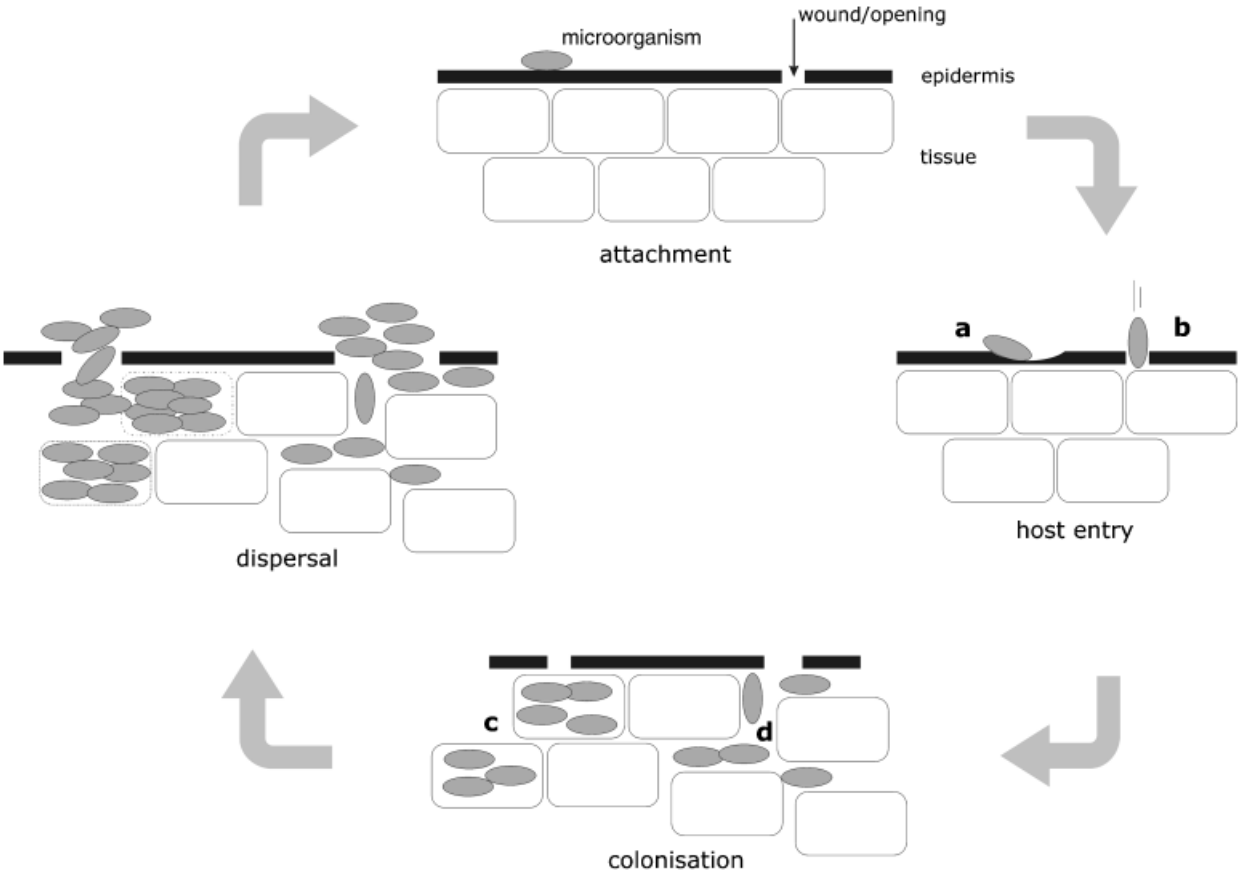

Fig. 2. General blueprint of a microbial infection. After attachment of a pathogenic microorganism, pathogens may enter host tissues (a) through active penetration processes, or (b) through wounds or natural openings. Subsequently, pathogenic microorganisms colonize host tissues. This colonization occurs either (c) intercellularly or (d) extracellularly. In a next stage, progeny of the pathogen is released and dispersed after which new infections can take place.

After attachment, the pathogenic microorganism may physically penetrate or enter the host. Mammalian pathogens can infect either intracellularly, as occurs with *Salmonella*, *Mycoplasma, Mycobacterium* spp., *Legionella* spp., *Shigella* and *Chlamydia*, or extracellularly, as occurs with *Pseudomonas aeruginosa*. Intracellular bacterial pathogens enter the host cells by an active invasion process that is often facilitated by surface-exposed proteins (Isberg *et al*., 1987), by microbial effector molecules that are delivered by the type III secretion system (Galan & Collmer, 1999), or by microbial toxins that mediate cytoskeleton remodelling (Takenouchi *et al*., 2004). Some vertebrate pathogens thrive within the host after being taken up by macrophages. Plant-pathogenic bacteria are predominantly extracellular microorganisms that multiply in the intercellular spaces between host cells. They enter host tissues either through wounds or through natural openings such as stomata, microscopic pores in the leaf epidermis essential for gas exchange. Although most mammalian fungal pathogens are extracellular, some invade the host cytoplasm, a process that is poorly understood (Tsarfaty *et al*., 2000; Wasylnka & Moore, 2002). The unicellular phase of *Candida albicans*, for example, has been shown to induce phagocytosis in host cells through the induction of polymerization of host microfilaments and microtubules (Filler *et al*., 1995), possibly through phosphorylation of specific endothelial cell proteins (Belanger *et al*., 2002). *Candida* cells can also transform into hyphae, which are harder to be phagocytosed, but are capable of entering host tissues by exerting mechanical pressure (Kumamoto & Vinces, 2005). Although fungal plant pathogens may be specialized to detect stomata or wounds and to enter plant tissues through these openings, many highly specialized fungal plant pathogens forcibly enter the host by piercing the leaf with an infection peg that arises from a so-called appressorium that facilitates the exertion of pressure (Bechinger *et al*., 1999).

In the next stage, microorganisms need to colonize the host in order to feed and replicate. Based on their interaction with the host, plant-pathogenic microorganisms are often divided into biotrophs and necrotrophs, although many intermediate forms exist (Thomma *et al*., 2001). Biotrophic pathogens feed on living plant cells, often by means of specialized feeding structures termed haustoria. Signals released from the haustorium are believed to suppress host defence and thus to maintain the pathogenic interaction (Vögele & Mendgen, 2003). In contrast, necrotrophic pathogens secrete toxins and lytic enzymes and absorb nutrients from necrotic tissues. The mode of infection of such pathogenic microorganisms is somewhat unsophisticated, as they apparently do not avoid triggering the host defence system and tend to be adapted to kill host cells as quickly as possible. This situation actually resembles human and animal infections by microbial pathogens. These infections are classified either as chronic, where a persisting pathogen causes a long-term infection, or as acute, where the pathogen quickly grows and spreads within the host. It has been suggested that the difference between chronic and acute infections is correlated with the mode of microbial growth, with acute infections associated with planktonic growth and chronic infections caused by microorganisms that form a biofilm (Furukawa *et al*., 2006).

Biofilms are structurally complex, multicellular communities produced through the production of an extracellular matrix by extracellularly growing microorganisms that adhere to abiotic as well as to living surfaces (Lam *et al*., 1980). This slimy matrix can be very diverse in composition, but generally consists of macromolecules that include polysaccharides and proteins. Biofilms offer their member cells protection from adverse environmental conditions, including host defence components and externally administered antimicrobial agents. Their formation is initiated by the interaction of cells with a surface, as well as by the interaction with each other (quorum sensing; Abraham, 2006), and is regulated by various environmental signals (O'Toole & Kolter, 1998). Foreign bodies implanted into human hosts form an excellent attachment matrix for the development of biofilms. Such biofilms incur costs for the medical system (replacing any catheter is expensive and risky) and predispose patients to the development of more serious infectious syndromes because biofilms can act as reservoirs for recurrent infections (Jones, 2006). When nutrient conditions become limiting, cells are released from the biofilm and enter a free-living, planktonic phase. These cells can spread, colonize new habitats, and form new biofilms (Costerton *et al*., 1995; Fux *et al*., 2005). It was recently discovered for uropathogenic *Escherichia coli* that biofilm-like structures can even occur intracellularly, embedding bacteria in a matrix shell in the host cell cytoplasm, which may facilitate resistance to host defence responses (Anderson *et al*., 2003).

Requirements for cross-kingdom host jumps

Even today, a set of five postulates formulated by Koch, and later adapted to fit viral infections, is the gold standard to prove that a specific microorganism is the cause of a specific disease (Koch, 1882; Rivers 1937). Essentially, it has been stated in these postulates that the microbial pathogen must be found in all diseased individuals and not in healthy ones. Furthermore, the microbial pathogen must be isolated and demonstrated to cause similar infections in naïve host species, whereupon it must be isolated again. Even in Koch's time it was noted that certain elements of the postulates may be problematic in specific cases. For instance, some microorganisms cannot be grown in a cell-free culture outside the host, reinoculation with a strictly human-pathogenic

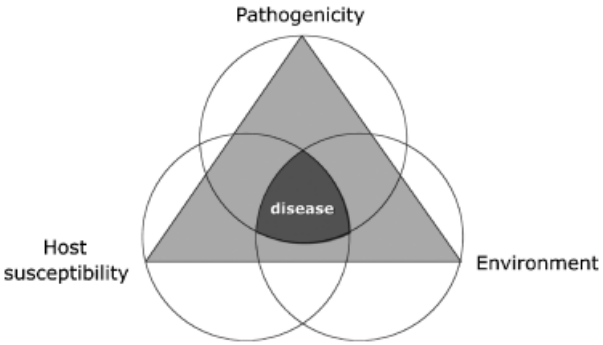

Fig. 3. Disease triangle. Compatible interactions between a pathogen and a host will only result in disease when environmental conditions are also fulfilled. The interactions not fulfilling all requirements will not result in disease.

microorganism is often considered unethical and therefore impossible, and pathogens may have a carrier state in which they do not necessarily cause disease in all individuals that harbour them. For reasons like this, failure to obey to Koch's postulates does not eliminate the microorganism from being the cause of disease (Fredricks & Relman, 1996). Nevertheless, such criteria are important in defining diseased state and pathogenicity from the perspectives of the host and pathogen.

Whether or not a particular microorganism infects a particular target organism depends on multiple conditions. First of all, an infection can only occur if both partners are compatible. Compatibility depends on the genetic constitution of the microbial pathogen as well as on that of the target organism. In addition, environmental circumstances influence the interaction. The necessary preconditions for successful pathogenesis have traditionally been depicted in the so-called 'disease triangle' (Fig. 3), which illustrates the concept that disease will occur only if specific requirements regarding pathogen, host and environment are met. The most important requirements for establishing a pathogenic relationship will be discussed below.

Microorganism and host are in close proximity

It is self-evident that the possibility of a microorganism developing a relationship with an organism will increase the more the two organisms meet. The most intimate type of relationship between a microorganism and a host is called a symbiosis ('living together'). The actual circumstances entailed by this word can be very diverse, as it ranges from mutualistic symbiosis (both species benefit) through commensalism (one organism benefits and the other is not significantly harmed or helped), to parasitism (one species benefits at the expense of the other). The distinction between these relationships is not always clear-cut. Moreover, especially in mutualistic and commensalistic relationships, a fine balance is maintained between the host and the microorganism, and a small disturbance can lead to a change in the relationship whereby mutualists or commensals can become pathogenic (Tanaka *et al.*, 2006). This has been well documented, for example, for *Candida albicans*, a commensal yeast of human gastrointestinal and genital mucosa that can become pathogenic and infect many body sites if the person hosting it becomes immunocompromised (Hube, 2004). Another clear example of opportunistic pathogenicity is provided by the bacterial species *Staphylococcus aureus*. These bacteria can be encountered in the nasal cavity of *c.* 1 in 3 humans, where they reside in a seemingly resting population without any obvious pathogenic effect exerted. Such nasal colonization, however, predisposes the carrier to opportunistic bacterial infection in times of waning immunity (Wertheim *et al.*, 2005). Furthermore, the primary host may bring a pathogenic microorganism into contact with a potential new host, with which it may go on to develop a novel pathogenic relationship. In this way, normally mutualistic or commensal plant endophyte bacteria and fungi, which may build upon their capacity to enter hosts in which they do not cause disease, may develop into pathogens on or in other hosts. Bacterial species such as *Pseudomonas aeruginosa, Staphylococcus aureus* and *Burkholderia cepacia* are known as beneficials, occurring as endophytes on some plant hosts, while functioning as pathogens on others (Berg *et al.*, 2005).

The future host can act as substrate

In order for a pathogenic microorganism to become stabilized and to persist in time, the host should be able to provide all required conditions for the microorganism to complete its life cycle. Each host presents different nutritional conditions that nonetheless must be relied upon to provide essential cellular requirements such as water, amino acids, micronutrients and energy. Auxotrophy for nearly any amino acid or fatty acid will diminish or abolish microbial pathogenicity, and therefore quorum-sensing of nutrient availability is essential for successful pathogenicity (Guerinot, 1994; Ratledge & Dover, 2000; van Burik & Magee, 2001).

