Invertebrates as a study model of anaerobic infections

Nevretenčarski modeli za proučevanje anaerobnih infekcij

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Abstract: Experiments with invertebrates have recently gained increased attention as a practicable substitute to traditional mammalian models in the study of host-bacterial interactions. Using an invertebrate study model has a number of advantages over traditional mammalian model including simple growth condition, short life-time, can be easily maintained, infected without anesthesia and with a smaller extent of ethical limitations. From a microbiological viewpoint, importance of anaerobic bacteria as agents for various diseases remains an interesting field for research. The study of the interaction between invertebrate model host and anaerobic bacteria therefore provides insights into the mechanisms underlying pathogen virulence and host immunity and complements or even compensates the use of mammalian model in assay for infectious disease. This review offers to consider about the appropriate invertebrate model select for the study of particular aspects of anaerobic bacterial pathogenesis.

Keywords: invertebrate model, anaerobic bacteria, virulence factors, disease


Ključne besede: nevretenčarski modeli, anaerobne bakterije, virulenčni dejavniki, bolezen
Introduction

The molecular basis of the pathogenicity of infectious agents, and of the corresponding mechanisms of host defence can be studied using model systems (Couillault and Ewbank 2002). There is a continuing need for the development of a simple animal model for the study of host pathogen interactions (Finlay 1999).

A number of different invertebrate host model systems have been described in the past few years that allow multidisciplinary studies of host–bacterial interactions from the perspectives of both the pathogen and the host. Consequently, many researchers have turned to invertebrates as effortless, practicable, simple, and inexpensive hosts to model a variety of human infectious diseases. It is important to select the model host that is best suited for testing a specific hypothesis (Mylonakis et al. 2007) including ethical, procedural and financial characteristics.

A number of different model systems, including amoeba, nematodes, crustaceans and insects, have been introduced, and it was observed that different bacteria responded in different ways to presumptive alternate hosts, and specific model systems might be more or less advisable for a defined pathogen (Ott et al. 2012). Aerobic and obligate anaerobic bacteria are successfully isolated but to isolate anaerobic bacteria from invertebrate models is often impracticable (Bergan 1984), even though, anaerobic bacteria in invertebrates cannot be excluded. The subsequent isolation of strictly anaerobic bacteria resulting in anaerobic micro niches within an oxic environment. This finding of anaerobic micro niches presents microhabitat of various microbes, despite the fact of its obviously inappropriate environment (König 2006).

This review presents five model hosts, the amoeba Acanthamoeba polyphaga, the nematode Caenorhabditis elegans (C. elegans), the fruit fly Drosophila melanogaster (D. melanogaster), the greater wax moth Galleria mellonella (G. mellonella) and the isopod Porcellio scaber (P. scaber). Predominant empirical advantages of each model are well developed genetic, biochemical and biological functions, the precision evaluation of an assay, conserved innate immune response, handling experiments at 37°C and ease of inoculation of an explicit amount of pathogen (Borner 2016).

Anaerobic bacteria are significant clinical pathogens

Anaerobes and their pathogenicity factors can affect common hosts and hosts with compromised resistance of harmed tissue. Their complex metabolism, the capability to produce pathogenicity components like extracellular toxins, superoxide dismutase, catalase, the abscess inducing capsular polysaccharide, proteases, lipases, heparinase, nucleases hyaluronidase, haemolysin, lipolysin, and neuraminidase, enzymes inactivating antibiotics, and resistance against phagocytosis, are responsible for local and systemic expansion of the endogenous bacterial infection during antimicrobial therapy (Bergan 1984, Brook 2011, Dorer and Isberg 2006). Avoidance and early healing treatment of circumstances that can lead to anaerobic infection can reduce their amount.

Anaerobic bacteria are found on the skin, on mucosal surfaces, in the mouth, pharynx and intestinal tract or genital tract as a part of the normal microbiota. Additionally, anaerobes can be isolated in all types of anaerobic infection including respiratory infection, subcutaneous and soft-tissue infections, endogenous infections in the central nervous system, oral cavity, head and neck, chest, abdomen, pelvis, skin, and soft tissue (Brook 2016). Infections results when anaerobes and other bacteria of the normal flora weaken and deceive immune system to avoid detection (Borner 2016), or permeate integumentary barriers. The infections are often polymicrobial, with other anaerobes, facultative anaerobes, and aerobes (Brook 2016). Several important diseases, botulism, tetanus, gas gangrene, food poisoning, and pseudomembranous colitis are caused by anaerobic Clostridium species from the environment or from normal flora (Brook 2016, Brooks et al. 2010). Their individual pathogenicity factors serum-independent chemotactic factors that attract polymorphonuclear cells, superoxide dismutase, catalase, capsular structures, proteases, lipases, heparinase, and nucleases, exotoxins of histotoxic clostridia and the ability of different anaerobes to produce enzymes inactivating antibiotics has become well established. Pathogenicity factors like hyaluronidase, haemolysin, lipolysin, and neuraminidase have been isolated (Brooks et al. 2010). Inspite of all that, the importance of
other virulence factors that may contribute to the pathogenicity of the anaerobic bacteria remains unclear (Brooks et al. 2010, Harding et al. 2013).