Iron is an important example of an essential host factor that can determine whether the interaction of a microorganism with a possible future host will be successful. It is essential for many cellular processes in all microorganisms except, possibly, lactobacilli (Archibald, 1983). Because of its two stable ionization states (Fe^{2+} and Fe^{3+}), iron is a suitable cofactor for many proteins mediating electron transfer and redox reactions. Plants generally have a relatively high iron content of about $100\,mg\,kg^{-1}$, but the mobility of iron between different plant organs is relatively low (Ma, 2005). Microorganisms living in or on plants have developed specialized mechanisms for acquiring iron from

the host (Expert, 1999). Animals, on the other hand, have levels of available iron between 20 and 100 times lower than those found in plants (Lux *et al*., 1978; Mateos *et al*., 1998), and the iron is tightly complexed in diverse types of molecules. At the same time, animals are generally characterized by high extracellular iron content in iron complexes transported in the blood stream. Intracellular iron is complexed in heme, iron-sulphur proteins, and ferritin, as well as in other proteins, whereas extracellular iron is bound to transferrin and lactoferrin proteins (Weinberg, 1992; Payne, 1993). Invasive microbial pathogens that multiply in the extracellular spaces of the host need to employ different strategies for iron acquisition from those used by microorganisms that grow within host cells. A prominent mechanism for the acquisition of extracellular iron is the synthesis and secretion of small iron-chelating molecules called siderophores (Byers & Arceneaux, 1998). After iron has been chelated outside the microbial cell, the iron-siderophore complex is transported back into the cell, where the iron is removed and utilized. An alternative strategy for iron acquisition is the direct use of host iron compounds, including heme, hemoglobin, transferrin, and lactoferrin. Uptake of all these materials involves specific, high-affinity outer membrane receptors (Byers & Arceneaux, 1998). In a few cases, such as for the plant pathogen *Erwinia chrysanthemi*, siderophores have been shown to serve as pathogenicity factors (Enard *et al*., 1988; Franza *et al*., 2005). On the other hand, microbial pathogenicity mechanisms may be influenced by changes in iron availability in the environment (Guerinot, 1994). For example, in *Erwinia chrysanthemi* low iron availability triggers the secretion of pectinolytic enzymes (Stoebner & Payne, 1988; Henderson & Payne, 1994). Furthermore, synthesis of a hemolysin toxin by *Vibrio cholerae*, a bacterium well known as a human gastrointestinal pathogen, is regulated by iron (Masclaux *et al*., 1996; Franza *et al*., 2002). For *Cryptococcus neoformans*, a facultative pathogen and causal agent of meningitis and pneumonia, it was recently shown that the iron-responsive transcription factor Cir1 controls regulation of genes involved in iron acquisition, and in addition controls various other important virulence characteristics (Jung *et al*., 2006). It is obviously the case that availability of iron and metals in general will be low in the acid stomach niche, and various methods for iron acquisition have been developed (Pflock *et al*., 2006). The gastrointestinal pathogen *Helicobacter pylori* is able to acquire rare metals efficiently (Perez-Perez & Israel, 2000). Interestingly, nickel signalling is coregulated with urease production. The latter involves a mechanism useful for modulating environmental conditions, especially the pH, implicating a link between iron sequestration and pH modulation (Van Vliet *et al*., 2004).

Because iron sequestration is crucial for pathogenic microorganisms, vertebrate hosts have developed iron-withholding defence mechanisms. One such mechanism is the complexing of iron with ferritins as soon as microbial invasion is sensed; the result is rapidly reduced levels of serum iron (Weinberg, 2000). Interestingly, a similar mechanism involving ferritins seems to be employed by plants in response to pathogen attack (Neema *et al*., 1993; Dellagi *et al*., 2005). In general, there seems to be a correlation between the capacity to acquire iron from diverse environments and cross-kingdom pathogenicity. For instance, the bacteria *Pseudomonas aeruginosa, Erwinia* and *Burkholderia* as well as the fungus *Rhizopus* produce several types of siderophores that function in invasions of many different organisms (Guerinot, 1994; Expert, 1999; Howard, 1999; Ratledge & Dover, 2000). These microorganisms are pathogenic to hosts from more than one kingdom (see Table 1).

Factors that promote infection need to be compatible with future hosts

All living pathogenic microorganisms produce molecular components that, upon secretion within the host or upon injection into host tissue, play a role in the establishment of an infection. These components may play a role in the release or uptake of nutrients or in the evasion or suppression of the host immune system. Early in the evolution of pathogenic relationships, successful factors will tend to become fixed in populations through positive selection (Read, 1994). Molecules that target specific components of a specialized cell type are far less likely to facilitate cross-kingdom host jumps than molecules that target a generally conserved host component, and therefore microorganisms displaying a capacity for cross-kingdom host jumps are likely to express factors that act upon a wide range of organisms. Indeed, an examination of microorganisms that possess cross-kingdom pathogenicity (Table 1) appears to support this scenario. An overview of broadly effective factors conferring the capacity for pathogenicity towards hosts from different kingdoms is shown in Table 2. Examples of such factors are the subtilisin-like proteases produced by several *Aspergillus* species (Kolattukudy *et al*., 1993; St Leger *et al*., 2000; Rementeria *et al*., 2005) and the phospholipase C (plcS) and exotoxin A (toxA) from *Pseudomonas aeruginosa* (Rahme *et al*., 1995). These compounds all show activity against plant, insect and human tissues (see Table 2). Furthermore, secondary metabolites (low-molecular-weight molecules that are not directly necessary for growth but instead are a by-product of regular metabolism) can have targets in hosts belonging to different kingdoms. These compounds function in microbial defence and in antibiosis (Wicklow, 1988). For instance, helvolic acid produced by some *Aspergillus fumigatus* isolates can suppress the respiratory oxidative burst that is essential to the innate immune response in both plants and animals (Rementeria *et al*.,

Table 1. Cross-kingdom pathogens with their cognate hosts

Species*	Aliases, including alternative morph names† and obsolete but widely used synonyms	Human/mammalian host	Plant host‡	Comments§
Absidia corymbifera	*Absidia ramosa*	Opportunistic infection	Stored plant products	(M) Domsch *et al*. (1993), Rollan *et al*. (1999), De Hoog *et al*. (2000a)
Acremonium potronii		Ocular, wound	Grapevine (E)	(A/M) De Hoog *et al*. (2000a)
Agrobacterium radiobacter (E)	*Agrobacterium tumefaciens*	Opportunistic infection	Wide host range	Host: fungi; Paphitou & Rolston (2003), Lacroix *et al*. (2006)
Alternaria alternata	*Alternaria tenuis*	Wound and sinus colonization and opportunistic infection	Wide host range	(A) Domsch *et al*. (1993), De Hoog *et al*. (2000a), Samson *et al*. (2004)
Alternaria infectoria	*Lewia infectoria*	Wound and sinus colonization and opportunistic infection	Wide host range	(A) De Hoog *et al*. (2000a), Samson *et al*. (2004)
Aspergillus flavus	*Aspergillus oryzae*	Opportunistic infection	Cotton, peanut, maize seed	(A) Host: insects; Domsch *et al*. (1993), De Hoog *et al*. (2000a), Samson *et al*. (2004), St Leger *et al*. (2000)
Bipolaris australiensis	*Drechslera australiensis* *Cochliobolus australiensis*	Wound and sinus colonization and opportunistic infection	Rice, tomato, millet	(A) Ellis (1971), De Hoog *et al*. (2000a)
Bipolaris hawaiiensis	*Drechslera hawaiiensis* *Cochliobolus hawaiiensis*	Wound and sinus colonization and opportunistic infection	Wide host range	(A) Ellis (1971), De Hoog *et al*. (2000a)
Bipolaris spicifera	*Drechslera spicifera* *Cochliobolus spicifer*	Wound and sinus colonization and Opportunistic infection	Wide host range	(A) Ellis (1971), De Hoog *et al*. (2000a)
Blastomyces dermatitidis		Opportunistic infection		Host: amoeba; Steenbergen *et al*. (2004)
Burkholderia spp. (E)		Opportunistic infection	Onion (*B. cepacia*)	Host: *C. elegans*, protozoa; Jones *et al*. (2001), Baldwin *et al*. (2004)
Chaetomium globosum (E)		Subcutaneous disease, opportunistic systemic infection	Barley (E)	Guarro *et al*. (1995), Reissinger *et al*. (2003), Yu *et al*. (2004), Paterson *et al*. (2005)
Cladophialophora carrionii	*Cladosporium carrionii*	Subcutaneous disease, wound	Cactus (E)	(A?) Zeppenfeldt *et al*. (1994), De Hoog *et al*. (2000a)
Colletotrichum gloeosporioides	*Glomerella cingulata*	Wound and opportunistic subcutaneous/eye infection	Leaf and fruit	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a), O'Quinn *et al*. (2001)
Corynespora cassiicola		Wound and opportunistic infection	Tropical hosts	(A) Ellis (1971), De Hoog *et al*. (2000a)
Cryptococcus neoformans	*Filobasidiella neoformans*	Opportunistic infection		Host: amoeba, insects, *Caenorhabditis elegans*; Mylonakis *et al*. (2002), Steenbergen *et al*. (2001), Apidianakis *et al*. (2004), Mylonakis *et al*. (2005), Steenbergen *et al*. (2003)
Curvularia lunata	*Cochliobolus lunatus*	Wound and sinus colonization and opportunistic infection	Grains, sugarcane, etc.	(A) Domsch *et al*. (1993), De Hoog *et al*. (2000a), Movil & Camacho-de-Torres (2000), SenGupta *et al*. (2001), Raviraja (2005)
Curvularia geniculata	*Cochliobolus geniculatus*	Wound and sinus colonization and opportunistic infection	Wide host range	(A) De Hoog *et al*. (2000a), Domsch *et al*. (1993), Samson *et al*. (2004)
Curvularia pallescens	*Pseudocochliobolus pallescens*	Wound and sinus colonization and opportunistic infection	Sugarcane, corn (E/P)	(A) De Hoog *et al*. (2000a)

Drechslera biseptata		Wound and sinus colonization	Grasses	(A) Leach & Tulloch (1972), Ellis (1971), De Hoog *et al*. (2000a)
Enterococcus faecalis (E)		Nosocomial infection	Arabidopsis	Host: *Caenorhabditis elegans*; Moellering (1992), Garsin *et al*. (2001), Jha *et al*. (2005)
Exserohilum rostratum	*Drechslera rostrata* *Setosphaeria rostrata*	Wound and sinus colonization and opportunistic infection	Wide host range	(A) Ellis (1971), De Hoog *et al*. (2000a), Broschat & Elliott (2005)
Fusarium oxysporum		Opportunistic infection	Wide host range	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a), Ortoneda *et al*. (2004)
Fusarium proliferatum	*Gibberella* sp.	Opportunistic infection	Wide host range	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a)
Fusarium solani	*Haematonectria haematococca*	Wound and opportunistic infection	Wide host range	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a)
Fusarium verticillioides	*Gibberella moniliformis*	Opportunistic infection	Corn	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a)
Fusicoccum dimidiatum	*Scytalidium dimidiatum* *Hendersonula toruloidea* *Nattrassia mangiferae*	Superficial skin disease in healthy individuals	Tropical trees	(A/M) human-to-human transfer; Sutton & Dyko (1989), De Hoog *et al*. (2000a), Crous *et al*. (2006)
Histoplasma capsulatum		Opportunistic infection		Host: amoeba; Steenbergen *et al*. (2004)
Hortaea werneckii	*Phaeoannellomyces werneckii* *Cladosporium werneckii* *Exophiala werneckii*	Superficial skin disease in healthy individuals	Salt stressed plants	(M) De Hoog *et al*. (2000a), Middelhoven (1997)
Lasiodiplodia theobromae	*Botryodiplodia theobromae* *Diplodia gossypina* *Botryosphaeria rhodina*	Eye/nail/subcutaneous infection, wound	Wide plant host range	(M) Maslen *et al*. (1996), De Hoog *et al*. (2000a), Summerbell *et al*. (2004), Crous *et al*. (2006)
Legionella pneumophila (E)		Opportunistic infection		Amoeba Rowbotham (1980), Greub & Raoult (2004)
Mycobacterium avium (E)		Opportunistic infection		Host: amoeba; Cirillo *et al*. (1997), Steenbergen *et al*. (2004)
Mucor circinelloides		Opportunistic infection	Vegetables, fruits	(M) Domsch *et al*. (1993), De Hoog *et al*. (2000a), Samson *et al*. (2004)
Phaeoacremonium alvesii		Wound and subcutaneous and joint infection	*Dodonaea viscosa*	(M) Mostert *et al*. (2005)
Phaeoacremonium krajdenii		Subcutaneous disease, wound	Grapevine, woody plants (E/W)	(M) Mostert *et al*. (2005)
Phaeoacremonium parasiticum	*Phialophora parasitica* *Togninia parasitica*	Subcutaneous disease, wound and opportunistic systemic infection	Grapevine, woody plants (E/W)	(M) De Hoog *et al*. (2000a), Mostert *et al*. (2005)
Phaeoacremonium venezuelense		Subcutaneous disease, wound	Grapevine, woody plants (E/W)	(M) Mostert *et al*. (2005)
Pectobacterium spp. (E)	*Erwinia* spp.	Opportunistic infection	Wide host range	Host: *Caenorhabditis elegans, Drosophila melanogaster*, pea aphid; Starr & Chatterjee (1972), Chatterjee & Starr (1980), Hao *et al*. (1990), O'Hara *et al*. (1998), Duarté *et al*. (2000), Grenier *et al*. (2006)

Table 1. Continued.

Species*	Aliases, including alternative morph names† and obsolete but widely used synonyms	Human/mammalian host	Plant host‡	Comments§
Pleurostomophora richardsiae	*Phialophora richardsiae*	Subcutaneous disease, wound	Woody plants (E)	(M) Hutchison & Reid (1988), De Hoog *et al*. (2000a)
Pseudomonas aeruginosa (E)		Opportunistic infection, cystic fibrosis patients; burns	Wide host range, soft rot	Host: insects, *Caenorhabditis elegans*; Elrod & Braun (1942), Mahajan-Miklos *et al*. (1999), D'Argenio *et al*. (2001)
Pythium insidiosum (C)	*Hyphomyces destruens*	Wound	Water lilies, grasses	Host: insects; De Cock *et al*. (1987), Mendoza *et al*. (1993), Schurka *et al*. (2003)
Rhizopus oryzae	*Rhizopus arrhizus*	Opportunistic infection	Vegetables, fruits	(A) Requires high blood iron levels not found in healthy host; Domsch *et al*. (1993), De Hoog *et al*. (2000a), Samson *et al*. (2004)
Schizophyllum commune		Nasal sinus infections	Tree pruning wounds	(A) De Hoog *et al*. (2000a), Crous *et al*. (2004)
Serratia spp. (E)		Opportunistic (hospital-acquired) infection, keratitis	*Cucurbitae*	Host: insects, *Caenorhabditis elegans*; Hejazi & Falkiner (1997), Kurz *et al*. (2003)
Sporothrix schenckii	*Sporotrichum schenckii*	Subcutaneous mycosis, wound	Moss, tropical grasses, Eucalyptus	(A?) Host: amoeba; Cooper *et al*. (1992), Hajjeh *et al*. (1997), De Hoog *et al*. (2000a), Steenbergen *et al*. (2004)
Staphylococcus aureus (E)		Superficial to systemic infections	Arabidopsis	Host: *Caenorhabditis elegans*; Garsin *et al*. (2001), Prithiviraj *et al*. (2005a)
Trichoderma longibrachiatum	*Hypocrea* sp.	Opportunistic infection		(M) Host: fungi; Vajna (1985), Domsch *et al*. (1993), Rollan *et al*. (1999), De Hoog *et al*. (2000a)

*Species are indicated and belong to the kingdom Fungi unless otherwise indicated in brackets: (C) kingdom Chromista, (E) kingdom Eubacteria.
†Alternative names. The pleomorph (teleomorph) is underlined.
‡(E) endophyte, (E/P) endophyte and pathogen, (E/W) endophyte and weak pathogen.
§(A) air-borne spores, (M) mucoid spores. Hosts other than mammalian and plant are provided.

Table 2. Effectors that play a role in pathogenicity with cross-kingdom targets

Pathogen	Pathogenicity factors	Characteristics	Mode of action	References
Enterococcus faecalis	Hemolysin, gelatinase, AS, hemagglutinin, Lipase	Extracellular proteases; aggregation, clumping, cytotoxin	Adhesion, tissue degradation, toxin	Elsner *et al*. (2000), Sifri *et al*. (2002)
Pectobacterium spp. *Erwinia* spp. *Dickeya* spp.	PATE, PGs, PELs, phosphatidase intimin-like proteins	Extracellular proteases, cell wall degrading enzymes	Cell wall degradation, cellular leakage and death, hemorrhage	Starr & Chatterjee (1972), Chatterjee & Starr (1980), Duarté *et al*. (2000)
Pseudomonas aeruginosa	Exotoxin A, proteases, phospholipase C, alginate, quorum-sensing, LPS, type III secretion	Protease; chaperone	Cytotoxin; inhibition of protein synthesis; cytolytic activity; stimulation of extracellular toxin production and heat stress protection during *in vivo* growth	Vasil & Iglewski (1978), Wick *et al*. (1990), Yorgey *et al*. (2001)
Serratia spp.	Proteases, hemolysin (ShlA)	Chitinase; lipase cytotoxin	Cell wall and membrane degradation, pore-forming toxin	Hejazi & Falkiner (1997), Kurz *et al*. (2003)
Burkholderia cepacia, *Burkholderia* spp.	LPS AHL synthase, porin exopolysaccharides	Endotoxin amino acid transport, secretion	Induces necrosis via TNFR induction especially in lung tissue; amino acid metabolism during parasitic growth, evasion of immune system, tissue invasion and damage	Jones *et al*. (2001), Baldwin *et al*. (2004), Bylund *et al*. (2006)
Aspergillus flavus *Aspergillus fumigatus* *Aspergillus* spp.	Proteases (subtilisin type); secondary metabolites	Toxins	Lysis and degradation of diverse tissues (depending on – kingdom affected); necrosis induction, suppression of immune system	Denning (1998), Leger *et al*. (2000), Rementeria *et al*. (2005), Nierman *et al*. (2005)
Alternaria alternata *Alternaria* spp.	Secondary metabolites, conidial diffusible factors	Toxins	Disruption of membrane function, suppression of innate immune response, toxic activity against susceptible cell organelles, disruption of cell physiology, ceramide signaling and cell cycle	Nishimura & Kohmoto (1983), Gilchrist (1997)
Rhizopus sp.	Fumaric acid			

2005). The infection-promoting factors produced by microbial cross-kingdom pathogens tend to be toxins that directly target cellular membranes, which are among the most conserved cellular components. The direct mode of toxin action, the induction of necrosis, is to be expected from pathogens that benefit from inducing cellular lysis and then taking up cellular components as nutrient source (after preprocessing them with extracellular enzymes).

The microorganism can contend with host immunity

In order to develop stable pathogenicity as opposed to occasional chance infection, the future pathogen must be able to suppress or avoid host immune responses. Invertebrate animals and plants rely completely on innate immunity for their self-defence (Thomma *et al.*, 2001; Da Cunha *et al.*, 2006; van Baarlen *et al.*, 2007). Plants have a more or less rigid vascular system that transports dissolved compounds in an aqueous solution throughout the plant, although they lack a system with circulating cells that can swiftly carry host defence components to distal parts of the organism. Invertebrates such as arthropods possess an 'open' system in which the so-called hemolymph can flow freely within the body cavity, making direct contact with all tissues and organs. In addition to containing antimicrobial proteins and peptides, the hemolymph contains circulating cells known as hemocytes. This category of cell includes plasmatocytes and granulocytes, which are capable of phagocytosis and encapsulation of invading microorganisms.

In contrast to the open hemolymph system of invertebrates, vertebrate animals have a closed circulatory system in which blood is contained within vessels. One unique feature of vertebrate self-defence is a very efficient adaptive immune system. This system utilizes T- and B-cells, outfitted with a diversity of antigen-specific receptors, which travel in the blood stream and detect and combat components recognized as potential pathogens. The antigen-specific receptors are quickly generated through somatic recombination, which gives the immune system an extensive capacity to mount large numbers of different but specific defence reactions. Despite the absence of an adaptive immune system in invertebrates and plants, the innate immune system in these organisms still possesses some degree of specificity, as specialized defence mechanisms are activated in cases of attack by particular types of pathogens (Lemaitre *et al.*, 1997; Thomma *et al.*, 1998, 2001). In addition, as with the genes encoding mammalian antigen-specific receptors, plant genes involved in the recognition of specific pathogen types have been found to occur in clusters on the genome. This arrangement allows recombination that potentiates the development of new recognition specificities (Michelmore & Meyers, 1998). Despite the presence of an adaptive immune system, innate immunity still plays a central role in vertebrate host defence. Apart from serving as a first line of defence against potential pathogens, especially at the body's natural openings, innate immunity is involved in the activation of adaptive immune responses (Yang *et al.*, 1999, 2000).

Despite fundamental differences, the innate immune systems in different higher eukaryotic kingdoms share a number of common features (Fig. 4). These include molecular structures involved in microbial recognition, mitogen-associated protein kinase-based downstream signalling pathways, and the defensive use of reactive oxygen species (respiratory burst) and antimicrobial peptides and proteins. However, clear differences can be observed as well. For instance, the vertebrate complement system does not seem to have a counterpart system in the other kingdoms. This complement cascade poses a barrier to infection, resulting in

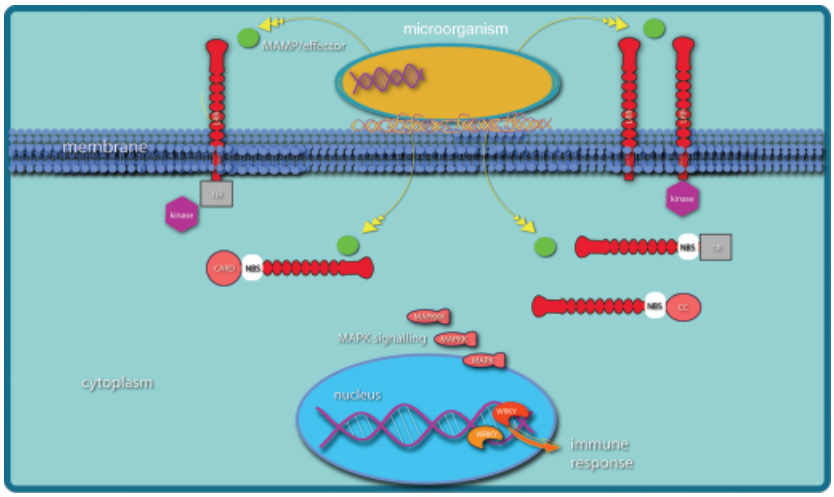

Fig. 4. Similarities in molecular components that play a role in the innate immune systems in different higher eukaryotic kingdoms. Pathogens are detected through various pattern recognition receptors (PRRs) that perceive microbial-associated molecular patterns (MAMPs) or effector proteins that may be released either extracellularly or in the host cytoplasm. PRRs on the left-hand side occur in mammalian hosts, while PRRs on the right-hand side occur in plants. Upon microbial recognition, MAPK signalling generally leads to transcriptional responses and the activiation of host immunity.

pathogen elimination, and, obviously, human pathogens have developed means of circumventing this. Multifactorial complement resistance has been observed for *Moraxella catarrhalis*, a human pathogen causing otitis media (Verduin *et al.*, 2002). Even more indicative of adaptation, *Staphylococcus aureus* has developed sophisticated means of interference in the complement-mediated opsonization. An inhibitor of one of the convertases involved in complement activation has been acquired through a bacteriophage (Foster, 2005; Rooijakkers *et al.*, 2005a, b). All of the above implies that pathogenic microorganisms are likely to meet similar molecular host defence components if they invade different hosts. A cross-kingdom comparison of disease-associated genes shows that, of 289 such genes identifiable in the human genome, nearly 80% have a clear orthologue in the insect *Drosophila melanogaster*. Furthermore, about 70% have an orthologue in another invertebrate, the nematode *Caenorhabditis elegans* (Rubin *et al.*, 2000). Even the plant species *Arabidopsis thaliana* shares 60% of these human disease-associated genes (MIPS *Arabidopsis thaliana* Genome Database, 2006). This all suggests that the molecular processes required for host defence have a high degree of fundamental similarity, and the ability to overcome a particular defence mechanism in one host could have implications for the ability to overcome similar mechanisms in other hosts. Apparently, the ability of a pathogen to infect multiple hosts might be very well reflected by homologies in their (innate) defence methodologies (van Baarlen *et al.*, 2007).

Microbial perception across kingdoms

Innate immunity is activated by the initial recognition processes of microbial invaders. This recognition can occur through so-called pathogen-associated molecular patterns (PAMPs), which include molecules of various natures such as the lipopolysaccharides of Gram-negative bacteria and the peptidoglycans of Gram-positive bacteria, as well as bacterial flagellin, microbial DNA and fungal cell-wall constituents (Girardin *et al.*, 2002; Nürnberger *et al.*, 2004). Although the term PAMP suggests that these molecules are unique to pathogenic microorganisms, they are in fact produced by both pathogenic and nonpathogenic microorganisms, and are therefore also frequently called microbial-associated molecular patterns (MAMPs). They are not, however, produced by the host, and are often required for microbial fitness (Medzhitov & Janeway, 2002). In addition to MAMPs, microbial effector molecules are also detected by the host. For instance, hosts are able to detect bacterial or fungal effector molecules that may be secreted into the intercellular space or are injected into cells by bacterial type III secretion systems (Nürnberger *et al.*, 2004).

The actual microbial recognition is mediated by so-called pattern recognition receptors (Fig. 4), which, in both insects and mammals, include the Toll-like receptors (TLRs). TLRs are a family of conserved transmembrane proteins with an extracellular leucine-rich repeat (LRR) domain and a cytoplasmic TIR domain [homologous to the cytoplasmic domain of Toll and the human Interleukin (IL)-1 Receptor] (Lemaitre *et al.*, 1996; Medzhitov *et al.*, 1997). In plants, homologous pattern recognition receptors containing extracellular LRRs have been identified as receptor-like proteins (RLPs) and receptor-like kinases (RLKs) (Jones *et al.*, 1994; Song *et al.*, 1995). Whereas both types of proteins are anchored in the cell membrane, neither of them contains a cytoplasmic TIR domain. The RLPs are characterized by a short cytoplasmic tail lacking obvious signalling domains, while RLKs contain a cytoplasmic kinase domain. In addition to these extracellular receptors for MAMP recognition, intracellular pattern recognition receptors have been identified. These include the mammalian NLR (NACHT-LRR) and plant NBS-LRR proteins (Belkhadir *et al.*, 2004; Kufer *et al.*, 2005; Martinon & Tschopp, 2005). The proteins in this class contain a central nucleotide-binding region, which in mammalian systems is called NACHT-ARC and in plants is called NB-ARC (van der Biezen & Jones, 1998; Koonin & Aravind, 2000). Apart from the central nucleotide-binding domain they contain a C-terminal LRR region. A specific subclass of the plant NBS-LRR proteins carries an N-terminal TIR domain similar to the mammalian Toll-like receptors. Remarkably, neither nematodes nor insects have homologues of the mammalian NLR or plant NBS-LRR proteins, suggesting independent evolutionary origins for those proteins in plants and mammals. Such convergent evolution is not uncommon, as it has been demonstrated that cross-kingdom recognition of flagellin by extracellular LRR proteins is probably brought about by convergently evolved receptor molecules and not by receptors derived from a common ancestor molecule (Nürnberger *et al.*, 2004).

Another class of pattern recognition molecules involved in host–pathogen interactions consists of the so-called sialic acid-specific lectins (siglecs), which belong to a group essentially comprising all proteins other than antibodies and T cell receptors that are capable of binding glycans (Mandal & Mandal, 1990; Varki & Angata, 2006). The siglecs can adhere to surface-expressed complex microbial sugar molecules and initiate downstream activation of, for instance, B cell differentiation (Crocker, 2005). Siglecs are also suggested to provide portals of entry for various viruses and bacteria. This siglec system is best studied in mammalian systems, but the first sialic acid-specific receptors were identified on plant cell surfaces (Muthing *et al.*, 2004). This might render siglec activity as yet another cross-kingdom conserved feature involved in the signalling upon detection of microorganisms. However, this involvement has yet to be confirmed for the plant sialic acid-binding proteins.

Host defence signalling across kingdoms upon pathogen perception

Once a pathogenic microorganism has been recognized, the host response often requires kinase activity that is mediated by a conserved family of serine-threonine kinases. The kinase domain that occurs in the cytoplasmic component of plant RLKs bears a resemblance to PELLE and Interleukin-1 Receptor-associated Kinases (IRAK) kinases from Drosophila and mammals, respectively (Shiu & Bleecker, 2001). Further downstream of these initial serine-threonine kinases, MAP kinase cascades are activated. Calcium fluxes then occur, reactive oxygen is produced, and transcriptional factors including NF-κB are activated that play a role in the inducible expression of defence response effectors such as antimicrobial peptides and proteins. Although the general patterns of these immune responses in different hosts are highly similar, there is little conservation between the individual components of these signalling cascades. It is likely that pathogen recognition capabilities and the ensuing cascade of responses have evolved independently in diverse members of different kingdoms, even though the evolutionary process may have built upon general signalling cascades that originated very early in evolution. The origin of such ancient signalling systems is likely to predate even the occurrence of multicellularity, because homologous general signalling blueprints are found in unicellular yeast species that are unlikely to have had multicellular ancestors (Ausubel, 2005). Interestingly, it was recently shown that *Arabidopsis* plants respond similarly to inoculation with human and plant-pathogenic bacteria, with the induction of many genes that correspond to transcription factors, signalling components, and cell wall- or secretion-associated components (Thilmony *et al.*, 2006).

The production of effectors of defence: antimicrobial proteins

A central aspect of innate immunity is the production of antimicrobial proteins, most of which are cationic, polar molecules with spatially separated, charged and hydrophobic regions (Boman, 1995). To date, hundreds of such proteins and peptides have been identified (Brahmachary *et al.*, 2004). They are organized in families that differ in size, sequence and structural motifs. Most of these peptides exert their antimicrobial activity by destabilizing negatively charged phospholipids in the plasma membranes of invading microorganisms, resulting in pore formation and membrane permeabilization (Kagan *et al.*, 1990; Ludtke *et al.*, 1996). Alternatively, cationic peptides may exert antimicrobial activity in part by affecting specific cytoplasmic targets. For instance, DNA, RNA or protein synthesis may be inhibited (Bals, 2000). For these cases, the ability of cationic peptides to permeabilize cytoplasmic membranes might provide a means for inhibitory components to reach an intracellular target. Apart from displaying direct antimicrobial properties, some of the antimicrobial proteins are known also to have signalling functions (Scott & Hancock, 2000; Yang *et al.*, 2004).

One class of antimicrobial peptides that is found to be conserved across kingdom boundaries comprises the defensins (Thomma *et al.*, 2002). Representatives of this peptide family isolated from plants, insects, invertebrates and vertebrates display remarkable structural homology (Fig. 5). Recently, the first defensin of fungal origin was identified and structurally characterized (Mygind *et al.*, 2005). In contrast to most other cationic peptides, it has been demonstrated that defensins, in at least some cases, do not undergo electrostatic binding to membrane phospholipids but interact specifically with membrane sphingolipid targets (Thevissen *et al.*, 2000, 2004). Interestingly, in one case it was found that an identical target was shared by a plant defensin from radish seeds and an insect defensin from a moth (Thevissen *et al.*, 2004). Insensitivity of fungal mutants to the plant defensin renders them insensitive to the insect defensin as well. Also here, the 'lock-and-key' interaction between pathogenic microorganism and host might apply.

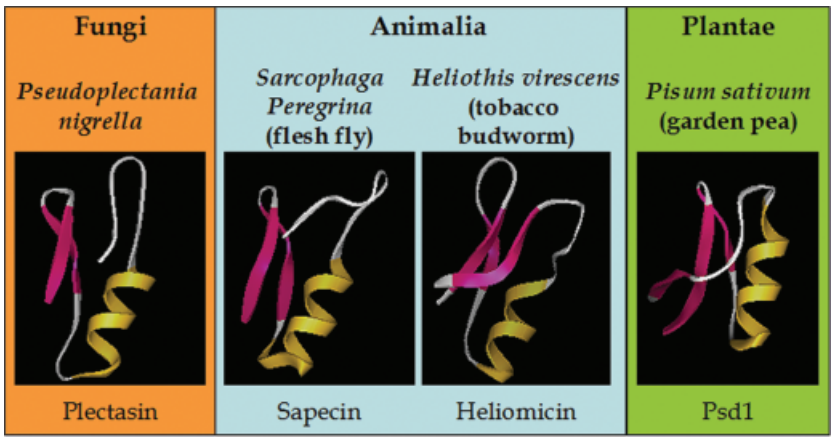

Fig. 5. Three-dimensional structure of defensins of fungal, animal and plant origin from the saprotrophic fungus *Pseudoplectania nigrella*, flesh fly, tobacco budworm and garden pea as examples. Structures were downloaded from the protein data bank (http://www.rcsb.org/pdb; PDB accession ID numbers: Plectasin: 1ZFU, Sapecin: 1L4V, Heliomicin: 1I2U, Psd1: 1JKZ). Pictures were generated using Swiss-PDB viewer. Alpha-helices and beta-sheets are shown in yellow and red, respectively.

It has recently been shown that the phenolic plant metabolite salicylic acid (SA) that mediates the expression of a number of genes encoding antimicrobial proteins acts against the virulence of *Pseudomonas aeruginosa* by repressing attachment, biofilm formation, and the production of virulence factors (Prithiviraj *et al*., 2005b). Inside the gut of *Caenorhabditis elegans* nematodes, SA-treated bacteria accumulate to densities similar to those attained by untreated bacteria, but they are less capable than the untreated bacteria of killing the nematodes. This difference supports the hypothesis that SA directly influences virulence factors (Prithiviraj *et al*., 2005b). Similar results were obtained in SA trials in which *Arabidopsis* and *Caenorhabditis elegans* were used as infection models for *Staphylococcus aureus* (Sifri *et al*., 2003; Garcia Lara *et al*., 2005; Prithiviraj *et al*., 2005a, c; Sifri *et al*., 2006; van Baarlen *et al*., 2007). Interestingly, intravenous aspirin [acetylsalicylic acid (ASA)] was shown to result in a reduction of *Staphylococcus aureus* densities on the endocardial surface in a rabbit model of invasive endocarditis. This effect could be attributed to the retention by ASA of the inhibitory properties of its precursor molecule, SA. Also in this interaction, pretreatment of bacteria with SA significantly reduced attachment to the cardiac epithelium (Kupferwasser *et al*., 1999).

How pathogens deal with host defence responses

If a pathogenic microorganism is sensitive to defence components released by the host, it has to find ways to evade or suppress them in order to cause infection. In several cases, similar strategies have been developed by different pathogens affecting hosts from different kingdoms. A first strategy to deal with host defence is self-protection. Pigments deposited in microbial cell walls, such as carotenoids and melanin, have been shown to be important for microbial survival and pathogenicity. Melanins are dark, brown to black, high-molecular-weight pigments that are formed by the oxidative polymerization of phenolic or indolic compounds by organisms that range from microorganisms to plants and animals (Nosanchuk & Casadevall, 2006). Melanins may play direct as well as indirect roles in microbial virulence. They act in protection, often of propagative structures such as spores and resting structures. These specialized structures are shielded by melanin against adverse conditions and environmental stresses of various kinds, such as extreme temperatures, UV radiation and detrimental compounds, and thus attain extended survival times (Frye *et al*., 1984; Rehnstrom & Free, 1996). For some plant-pathogenic fungi such as *Magnaporthe grisea*, melanin has also been directly implicated in virulence, as melanization of the appressorium was found to be required for tissue penetration (Howard & Valent, 1996). In the human-pathogenic fungi *Cryptococcus neoformans* and *Exophiala dermatitidis*, melanin-deficient strains were shown to exhibit decreased virulence (Dixon *et al*., 1987; Wang & Casadevall, 1994). The precise means by which melanin may contribute to virulence is only partially understood. Several studies have shown that melanin can act as a scavenger of reactive oxygen species that are produced during host defence reactions (Fels *et al*., 1987; Wang & Casadevall, 1994; Jacobson *et al*., 1995; Wang *et al*., 1995; Schnitzler *et al*., 1999). Melanized cells are less sensitive to killing by reactive oxygen species than are unmelanized mutants or other unmelanized cells. Moreover, melanized cells are more resistant than unmelanized cells to lysis, and, unlike unmelanized equivalents, can prevent phagocytosis (Wang *et al*., 1995; Rosas & Casadevall, 2001). Melanin also protects against the activity of clinically used antifungal agents in both *Cryptococcus neoformans* (Martinez & Casadevall, 2006) and *Madurella mycetomatis* (unpublished observations). In humans, immunosuppressive effects such as downregulation of tumour necrosis factor-α (TNF-α), a signalling molecule in the human inflammatory response, have been attributed to melanin derived from fungal pathogens such as *Cryptococcus neoformans* (Huffnagle *et al*., 1995).

A second strategy to deal with host defence is suppression. Several microbial pathogens are known to produce metabolites that display immunosuppressive effects, of which gliotoxin is probably the best characterized. Gliotoxin is involved in diverse animal mycoses, causing macrophage inhibition and induction of apoptosis in various cells, including neutrophils (Müllbacher & Eichner, 1984; Sutton *et al*., 1994), through inhibition of the assembly of the NAPDH oxidase enzyme complex that is involved in the respiratory burst (Tsunawaki *et al*., 2004). It also promotes TNF-α-induced apoptosis (Ward *et al*., 1999) and specifically inhibits the nuclear transcription factor NF-κB that regulates the inflammatory response (Pahl *et al*., 1996). Gliotoxin is produced by several phylogenetically separated fungi including *Aspergillus fumigatus*, *Trichoderma virens*, *Candida albicans* and some *Penicillium* spp. (Gardiner *et al*., 2005). It belongs to the class of epipolythiodioxopiperazine (ETP) compounds, of which some members, such as sirodesmin, which is produced by the plant pathogen *Leptosphaeria maculans* (Rouxel *et al*., 1988), display antibacterial and antiviral effects. The putative gliotoxin biosynthetic gene cluster identified in the *Aspergillus fumigatus* genome (Gardiner *et al*., 2004) strongly resembles the sirodesmin biosynthetic gene cluster.

Many Gram-negative bacterial pathogens possess type III secretion systems that are employed to inject bacterial effector proteins into host cells (Jin & He, 2001). Bacterial mutants that are incapable of injecting these effectors are nonpathogenic (Lindgren *et al*., 1986). The type III secretion system is not restricted to pathogenic bacteria, as symbiotic plant and insect bacteria also use type III secretion systems to

interact with their hosts (Viprey *et al.*, 1998; Dale *et al.*, 2001). For the plant pathogen *Pseudomonas syringae* it has been determined that, depending on the strain, about 20–30 effectors are injected into the host cells (Chang *et al.*, 2005). Many type III effector proteins produced by diverse pathogens have been shown to display protease activity (Alfano & Collmer, 2004). For plant pathogens in particular, the biological function of a growing number of bacterial effectors has been elucidated (Nomura *et al.*, 2005), and several of them inhibit host defence responses (Hauck *et al.*, 2003; DebRoy *et al.*, 2004). Two *Pseudomonas syringae* effectors, AvrRpt2 and AvrRpm1, target the same host factor RIN4, which is a regulator of MAMP signalling and basal defence in *Arabidopsis*, through different modes of action (Kim *et al.*, 2005). While AvrRpt2 degrades RIN4 through cysteine protease activity, AvrRpm1 interaction with RIN4 leads to its phosphorylation, thereby inactivating the protein. The emerging view is that many plant pathogen effector molecules act to suppress the MAMP-triggered immune response (Chisholm *et al.*, 2006; Jones & Dangl, 2006). For instance, the *Pseudomonas syringae* effectors AvrPto and AvrPtoB were found to intercept multiple mitogen-associated protein kinase (MAPK) signalling cascades that act in nonhost immune responses (He *et al.*, 2006). Bacterial virulence factors other than type III effectors may also play a role in the suppression of basal host defences. Several *Pseudomonas syringae* pathovars produce the phytotoxin coronatine, which mimics the action of jasmonic acid (JA), a plant compound involved in basal defence against pathogenic microorganisms (Thomma *et al.*, 1998; Zhao *et al.*, 2003). Coronatine activates the JA signalling pathway in tomato, thereby suppressing SA-dependent defence responses (Zhao *et al.*, 2003).

Two newly discovered immune modulators from *Staphylococcus aureus*, namely staphylococcal complement inhibitor (SCIN) and chemotaxis inhibitory protein of *Staphylococcus aureus* (CHIPS), are important virulence factors that protect *Staphylococcus aureus* from host innate immune responses. While SCIN is a C3 convertase inhibitor that inhibits the ability of host neutrophils to phagocytose *Staphylococcus aureus* by blocking the formation of C3b on the surface of the bacterium, CHIPS specifically binds two chemokine receptors, resulting in the inhibition of neutrophil chemotaxis (de Haas *et al.*, 2004; Rooijakkers *et al.*, 2005a, b).

Besides modulating basal defence responses, the programmed cell death (PCD) or apoptosis response may also be targetted as part of the modification of host defences. In some such cases cell death is triggered, while in other cases it is suppressed. These modulating actions can be achieved by various effectors. The involvement of ubiquitination in PCD has been well demonstrated in many organisms (Hershko & Ciechanover, 1998). YopJ is an effector protein produced by *Yersinia* spp. that suppresses host defence by inducing the death of infected macrophages and by blocking host inflammatory responses. The latter effect is accomplished by the inhibition both of the nuclear factor NF-κB and of MAPK signalling. YopJ achieves its effect through its activity as a promiscuous deubiquitinating enzyme that negatively regulates signalling by removing ubiquitin moieties from critical signalling proteins (Zhou *et al.*, 2005). On the other hand, the effector molecule AvrPtoB that displays ubiquitin ligase activity and that is produced by diverse plant pathogens is found to inhibit PCD initiated by disease resistance proteins (Janjusevic *et al.*, 2006). Remarkably, AvrPtoB is also able to suppress PCD induced by various triggers in the nonpathogenic baker's yeast *Saccharomyces cerevisiae*, indicating that AvrPtoB acts on PCD components apparently conserved across different kingdoms (Abramovitch *et al.*, 2003). Other ways to modulate PCD have also been reported. The pathogenicity factor tomatinase, produced by the tomato leaf spot fungus *Septoria lycopersici* during infection, was found to suppress host PCD by degrading preformed antimicrobial host saponins into PCD-suppressive degradation products (Bouarab *et al.*, 2002).

The capacity to grow, colonize and persist in the future host

Most pathogenic microorganisms have a free-living life style too, or at least have free-living nonpathogenic sister species or genera. For instance, many *Pseudomonas* spp. occur as saprotrophic soil bacteria and do not cause disease, although some species can cause opportunistic infection of immunocompromised humans (Gilardi, 1972; von Graevenitz, 1973). Similarly, fungi belonging to the genus *Penicillium* are ubiquitous saprotrophs, but the species *Penicillium marneffei* can cause systemic opportunistic infection of people suffering from AIDS (Kaufman, 1998). Survival upon entering a living organism is associated with an innate capacity for rapid modification of metabolic activity or adapting to (and taking advantage of) host metabolism. A difference in the capacity for pathogenicity may be decided for at the transcriptional level (Berg *et al.*, 2005). As can be expected, the presence of virulence factors is an additional prerequisite (Foster, 2004; Mendes-Giannini *et al.*, 2005; Poly *et al.*, 2005), although the individual or combined contributions of these factors to pathogenicity may be elusive. For example, virulence factors can appear to be correlated with host preference (Melles *et al.*, 2004), or can appear to be redundant (Rementeria *et al.*, 2005). The lifestyle and environmental niche are among a number of factors determined by fundamental requirements with respect to humidity, nutrients, pH and temperature optima of a species. These basic growth conditions may be similar in both the pathogenic and the harmless members of clades

containing pathogens (Xu, 2004; Heurlier *et al.*, 2005). Pathogens usually have additional features that are lacking in their nonpathogenic relatives, such as tolerance of elevated temperatures (Araujo & Rodrigues, 2004). When these pathogens enter mammals, this tolerance may enable them to persist at least temporarily. Such tolerance may have evolved in the environment: for instance, thermotolerance is clearly favoured in microorganisms that occur in composting plant debris or in those occurring in tropical regions. Such traits can be considered to be pre-existing adaptations to relatively extreme environmental conditions outside of the natural host, and need not have evolved primarily as traits selected in the development of pathogenicity (van Burik & Magee, 2001).

Many fungi occur mainly in the soil or on decaying plant materials, but some species or isolates can nonetheless infect various hosts upon contact. Several species of the genus *Aspergillus* are good examples of this; for instance *Aspergillus fumigatus* and *Aspergillus terreus* are important pathogens of humans, whereas *Aspergillus flavus* has an even wider host range including plants. A shift from an environmental state into a clinical, pathogenic state inside a host requires adaptation to or tolerance of the new environment. Not all organisms are able to survive this shift. One decisive moment is when fungal spores enter a mammalian host and experience substantially increased temperature. Germination of *Aspergillus fumigatus* conidia is stimulated at normal mammalian body or fever temperatures, whereas these temperatures decrease or completely inhibit germination of conidia of various other *Aspergillus* species (Araujo & Rodrigues, 2004). For *Cryptococcus neoformans* it is suggested that sugars such as trehalose and sorbitol act as stress protectants during growth at high (37 °C) temperatures (Petzold *et al.*, 2006). Furthermore, the iron-responsive *Cryptococcus neoformans* transcription factor Cir1 was determined to control the ability to grow at human body temperature (Jung *et al.*, 2006). Another example is the opportunistic human pathogen *Pseudomonas aeruginosa*, which can grow at temperatures ranging from 11 up to 44 °C (Health Protection Agency, 2006). It can also use over 80 organic compounds for growth, and has a good ability to survive under a variety of conditions including oligotrophic aquatic habitats and high-redox stress environments such as wounded or necrotic tissue (Wick *et al.*, 1990).

Especially for opportunistic and facultative pathogens, pathogenicity mechanisms may be selected for in alternative hosts or in the environment. The biotic and abiotic environment has direct and indirect effects on the success of host–pathogen interactions (Parker & Gilbert, 2004). Interactions between microorganisms may lead to the development of pathogenicity towards other organisms. *Cryptococcus neoformans* pathogenicity towards humans and the capacity to persist within mammalian macrophages may have arisen as a result of adaptations necessary for survival in interactions with soil protozoa or other soil organisms (Steenbergen *et al.*, 2001). The primary niche for *Cryptococcus neoformans* is not well understood but includes microorganism-rich sites such as bird and bat guano and animal-inhabited tree holes (Randhawa *et al.*, 2003), although the organism seems to have a clear preference for eucalypt trees. *Cryptococcus neoformans* has been shown to be capable of surviving phagocytosis by amoebae and can even replicate within, and then escape from, these organisms (Steenbergen *et al.*, 2001). Macrophages and amoebae share common properties that include the phagocytosis of particles into vacuoles where lytic enzymes are secreted for digestion. It is thought that pathogens escape killing in both cell types through mechanisms that are similar at the cellular level.

A bacterial parallel to *Cryptococcus neoformans* is the pathogen *Legionella pneumophila*, which persists in both macrophages and amoebae (Rowbotham, 1980). The genes required for survival and replication in the two hosts have been shown to share a remarkable overlap (Gao *et al.*, 1997). There is a dynamic dimension to these interactions, as in an experimental slime mould model passage of *Cryptococcus neoformans* through the microorganism-digesting myxomycete not only failed to kill the yeast, but also increased its virulence on a mammalian host (Steenbergen *et al.*, 2003). A similar increase in virulence was observed by passage of the bacterium *Mycobacterium avium* and the yeast *Histoplasma capsulatum* through amoebae (Cirillo *et al.*, 1997; Steenbergen *et al.*, 2003, 2004), while repeated reproduction of *Aspergillus flavus* on moth larvae led to a decreased capacity for saprobic growth (Scully & Bidochka, 2005; see below). All these finding support the idea that key aspects of pathogenesis in these microorganisms are derived from mechanisms employed for survival in the environment external to the host (Steenbergen *et al.*, 2001). This might represent a general mechanism: a wide variety of pathogenic and potential future pathogenic fungi may be under selection by predatory soil organisms such as nematodes and amoebae. It has been shown that the yeast forms of the facultative mammalian pathogens *Blastomyces dermatitidis*, *Sporothrix schenckii* and *Histoplasma capsulatum* possess the capability to infect amoebae (Steenbergen *et al.*, 2004). It has also been suggested that protozoans act as a reservoir for microbial pathogens such as *Listeria monocytogenes*, *Chlamydia pneumoniae* and *Pseudomonas aeruginosa* (Greub & Raoult, 2004). Amoeba-like protozoans were also suggested to be an ancestral host to *Rickettsia* spp., intracellular Gram-negative bacteria (Ogata *et al.*, 2006). One species, *Rickettsia bellii*, was found to be able to survive in the vacuole of amoebae as well as to replicate in nuclei of eukaryotic cells. Interestingly, this species expresses sex pili-like appendages on its surface and contains genes enabling transfer of DNA.

Ogata *et al.* (2006) suggested that protozoans hosting *Rickettsia* spp. may have served as a gene pool-like reservoir where gene exchange may have occurred between *Rickettsia* spp. and possibly also other genera. Such a genetic reservoir within protozoans may have contained factors enabling the survival, adaptation and reproduction of *Rickettsia* within the protozoans and eukaryotic cells (Ogata *et al.*, 2006). In general, the survival of microorganisms in protozoans (which often display a predatory-like behaviour towards bacteria) may well have driven the evolution of bacterial virulence factors, some of which may eventually be employed in mammalian infection.

Adaptation to a specific environment or host may lead to a decreased capacity to prosper in other environments or hosts. To test this possible negative effect of specialization, Scully & Bidochka (2005) tested mycelial growth and conidial production in *Aspergillus flavus* strains grown on culture media and on living insects. Radial growth was constant when *Aspergillus flavus* isolates were subcultured multiple times on culture media. After repeated passage of *Aspergillus flavus* through an insect host, increasing numbers of conidia were formed on cadavers after the infected insects had died. However, radial growth of the insect-adapted isolates on artificial culture media decreased steadily, demonstrating that continued pathogenicity was selecting for a decrease in the capacity for saprotrophic growth (Scully & Bidochka, 2005).

Interestingly, continued propagation of *Aspergillus flavus* on wax moth larvae resulted in a strain that was affected in host range as well as conidia production *in vitro*, while pathogenicity and conidial production on the insect host was maintained. This host restriction was found to correlate with cysteine/methionine auxotrophy and shows how a shift from being an opportunistic pathogen to becoming an obligate pathogen can occur. This seems to be an opportunistically driven adaptive process. Possibly, through further adaptation, increased virulence could occur (Scully & Bidochka, 2006), although virulence attenuation could occur once adaptive pressure was alleviated (Bartley *et al.*, 2006). This could similarly have occurred in *Yersinia pestis*, an obligate pathogen that causes plague. The facultative pathogen *Yersinia pseudotuberculosis* is thought to be the ancestor of the obligate pathogen *Y. pestis*, and compared with *Y. pseudotuberculosis, Y. pestis* has a smaller host range and has lost a number of metabolic enzymes (Chain *et al.*, 2004). This has been corroborated by estimating the evolutionary history and its timing for *Y. pestis*: it was convincingly demonstrated that *Y. pestis* represents a relatively juvenile clone (Achtman *et al.*, 2004). Not all fungi show an evolution towards increasing pathogenicity after serial passage through insect hosts (Hall, 1980). Understanding the differences between taxonomic groups in this capacity is important if we are to understand the evolution of pathogenicity.

Adaptation is a very important feature of host–pathogen interaction, and bacteria have developed various mechanisms to adapt. In simple molecular terms these mechanisms could all be traced back to genomic differences: nucleotide mutation, deletion, insertion, and target multiplication can all lead to differences in ecological fit. A very interesting example is provided by variation in tandem repeat loci. In many bacteria, regions of repetitive DNA are likely to undergo changes during replication. The number of repeat units may vary locally, and when the repeat is within a coding region or gene promoter differential gene expression may be the consequence of the repeat variation (van Belkum *et al.*, 1998). This results in phase variation and is a widespread phenomenon in the bacterial kingdom. *Haemophilus influenzae*, for instance, uses arbitrary repeat variation to introduce variability into its lipopolysaccharide (Schweda *et al.*, 2003). This may affect survival in given niches or the capacity to overcome host responses. Many other bacterial species use the same trick to overcome host defence, so phase variability may be an important feature both for pathogenic microorganisms that 'cling to their host' and for pathogens making host or kingdom jumps.

Species such as the opportunistic pathogen *Aspergillus fumigatus* not only produce factors that induce necrosis and cell death in human tissue (Rementeria *et al.*, 2005) but also produce so-called fitness factors. In contrast to virulence factors, fitness factors contribute to microbial survival, for instance through protection of the fungus from adverse conditions during growth inside the host, or outside in the environment. For *Aspergillus*, these factors are generally found in nonpathogenic *Aspergillus* species as well as in the opportunistic aspergilli (Nierman *et al.*, 2005). Some widely distributed fitness factors may, however, predispose the fungi possessing them to develop true pathogenicity. Fitness factors and other factors fortuitously contributing to pathogenic success may circulate among the members of microbial communities, including the soil-borne communities that many microorganisms belong to during at least some phase of their life histories. For example, the genes encoding the iron uptake system that contributes to the pathogenicity of *Yersinia* species infecting humans are also present in nonclinical *Klebsiella* soil bacteria (Hacker & Carniel, 2001).

The growth in soil or decaying vegetation of environmentally common opportunistic fungal pathogens such as *Aspergillus fumigatus* and *Aspergillus flavus* appears to have allowed genetic exchange and recombination among isolates, giving rise to a diverse gene pool that underlies the capacity of these organisms to tolerate an extremely wide range of environments. These fungi, although apparently asexual, are characterized by high genetic diversity (Debeaupuis *et al.*, 1997; Horn, 2003) that may reflect the existence

of meiotic recombination and sexual reproduction or the occurrence of parasexuality. Indeed, the complete genetic machinery for sexual reproduction has been found in *Aspergillus fumigatus* and it must be there for a reason (Debeaupuis *et al*., 1997; Paoletti *et al*., 2005). This highlights the fact that genome sequencing is an important tool for establishing microbial factors important not only for sexual reproduction but also for controlled and successful reproduction in the host environment. Nevertheless, sexual reproduction has not yet been observed for *Aspergillus fumigatus* in nature. Similarly, a soil-borne phase as part of the life history is found in bacteria that show the capacity for cross-kingdom infection (Berg *et al*., 2005). The high diversity engendered by soil-borne genetic exchange may influence the final outcome of an infection, because host invasion by multiple genotypes may increase the virulence of all the pathogens present (Ewald, 1996; Read & Taylor, 2001; Galvani, 2003).

The possibility of genome perpetuation by means of reproduction on the host or on its byproducts

Reproduction of the pathogenic microorganism on the host or in its immediate environments is essential in the establishment of evolutionarily stable host–pathogen relationships (as opposed to incidental or chance infection). This process requires coevolution of host and pathogen, which ultimately should lead to mutually better survival and/or increased fitness. Many fungal pathogens reproduce both sexually and asexually. While sexual reproduction is important in the generation of variation by means of meiotic recombination, asexual reproduction can efficiently generate massive amounts of one specific, successful genotype. The clonal mode of asexual reproduction ensures the preservation of successful genotypes, not just in completely clonal (bacterial) species but also in fungi that have both sexual and asexual reproduction (Hull, 1980; Anderson & Kohn, 1995). Different traits may be transmitted into different genetic backgrounds, either vertically through regular sexual or asexual processes or horizontally (Hacker & Carniel, 2001; Baldwin *et al*., 2004). When the necessary fitness and pathogenicity factors are recombined in a single genetic background, a future pathogen may become able to infect an otherwise resistant, novel host. However, stable pathogenicity will depend on two major features: (1) the preservation of the succesful infectious pathogenic genotype; and (2) multiplication in combination with dispersal of the successful genotype. In other words, the microorganism needs to reproduce on the host or on host products (faeces for instance). Bacteria and yeasts generally only undergo typical dividing or budding of unicells in a host medium that in some way can be disseminated (for example via aerosols, via surface contact, or via insect transmission). Filamentous fungi must undergo a relatively complex sporulation process, because hyphae themselves are harder to disseminate. Some fungi are also able to undergo a phase shift in their life cycle from a modular (filamentous) state to a particulate (yeast or spherule) state, known as dimorphism. A particularly significant number of ascomycetous fungi have the ability to alternate between hyphal and unicellular yeast-like forms (van Burik & Magee, 2001). The small and thus easily translocated yeast form is able to circulate in the bloodstream and, in susceptible hosts, cause disseminated disease. Size dependence in infectious propagules is also seen in the highly infectious conidia of *Aspergillus fumigatus* and *Aspergillus flavus*, which, because of their 2–4 μm size, are very well suited for efficient airborne dispersal and entrance into the human respiratory system (Walsh & Pizzo, 1988; Rementeria *et al*., 2005). Interestingly, many fungi with the capacity to form small air-borne conidia or to undergo dimorphism cluster together in a phylogenetic tree (Fig. 6). Recently, a hybrid histidine kinase was identified as a global regulator of dimorphism in several pathogenic fungi (Nemecek *et al*., 2006). In addition to the transition from filamentous to yeast, this kinase also regulates the expression of virulence genes.

Efficient sporulation is determined not only by the pathogenic microorganism, but also by the host and the environment. The extent to which tissue is colonized, yielding microbial biomass, influences the amount of potential sporulation generated, as does the extent to which tissue is necrotized (in necrotrophic pathogens) or tapped (in biotrophic pathogens) to provide nutrients. The final quantity of sporulation that can be produced is, however, also strongly influenced by the environment (Rotem *et al*., 1978). Particular triggers such as optimal light and humidity conditions may be required for maximal sporulation to occur. Facultative pathogens that combine a saprotrophic and a pathogenic lifestyle require sporulation-promoting conditions while growing on their hosts in addition to having their basic nutritional requirements met (Rotem *et al*., 1978). For example, sporulation of *Alternaria porri* is negatively correlated with increasing sugar content, high relative humidity (Bashi & Rotem, 1975, 1976) and conditions stimulating photosynthesis (Cohen & Rotem, 1970). Environmental triggers may only be necessary for a specific type of spore, for example those serving as survival structures. Such highly resistant spores frequently result from sexual processes. In some fungi, sexual sporulation may also depend on hormonal regulation by small molecules, such as terpenoids, or by peptides as the yeast alpha- and **a**-factor. Secondary metabolites and cyclic adenosine monophosphate (AMP) may also be involved (Dahlberg & van Etten, 1982). Host factors and local C:N

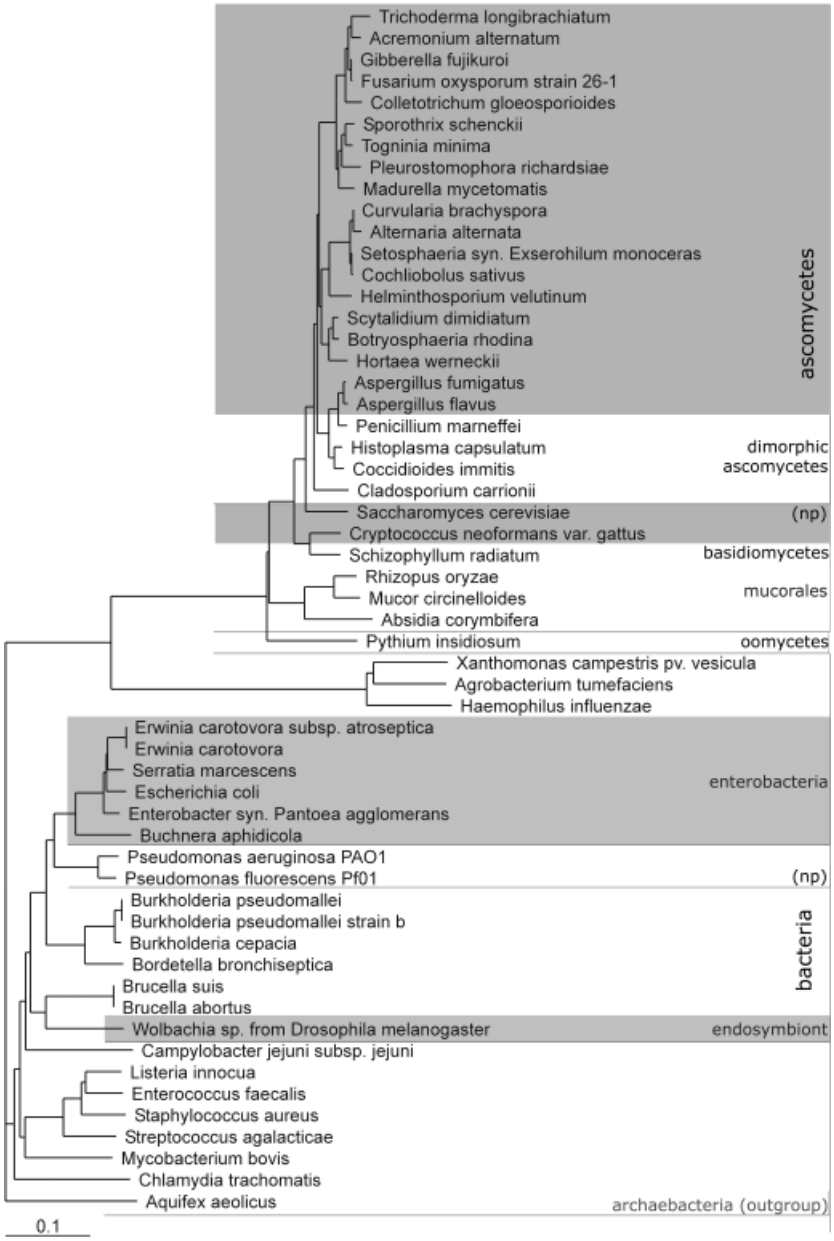

Fig. 6. Phylogenetic relationships between various cross-kingdom pathogens and a number of selected pathogenic as well as nonpathogenic (np) relatives.

imbalance may directly stimulate sporulation of the pathogen (Rotem *et al.*, 1978; Dahlberg & van Etten, 1982).

Dry-spored fungi typically sporulate at the substrate-to-air interface. This implies that, in order to sporulate, these fungi must be able to form conidiophores outside colonized host substrate. An example of this is seen in the sporulation of *Aspergillus fumigatus* inside the lungs (case studies listed at the Aspergillus website, The Fungal Research Trust, 2006). This fungus is not able to sporulate within colonized human tissue, but can sporulate in air pockets such as pulmonary cavities. Some fungi form spore types that have more relaxed sporulation prerequisites, such as the macroconidia of *Fusarium*: these macroconidia can be formed in liquid culture as well as on solid agar containing leaf extracts (Togawa, 1992; Ohara *et al.*, 2004).

For pathogenic bacteria, perpetuation of the genome depends on cell division, tissue destruction with lysis giving access to the immediate environment, and release of progeny into this environment. Pathogenic bacteria are thus most effectively transferred between hosts when there is frequent contact between hosts, or when there is contact between a recently sacrificed host and future hosts, or when the pathogenic bacteria are able to produce resistant but still infectious structures that can survive for prolonged periods

outside a sacrificed host before infecting a new host. Spores of *Clostridium difficile* can survive for prolonged periods in inanimate environments and can even withstand the aggressive action of a variety of cleansing detergents (Margosch *et al.*, 2006). All bacteria that have made cross-kingdom jumps fulfil one or more of these requirements. For at least some bacteria, including *Pseudomonas aeruginosa, Burkholderia cepacia, Pantoea* and *Enterobacter*, dispersion across distances in the kilometre range can be achieved by biofilm-derived, aerosolized particles. Environmentally recovered *Pseudomonas aeruginosa* strains that were transported by air in biofilm particles all had the properties of clinical strains, including hemolytic and proteolytic capacities (Morris & Monier, 2003).

Examples of microorganisms that have accomplished cross-kingdom host jumps

Several general aspects and requirements of microbial cross-kingdom pathogenicity have been discussed. Microorganisms that have been documented as pathogens on hosts belonging to different kingdoms are listed in Table 1. Some of these pathogen–host combinations are based only on laboratory experiments and have thus far not been encountered in nature. However, the ease with which such partially artificial infections are induced is highly suggestive of the existence of natural infectious syndromes crossing kingdom borders. The following section of this review will highlight a number of cross-kingdom pathogens.

The bacterial pathogen *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative saprotrophic bacterial species that is ubiquitously present in the environment. This microorganism is notorious for causing sepsis in burned patients and in immunodeficient patients including neonates, and for being the major cause of mortality in humans afflicted with cystic fibrosis (CF). It rarely causes infections in immunocompetent patients and is generally considered as an opportunistic pathogen. Remarkably, in a study investigating the genomes of *Pseudomonas aeruginosa* isolates from various clinical and environmental sources, it was found that the total genome content, including known virulence genes, was strongly conserved in all isolates. This suggests that *Pseudomonas aeruginosa* isolates essentially possess the basic machinery to cause human infections regardless of the habitat from which they are isolated (Wolfgang *et al.*, 2003). The increased virulence of pathogenic strains was found to be correlated with the presence of pathogenicity islands harbouring clusters of virulence genes that may have been acquired from other microorganisms or mobile genetic elements including bacteriophages through horizontal gene transfer (He *et al.*, 2004). One important feature of *Pseudomonas aeruginosa* isolates is their capacity to adapt to human hosts suffering from CF. They seemingly do so in direct competition with other microbial species, because they do appear to be conquering niches initially occupied by *Haemophilus influenzae* and *Staphylococcus aureus*. Most isolates successfully adapted to the human CF lung show a so-called mucoid phenotype upon cultivation. This is a result of the production of significant amounts of exopolysaccharides, which is thought to be associated with the increased intra-lung fitness of the microorganisms (Jain & Ohmann, 2005). *In vivo* mutation rates were found to be elevated such that isolates showed positive (or diversifying) selection across the whole genome, especially in those regions involved in pathogenicity (Smith *et al.*, 2006). Interestingly, those virulence factors that are necessary for acute infection were found to be negatively selected against in isolates causing chronic infections. Isolates that were recovered from CF patients after a period of eight years were found to be genetically different from the initial, clonal *Pseudomonas aeruginosa* population, and were also different from natural wild-type isolates (Smith *et al.*, 2006). *In vivo* adaptation towards a susceptible host is an important feature of pathogens and may be stress-related, for example in reaction to inflammatory host responses (Brown *et al.*, 2006).

In several laboratory studies, clinical isolates of *Pseudomonas aeruginosa* have been found to display cross-kingdom pathogenicity by successfully infecting the plant species *Arabidopsis thaliana*, as well as tobacco, lettuce, the nematode *Caenorhabditis elegans*, and the insects *Drosophila melanogaster* and *Galleria mellonella* (Elrod & Braun, 1942; Rahme *et al.*, 1995, 1997, 2000; Mahajan-Miklos *et al.*, 1999; D'Argenio *et al.*, 2001). The observation that different *Arabidopsis* ecotypes display differential degrees of resistance to different *Pseudomonas aeruginosa* isolates has been interpreted to suggest that this microorganism may infect *Arabidopsis* under natural conditions (Rahme *et al.*, 1997).

Many bacterial cell-associated and secreted factors that play a role in *Pseudomonas aeruginosa* virulence have been identified. These include flagella and type IV pili, type III secretion systems, lipopolysaccharides, proteases, endotoxins and exotoxins, and the mucous exopolysaccharide alginate. The production of these virulence factors is regulated by environmental stimuli and by quorum-sensing cascades (Rahme *et al.*, 2000). Remarkably, several bacterial mutants have been identified that display reduced virulence in multiple cross-kingdom hosts. Bacterial mutants at the exotoxin A gene (a protein synthesis inhibitor), the phospholipase C gene (involved in phospholipid degradation) and the *gacA* gene (a transcriptional activator of effector genes) were found to display pathogenicity reduced below wild-type levels in mice as well as in *Arabidopsis* (Rahme *et al.*, 1995).

Similar studies with these and additional mutants in other cross-kingdom hosts have shown that a number of virulence factors promote virulence on multiple hosts (Rahme *et al.*, 2000). Although the relative severity of the effects of particular mutations does not always correspond among the different hosts, at least a subset of the existing virulence factors is required for full pathogenicity in all hosts (Rahme *et al.*, 2000).

It is not only *Pseudomonas aeruginosa* that behaves as a cross-kingdom pathogen in various laboratory infection models. A similar situation is true for the vertebrate bacterial pathogens *Staphylococcus aureus* and *Enterococcus faecalis*. Both pathogens have been reported also to infect *Arabidopsis* and *Caenorhabditis elegans* (Garsin *et al.*, 2001; Jha *et al.*, 2005; Prithiviraj *et al.*, 2005a, c). Although these pathogens can be regarded as cross-kingdom pathogens, they have rarely or never been reported to cause cross-kingdom infections in nature. Interestingly, however, the same regulatory mechanisms that lead to attenuated virulence in humans seem to play key roles in diminishing the deleterious effect of plant infections (van Baarlen *et al.*, 2007). The following examples, however, concern pathogens that have been reported to cause natural cross-kingdom infections.

The *Burkholderia cepacia* species complex

Bacteria from the genus *Burkholderia* (formerly classified as rRNA group II of *Pseudomonas*), of which *Burkholderia cepacia* is the type species, are Gram-negative rod-shaped bacteria that, like *Pseudomonas* spp., are commonly found in soil, water, and the plant rhizosphere (Parke & Gurian-Sherman, 2001). Members of the *Burkholderia* complex were first classified as *Pseudomonas* species. The *Burkholderia cepacia* complex consists of ten closely related species, or genomovars, that have been identified as pathogens of hosts belonging to three kingdoms: Plantae, Animalia and Fungi. The capacity of members of the *Burkholderia cepacia* complex to infect fungi and to compete with other plant-pathogenic bacteria has resulted in use of these bacteria in the biological control of plant diseases (Parke & Gurian-Sherman, 2001). The species complex is especially predominant in the group of culturable bacteria that occur in the plant rhizosphere, not only on the outside of plant roots but also internalized in root tissues, where they can influence plant growth in many ways. Some endophytic *Burkholderia* strains are even capable of fixing atmospheric nitrogen (Gillis *et al.*, 1995). Clinical *Burkholderia cepacia* isolates are most often derived from CF patients and from patients with suppressed or deficient immune responses (Govan & Deretic, 1996; Jones *et al.*, 2001).

Originally, *Burkholderia cepacia* was described as the causal agent of sour skin of onion, also known as onion rot. When a large collection of *Burkholderia cepacia* strains collected from soil, clinical sources and rotting onions was studied, clinical strains were not able to induce rot in onion slices. For the other isolates, regardless of their origin, pathogenicity towards onion was found to correlate with pectinolytic activity (Gonzalez *et al.*, 1997; Yohalem & Lorbeer, 1997). This activity is based on the production of an endopolygalacturonase that is essential for the maceration of onion tissue; *Burkholderia cepacia* isolates that are not pathogenic towards plants do not produce this enzyme (Gonzalez *et al.*, 1997). Apart from this virulence factor, *Burkholderia cepacia* is known to produce several metabolites that not only function as antibiotics (Parke & Gurian-Sherman, 2001), but also contribute to plant pathogenicity (Hu & Young, 1998).

Pathogenicity towards humans features the establishment of lung necrosis by endotoxin activity of bacterial lipopolysaccharide. This reaction is mediated by the induction of heightened TNF-α levels and the stimulation of inflammatory responses (Shaw *et al.*, 1995; Govan & Deretic, 1996; Jones *et al.*, 2001). Additional *Burkholderia cepacia* complex virulence factors contribute to pathogenic processes but are not essential for pathogenicity. These include factors inducing necrosis in lung tissue, including porins and *N*-acyl homoserine lactones (usually abbreviated to AHL based on acyl-homoserine-L-lactone). Also included are factors that promote survival and persistence during *in vivo* growth, for example amidase, a protein involved in amino acid metabolism (Baldwin *et al.*, 2004). AHL is involved in quorum sensing, the regulation of protease production, and endopolygalacturonase secretion (Aguilar *et al.*, 2003; see below). Some strains also produce hemolysins and phospholipase C, both of which contribute to disease development (Govan & Deretic, 1996).

Overall, there is no clear correlation between the origin of isolates and their pathogenicity towards animals or plants, and different strains of the same species may display different patterns of pathogenicity. In an experimental model using the nematode *Caenorhabditis elegans*, the pathogenicity of *Burkholderia cepacia* strains isolated from different origins (clinical, onion or environmental) was found to be strain-dependent, but species- (or genomovar-) independent (Cardona *et al.*, 2005). It is, therefore, not surprising that the genetic diversity of *Burkholderia cepacia* is extremely high and that many isolates are non-clonal (Parke & Gurian-Sherman, 2001). There is, however, one case report in which a strain colonizing a CF patient appeared to be indistinguishable from (but not necessarily identical to) a soil isolate (Govan & Vandamme, 1998). Interestingly, plant-pathogenic isolates as well as environmental isolates recovered from wheat and maize rhizospheres were all found to belong to genomovar group III, in which the most virulent human-pathogenic isolates are

also found (Jones *et al.*, 2001). It is thought that the presence of numerous insertion sequences and at least one pathogenicity island enable the exchange and recombination of virulence factors (Parke & Gurian-Sherman, 2001; Baldwin *et al.*, 2004). The extreme virulence of this group was found to be associated with the presence of so-called cable pili. The pili form dense structures surrounding the cells, which apparently generates a selective advantage and proliferative microbial expansion (Sun *et al.*, 1995).

It is possible that specific *Burkholderia cepacia* serotypes occur intracellularly in human epithelial cells and phagocytes, surviving host chemical attack and immune responses when present (Govan & Deretic, 1996). This possibility is supported by the ability of some strains to occur as endophytes. It was recently demonstrated that *Burkholderia* spp. bacteria may occur as intracellular symbionts in plant-pathogenic *Rhizopus*. In this fungus, the bacteria produce a polyketide metabolite, rhizoxin, that confers pathogenicity of the fungus to rice seedlings (Partida-Martinez & Hertweck, 2005). Moreover, a *Rhizopus microsporus* strain isolated as a pathogen from human tissue was also found to contain rhizoxin-producing intracellular bacteria (Partida-Martinez & Hertweck, 2005). This bacteria–fungus relationship may be seen as an extreme form of the horizontal transfer of metabolite-encoding genes, in which the fungus may also transfer or introduce bacteria into novel hosts or niches. Clearly, symbiotic relationships of pathogenic bacteria and other organisms may have important implications for host ranges and may thereby modulate the clinical impact of these bacteria.

Pathogenic species of the genus *Alternaria*

Alternaria spp. are cosmopolitan fungi that are commonly isolated from plants and soil. In addition, because *Alternaria* spp. are capable of degrading cellulose, they are also frequently found indoors, for instance on wallpaper. Although the fungus generally occurs as a soil-borne saprotroph, some species are plant pathogens that, collectively, cause various diseases in a wide range of host plants (Thomma, 2003). *Alternaria* spp. are also well-known post-harvest pathogens that infect stored food and feed. They have, moreover, emerged as opportunistic pathogens of humans, particularly of immuno-compromised individuals (Viviani *et al.*, 1986). Most isolates found as pathogens in humans belong to *Alternaria alternata* and *Alternaria infectoria*, with some cases also credibly attributed to *Alternaria tenuissima* and *Alternaria dianthicola*. *Alternaria* spp. are well known for the production of toxic secondary metabolites, some of which are powerful mycotoxins. These include alternariol, altertoxin, tenuazonic acid and AAL-toxin, some of which have been implicated in the development of cancers (Shier *et al.*, 1991). *Alternaria* is also implicated in respiratory diseases. Spores belonging to this genus are among the most common air-borne allergens and are a widely prevalent cause of asthma (Fung *et al.*, 2000), which has been attributed to eosinophil degranulation (Inoue *et al.*, 2005).

Two major features of *Alternaria* species are the production of melanin, especially in the spores, and the production of toxins. Several toxins have been identified that have severe effects on a narrow range of plant species serving as hosts to the fungus. These toxins are indispensable for disease (Wolpert *et al.*, 2002; Thomma, 2003). Most plant-pathogenic *Alternaria* species are variants (pathotypes) of *Alternaria alternata* (Nishimura & Kohmoto, 1983). Remarkably, it has been shown that host-specific toxin-producing *Alternaria alternata* strains, i.e. specialized pathogenic strains, carry small extra chromosomes that are absent in nonpathogenic strains (Akamatsu *et al.*, 1999; Hatta *et al.*, 2002). These chromosomes feature physical clusters of toxin genes that are required for pathogenicity but not for normal growth. Combined with the observation that fungal isolates pathogenic to a specific host do not form monophyletic groups, this suggests that these chromosomes are acquired through horizontal gene transfer (Kusaba & Tsuge, 1994; Tanaka *et al.*, 1999; Thomma, 2003).

The most intensively studied *Alternaria* toxin is AAL-toxin, produced by the tomato pathogen *Alternaria alternata* f.sp *lycopersici*. AAL-Toxin is an aminopentol ester that structurally resembles the mycotoxin fumonisin B1. The latter toxin was originally identified in *Fusarium verticillioides* cultures (formerly *Fusarium moniliforme*), but is also produced by AAL-toxin-producing *Alternaria alternata* isolates (Chen *et al.*, 1992). It is highly toxic to animals, causing, for example, fatal leukoencephalomalacia (or 'Moldy Corn Poisoning') in horses that have ingested *Fusarium*-contaminated grain (Abbas *et al*, 1994). Interestingly, fumonisin B1 is also selectively toxic to AAL-toxin-sensitive plant genotypes (Gilchrist *et al.*, 1992). Both toxins are potent inhibitors of sphingolipid (ceramide) biosynthesis (Abbas *et al*, 1994), leading to accumulation of sphingoid base precursors and to a depletion of complex sphingolipids, eventually resulting in the death of plant and animal cells (Abbas *et al.*, 1994; Wang *et al.*, 1996a, b). This cell death is reminiscent of apoptosis and, indeed, transgenic tomato plants expressing a viral antiapoptotic gene were protected against AAL-toxin-induced cell death and pathogen infection (Lincoln *et al.*, 2002). As discussed earlier, the use of the host suicide program is a general pathogenicity strategy of microbial pathogens. Apparently, some species enhance apoptosis. *Chlamydia trachomatis* does it the other way around: apoptosis can be successfully inhibited (Fischer *et al.*, 2004). By the destruction of several preapoptopic proteins the intracellular bacteria can most probably reproduce more successfully.

The genus *Aspergillus*: *Aspergillus fumigatus* and *Aspergillus flavus*

Aspergillus spp. are globally distributed, soil- and compost-inhabiting ascomycetous fungi. Although none of the species causes a directly contagious disease of warm-blooded animals, several species are considered virulent opportunistic pathogens. They have been found to cause disease (aspergillosis) in humans and many animal species. In immunocompetent humans, disease occurs in various forms, ranging from an allergic reaction in people with asthma, to localized colonization of a pre-existing lung cavity (giving rise to an aspergilloma or fungus ball), to infestation of the nasal sinuses or outer ear canal epithelium (giving rise to otitis externae). In immunocompromised patients, more severe and invasive conditions arise, ranging from catheter-associated peritonitis to localized invasive pulmonary infection and disseminated aspergillosis (Kwon-Chung & Bennett, 1992). The latter is an invasive infection that spreads through the bloodstream from the lungs to other parts of the body. Various tissues can be affected, including ears and eyes, the cardiovascular and urinary system, and the brain. The most important human pathogen in this genus, *Aspergillus fumigatus*, commonly occurs on compost, plant debris, bird feathers and droppings, and crops. Its conidia are regularly found in house dust and, especially in warm areas, can seasonally become common in indoor and outdoor air (Mullins *et al.*, 1976; Denning, 1998; Latgé, 1999; Nierman *et al.*, 2005; Rementeria *et al.*, 2005). One cubic metre of air commonly contains 1–10 conidia which, when inhaled by immunocompromised humans, may lead to disease and sometimes to local epidemics of disease (Hospenthal *et al.*, 1998). The mortality associated with invasive aspergillosis can be up to 60–80%.

Aspergillus pathogenicity is largely determined by proteins and secondary metabolites. *Aspergillus fumigatus* produces metabolites that have various effects, including inhibition of the mammalian cell cycle, induction of toxic shock, induction of tissue necrosis, and initiation of tremor. The most well-known *Aspergillus fumigatus* metabolites include helvolic acid, ribotoxin, restrictocin, fumagillin and gliotoxin. The last three of these metabolites have been shown to play a role in the suppression of the mammalian immune system (Tomee & Kauffman, 2000; Rementeria *et al.*, 2005).

Compared with *Aspergillus fumigatus*, the equally virulent but less common clinically encountered *Aspergillus flavus* has a wide host range that encompasses not only vertebrate animals but also insects and plants (St Leger *et al.*, 2000). It frequently causes infections in patients who are minimally or not at all immunocompromised, and it is particularly common in mycotic sinusitis (Iwen *et al.*, 1997), although it can also cause the full range of opportunistic aspergilloses in immunocompromised patients. *Aspergillus flavus* grows on corn and peanuts and produces aflatoxins that are extremely poisonous to humans. As may be expected from a cross-kingdom pathogen, genetic diversity in *Aspergillus flavus* is high, possibly because of the occurrence of cryptic sex in natural populations (Geiser *et al.*, 1998). This genetic diversity probably accounts for the production of a wide range of proteases and other hydrolases that potentially act as toxins (St Leger *et al.*, 1997). *Aspergillus flavus* secretes over 50 distinct proteins *in vitro*. This secretion shows some substrate specificity, but at least two dipeptidyl peptidases and an alkaline protease that may function as a virulence factor are secreted on different media (Rementeria *et al.*, 2005). In a variety of *Aspergillus flavus* isolates collected from human, insect and plant sources, production of proteases was significantly higher in strains isolated from humans and insects than in those isolated from plants. However, low protease production did not abolish the ability of plant-derived strains to infect insects (St Leger *et al.*, 2000).

As has been found in other microorganisms (Table 2), proteases appear to be important pathogenicity factors for *Aspergillus* species. A 33-kDa subtilisin-like serine protease from *Aspergillus flavus* and *Aspergillus fumigatus* that shows cross-reactivity with an IgG antibody displayed proteolytic activity when tested on lung polymers (Kolattukudy *et al.*, 1993; St Leger *et al.*, 1993). Nitrosoguanidine mutants of the gene encoding the 33-kDa protease were significantly less able than the wild type to kill mice, although the mutagenization method used can also generate undetectable secondary mutations explaining the differential virulence levels (Kolattukudy *et al.*, 2000). The exact contribution of specific proteases to the establishment of disease has been a matter of debate because deletion mutants have not yet been tested, and because the presence of the genes encoding these proteins has not always been correlated with pathogenicity (discussed in Holden *et al.*, 1994; Tomee & Kauffman, 2000). In the various *Aspergillus flavus* isolates collected from humans, insects and plants, nearly all isolates were shown to produce pectinases, and gene-replacement mutants for pectinase genes were found not to be infectious on plants (St Leger *et al.*, 2000). This is in line with earlier experiments showing that pectinase activity, together with other factors, contributes to *Aspergillus flavus* pathogenicity on cotton (Shieh *et al.*, 1997). Regardless of source, then, *Aspergillus flavus* isolates appear to contain factors active in plant pathogenicity as well as important factors operative in animal pathogenicity.

It should be noted that *Aspergillus* spp. as mammalian and avian pathogens are not often found sporulating on or in the living host (with fungus balls in lung cavities and outer ear canal infestations as exceptions). However, they are among the first fungi to grow on corpses (Okudaira *et al.*,

1977) and may thus evolutionarily reinforce their pathogenicity towards animals by establishing themselves while the animals are in relatively good health and then growing aggressively when these hosts become weakened and their health deteriorates drastically, finally sporulating after host death.

Pathogenicity of *Rhizopus* spp. (zygomycetes)

The *Zygomycota* (previously known as zygomycetes) are a phylum of fungi that contain the orders *Mucorales*, *Mortierellales*, and *Entomophthorales*. Whereas *Entomophthorales* were originally identified as insect pathogens, several zygomycete species have been identified as pathogens of animals and plants. The most well-known pathogenic species are from the genus *Rhizopus* (Mucorales). Mucoralean species occur in soil, vegetable debris, manure and compost, but also occur indoors ('black bread mold'). In humans, they cause various types of zygomycoses (also referred to as mucormycoses). In immunocompetent individuals, zygomycoses mostly occur as dermal infections, especially in connection with open wounds or burns or similar lesions treated with unsterile bandages, but also as noninvasive nasal sinus infections. In immunocompromised patients, infections can occur internally and can arise from inhaled or ingested inoculum. The main categories of human zygomycosis in compromised patients are pulmonary, rhinocerebral, gastrointestinal, cutaneous and disseminated infection (Ribes *et al*., 2000). The secretion of proteolytic enzymes plays an important role during entry and invasive growth of *Rhizopus* in human tissues (Odds, 1991). Crude fungal extracts of *Rhizopus* have been shown to contain mycotoxins (Brook & White, 1966), although, as with a number of other important mycotoxins (Gupta *et al*., 2000), these have not yet been extensively investigated for their role in human infections. It is interesting to note that the combination of being black (i.e. producing melanin) and being frequently isolated from rotting plant material is quite common among opportunistic fungi (De Hoog *et al*., 2000b). A variety of such species have been identified, and it has been demonstrated that the biological diversity of the environmental isolates far exceeds that of clinical isolates. In combination with the somewhat cumbersome phylogenetic classification of such black fungi this suggests that only specific lineages of the environmental isolates may be capable of causing human infections. What the specific disease-invoking attributes of such strains are is currently not known but is certainly worth further investigation.

The pathogenicity of *Absidia corymbifera* (also *Mucorales*, sometimes referred to by the outdated name *Absidia ramosa*) and *Rhizopus* species towards plants has been shown to be mediated by the secretion of several proteins and metabolites, including auxin (indole acetic acid, IAA) (Gruen, 1959). Auxins are plant growth hormones that play a role in root and shoot development. Few microbial pathogens can produce auxins. Their role in pathogenesis is not restricted to the induction of gall and callus formation; rather, IAA is also implicated in the suppression of plant defence (Shinshi *et al*., 1987). Remarkably, IAA induces adhesion and filamentation of *Saccharomyces cerevisiae*, triggering differentiation into an invasive form (Prusty *et al*., 2004), as may occur in human opportunistic infections caused by certain strains of this organism. It is presently not known whether such an effect plays a role during the infection process of plant-pathogenic auxin-producing fungi.

Toxic metabolite production is also important for plant infection. Several *Rhizopus* species cause almond hull rot disease. These species produce fumaric acid and other toxins, causing leaf and twig necrosis distant from the actual infection site and without the presence of fungal structures (Woltz, 1978).

A completely different type of pathogenicity factor employed by zygomycetes is the utilization of iron-scavenging siderophores (Holzberg & Artis, 1983). Dialysis patients who received iron chelators often developed zygomycoses, and it has been demonstrated that zygomycetes are able to enhance their growth by utilizing iron bound to chelators (Boelaert *et al*., 1993, 1994).

Will more pathogens evolve cross-kingdom host jumps?

The discussion of whether or not more pathogenic microorganisms will ultimately be capable of cross-kingdom host jumps is not a trivial one. For many microorganisms, information on their natural habitats and native ecology is scarce, and information on the identity and distribution of host species may be scarce as well. Moreover, differences in terminology and diagnostics may have obscured certain aspects of host–pathogen relationships. Pathogenic microorganisms displaying cross-kingdom host ranges may be overlooked owing to taxonomical issues and the use of different species names for the same microorganism. For example, a specific subgroup of the plant-pathogenic *Erwinia* spp. produce a yellow pigmentation composed of carotenoids. This so-called *herbicola-lathyri* subgroup contains saprotrophic, epiphytic and weakly pathogenic plant-associated bacteria, but also *Erwinia* strains that have been isolated from animals and humans (Lind & Ursing, 1986; Beji *et al*., 1988). However, carotenoid-producing and related nonpigmented clinical *Erwinia* strains (Schneierson & Bottone, 1973) have traditionally been named *Enterobacter*. As a result, strains belonging to the same pathogenic taxon are now known under different species or even genus names, such as *Erwinia herbicola* or *Enterobacter*

agglomerans, depending on their plant or animal origin (Starr & Chatterjee, 1972; Schneierson & Bottone, 1973). The often completely separate developments of plant microbiology and animal microbiology are likely to have engendered more of these taxonomical disguises.

Furthermore, cross-kingdom pathogens may not have been recognized because given biovars of certain pathogenic microorganisms may simply be uncultivable on the currently available (semi)synthetic growth media. An example of this is *Madurella mycetomatis*, a member of the ascomycetes that causes mycetoma, a disease of humans in the Tropics that is particularly prevalent in the so-called mycetoma belt (between latitudes 15° south and 30° north) (Ahmed *et al.*, 2004). The infection is characterized by massive swelling and tumour formation, especially in the extremities. The source of the infectious *Madurella mycetomatis* strains, which are primarily clonal (Ahmed *et al.*, 2003b), has long been enigmatic, and a search for the ecological niche was only possible after the development of molecular diagnostic tools for the species (Ahmed *et al.*, 2003a). Despite the fact that the strains could not be cultured from soil and plant parts, these diagnostic tools very clearly indicated the presence of *Madurella mycetomatis* in the environment in soil samples and thorns from acacia trees (Ahmed *et al.*, 2002). Apparently, this fungus has to be cycled through a mammalian host prior to its being cultivable on the currently available synthetic culture media.

The list of cross-kingdom pathogens may also expand because humans are nowadays more often exposed to potential cross-kingdom pathogens. The contact of humans with different microbial species has increased substantially over recent decades for several reasons, including changes in dietary habits, long-distance transport of produce, methods of fruit and vegetable production, changes in food preparation, and, last but not least, enhanced intensity of human travelling. As a result of global trade and international travel, human populations and local environments are brought into close contact with pathogenic species to which they had never been previously exposed.

A trend that can be observed from the cross-kingdom pathogens listed in Table 1 is that nearly all cross-kingdom pathogens originate from terrestrial ecosystems that contain a very rich microbial community with all possible interactions (Whipps, 2001). One such ecosystem is the rhizosphere, the layer of soil that is influenced by the metabolism of plant roots. It has been shown that, within the rhizosphere, horizontal gene transfer can and does occur frequently, facilitating rapid changes in bacterial and fungal genomes and the exchange of specific traits, including the transfer of pathogenicity islands (Troxler *et al.*, 1997; Van Elsas *et al.*, 2003; Nakamura *et al.*, 2004). With regard to attachment, the production of a specific adhesin, HecA, has been shown to be required by the plant-pathogenic bacterial species *Erwinia chrysanthemi* for full virulence on tobacco plants (Rojas *et al.*, 2002). Homologues of the encoding gene were found to occur in many plant as well as animal microbial pathogens. When the phylogenetic relationships of genes homologous with HecA are examined for different bacterial species, they do not correlate with the phylogenetic history of the bacterial species themselves; therefore, horizontal gene transfer is thought to have contributed to the dispersal of this gene (Rojas *et al.*, 2002). Virulence as a genetic trait thus may be promoted and disseminated in such soil habitats. For horizontal gene transfer to occur at an ecologically significant probability level, frequent close contact between microorganisms is required. Therefore, bacterial biofilms are also known as hot spots for horizontal gene transfer (Molin & Tolker-Nielsen, 2003). Microorganisms co-occurring in a biofilm are likely to exchange particular traits (Sørensen *et al.*, 2005). Selection for pathogenicity, as may occur when various genotypes of a marginally pathogenic organism are inhaled by a human being, will tend to promote the numerical increase of those microorganisms that can express compatible pathogenicity factors and also successfully reproduce in the context of pathogenicity or pathogenicity-induced mortality. Pathogenicity as a regular feature of any microbial life cycle will become fixed when it contributes to the evolutionary success of the individual or the clonal lineage. All potential novel sources and introductions of microorganisms and increasing contacts and interactions will increase the probability that new microorganisms will develop into human pathogens. The increasing number of of immunocompromised people (Denning, 1998) is a factor that may significantly contribute to this effect. In association with, for instance, the AIDS pandemic, a variety of opportunists never seen before in clinical settings will become increasingly visible over the coming decades.

In light of an ongoing diversification of pathogenicity factors, it is not surprising that occasionally some pathogenic microorganisms may also undergo a change of host range. There are a few examples in the literature in which such tendencies may be seen. For instance, *Brucella suis* occurs worldwide as a pathogen of domestic and wild animals (Boschiroli *et al.*, 2001). Recently, the genome sequence of *Brucella suis* was determined and was found to display a remarkable similarity to that of plant-associated *Rhizobiaceae*, such as the plant-pathogenic bacterium *Agrobacterium tumefaciens* and the plant symbionts *Sinorhizobium meliloti* and *Metarhizobium loti* (Paulsen *et al.*, 2002). Again, full genome sequencing will reveal a plethora of novel genes present in a variety of organisms, and many of these will be involved in pathogenicity mechanisms. Furthermore, web-based catalogues of experimentally verified pathogenicity and virulence genes, such as PHI-base for fungal and

oomycete animal and plant pathogens, will further facilitate the identification of pathogenicity mechanisms (Baldwin *et al.*, 2006; Winnenburg *et al.*, 2006).

Interestingly, *Agrobacterium tumefaciens* has been recorded as a pathogen of humans, although mainly in immunocompromised patients (Paphitou & Rolston, 2003). The similarity at the genome level between *Brucella* and plant-associated bacteria has led to the speculation that *Brucella* evolved from soil- or plant-associated ancestral bacteria and that it may still be able to grow in the environment outside mammalian hosts (Paulsen *et al.*, 2002). However, no pathogenicity of *Brucella* towards plants has been documented, suggesting that pathogenicity towards plants may have been lost during the course of evolution. On the other hand, *Brucella* may be developing factors that favour (regaining) plant pathogenicity. Several virulence factors have been identified in *Brucella* whose counterparts in *Rhizobiaceae* are required for endosymbiosis or pathogenesis in plants (Tsolis, 2002). *Agrobacterium tumefaciens* bacteria deliver virulence factors into plant cells using a type IV secretion mechanism, parts of which are also found in *Brucella* spp. (Christie *et al.*, 2005). *Agrobacterium tumefaciens* and *Burkholderia cepacia* genomovar III, the genomovar accounting for the largest proportion of the species' pathogenic isolates, share a VirB/D4 type IV translocation system, whereas *Brucella* spp. lack the VirD4 protein. VirD4 is essential for DNA transfer to plants and functions as a coupler of the bacterial transport pore, bacterial DNA, and virulence proteins during plant infection (Kumar & Das, 2002; Christie *et al.*, 2005). The complex and highly variable genome composition of *Brucella* spp. shows features of large-scale rearrangements, genetic islands, insertion sequences and host-specificity genes (Boschiroli *et al.*, 2001; Paulsen *et al.*, 2002). Such genome characteristics are signatures of taxa capable of horizontal gene transfer. It is therefore reasonable to assume that horizontal gene transfer to *Brucella* has occurred in the past and might eventually lead to the acquisition of factors conferring the capacity to infect plants. For example, if *Brucella* were to acquire VirD4-like virulence factors, it might be able to extend its host range to plants. The phylogenetic distance of *Brucella* to *Agrobacterium* and *Burkholderia*, based on 16 rRNA gene sequences, is relatively large (Fig. 6). However, these taxa are remarkably similar with respect to their secretion systems (Christie *et al.*, 2005). In addition, genes involved in virulence and polysaccharide biosynthesis can complement each other across these three genera (Boschiroli *et al.*, 2001; Paulsen *et al.*, 2002; Tsolis, 2002). The latter features are characteristic of horizontal gene transfer. Full-genome sequencing and the use of metagenomic technologies (Tringe & Rubin, 2005) will further enhance our insight into the nature of the microbial gene pool in general.

Fungi may also be expected to develop cross-kingdom pathogenicity. As discussed above, *Aspergillus flavus* has a cross-kingdom host range while *Aspergillus fumigatus* apparently does not. Nevertheless, certain properties mark *Aspergillus fumigatus* as a potential cross-kingdom pathogen. Although the fungus displays optimal conidial germination and capacity for invasive growth ranging from *c.* 37 to 42 °C, it also grows well at lower temperatures, although its growth rate is reduced. Furthermore, the fungus occurs worldwide in composting plant debris and on plant surfaces (Hospenthal *et al.*, 1998). Interestingly, *Aspergillus fumigatus* may infect and display invasive growth in germinating *Vigna mungo* seedlings after application of a specific phytotoxin, namely phaseolinone, interfering with plant defence responses. This metabolite is produced by the plant pathogen *Macrophomina phaseolina* (Sett *et al.*, 2000). During growth of saprotrophs on infected plants, pathogenicity traits may be acquired on rare occasions from another organism, for example through horizontal transfer of a phytotoxin biosynthesis gene cluster. This scenario is similar to what has probably occurred for plant-pathogenic *Alternaria alternata* strains and in *Cochliobolus* species (Wolpert *et al.*, 2002; Thomma, 2003). The global distribution of *Aspergillus fumigatus* and its capacity for toxin and protease production coupled with its frequent occurrence in the soil and its possible cryptic sexual reproduction may enable it eventually to broaden its pathogenic spectrum.

Now that we have defined a number of requirements for such jumps to take place, can we predict which types of pathogenic microorganisms are the most likely to develop cross-kingdom host jumps? One obvious speculation would be that pathogenic microorganisms that currently have a broad host range are more likely than highly specialized organisms to become pathogenic on cross-kingdom hosts. Pathogenic microorganisms with narrow host ranges are more likely evolutionarily to optimize the intimate relationship that they have already established with their existing hosts and their descendants. Ongoing evolution will inevitably lead to host shifts for some pathogenic microorganisms, and some shifts will occur on cross-kingdom hosts.

Acknowledgements

B.P.H.J.T. is supported by a VENI grant from the Research Council for Earth and Life sciences (ALW) and by the Centre for BioSystems Genomics (CBSG), both of which are part of the Netherlands Organization for Scientific Research (NWO). P.v.B. is supported by the Technology Foundation (STW), also part of NWO. Work in the laboratory of A.v.B. is supported by grants from the Dutch Ministry of Economic Affairs (BTS and STW), the Human Frontier Science Program, EU FP6, Top Institute Pharma, Erasmus Trustfund and the Dutch Maag, Lever en Darm Stichting. We would

like to thank Dr M.D. Bolton and Dr A.J. Debets for valuable suggestions. All authors declare a lack of conflict of interest.

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