Important anaerobes that may cause human infection and/or are isolated in polymicrobial 
an aerobic infections are: i. Gram-negative bacilli Bacteroides spp., Prevotella spp., Porphyromonas 
spp., Fusobacterium spp., Bilophila spp. and Sutterella spp., ii. Gram-negative cocci mainly 
Veillonella spp. (Brook, 2011), iii. Gram-positive cocci Peptostreptococcus spp., Anaerococcus 
spp., Finegoldia spp., Parvimonas spp., and Peptoniphilus spp. (Murphy and Frick, 2013), iv. Gram-positive spore forming Clostridium spp., and no spore-forming bacilli Actinomyces 
spp., Propionibacterium spp., Eubacterium spp., Lactobacillus spp., Bifidobacterium spp., Egg-
erthella spp., Arachnida spp. (Brook 2011). The isolation and identification of anaerobic bacteria 
associated with specific infection are important as well as characterization of their virulence factors 
(Brook 2016, Murphy and Frick 2013).

Invertebrate as a model host for 
studying virulence of anaerobic bacteria

Invertebrate models have gained increased 
attention as a viable alternative to traditional 
mammalian models of infection (Mowlds et al. 
2008, Renwick et al. 2006) and are increasingly 
being used to study a number of important hu-
man pathogens. Using invertebrate models 
have number of advantages over traditional 
mammalian models, as invertebrates are not 
subject to the ethical limitations of mammalian 
models (Harding et al. 2013). No invertebrate 
model hosts reproduces all aspects of mammalian 
infection and any particular invertebrate is likely 
to have specific advantages. The selection of a 
model system for studying virulence of anaero-
bic bacteria is largely dependent on the specific 
pathogen virulence related factors, the specific 
host innate immune responses of interest, and 
the scientific question asked. If the goal is to 
study innate immune responses, the choice most 
likely will require the selection of a multicellular 
model genetic organism such as D. melanogaster 
or C. elegans. If the goal is to study phagocytosis 
and/or the outcome of ingestion, the choices 
include unicellular organisms such as amoebae 
and slime mold or invertebrates such as insects 
with phagocytic cells (Mylonakis et al. 2007). If 
the goal is to study gut microbe homeostasis and 
gut infection by the human pathogen anaerobic 
bacteria, a model with increasing evidence for a 
reciprocal relationship between beneficial and 
pathogenic bacteria in the gut and the intestinal 
immune system with suitable environment for 
developing of resident and anaerobic microbiota 
must be found and practiced (Glavis-Bloom et 
al. 2012).

Manipulating with alimentary, physiological 
and behavioral characteristics of different inver-
tebrate models might play an important role with 
an optimal adaptation of anaerobic bacteria to 
invertebrate’s environment through a completely 
different mechanism of interactions ranging from 
pathogenesis to obligate mutualism.

Acanthamoeba polyphaga as a study model for 
an aerobic bacteria

Protozoa are frequently used in laboratories 
as experimental organisms for studies of cell 
locomotion (Amoeba proteus), nonmuscle con-
tractile systems (Acanthamoeba), and the effects 
of removing and transplanting nuclei (Brusca and 
Brusca 2004).

Amoebae species are well established model 
systems for a number of pathogenic bacteria. 
Amoebae have been used as model organism to 
study the pathogenicity of bacterial strain, such as 
Pseudomonas aeruginosa (Pukatzki et al. 2002), 
as a biological tool for isolation of several amoeba-
resisting intracellular microorganisms (Adekambi 
et al. 2004, Greub et al., 2004, La Scola et al. 
2004) but it has not been an appropriate study 
model for anaerobic bacteria yet. Clostridium 
frigidicarnis was demonstrated to be lytic for 
amoebae (Pagnier et al. 2008).

Caenorhabditis elegans as a study model for 
an aerobic bacteria

The soil-living small size nematode C. elegans 
with rapid life cycle and transparent body, fully 
sequenced genome, and physiological and anatomical 
simplicity is a model host with excellent po-
tential for studying cell biology and pathogenicity
(Brusca and Brusca 2004, Glavis-Bloom et al. 2012). Both, aerobic and anaerobic metabolic pathways are found and worm is able to switch from one pathway to the other according to environmental oxygen concentrations. Facultative anaerobiosis is evidently meaningful in parasitic nematodes and those that live in additional anoxic environments (Brusca and Brusca 2004).

Bacteria that infect C. elegans are both Gram-negative and Gram-positive bacteria. *Salmonella typhimurium, Aeromonas hydrophila, Pseudomonas aeruginosa, Burkholderia pseudomallei, Burkholderia cepacia, Burkholderia cenocepacia, Yersinia pestis, Yersinia pseudotuberculosis* are Gram-negative bacteria that infect C. elegans and *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Microbacterium nematophilum, Enterococcus faecalis, Bacillus thuringiensis* are Gram-positive bacteria (Borner 2016).

Practical advantages, the particular bioinformatics approach and biological processes have increased the use of *C. elegans* in toxicological studies (Boyd et al. 2012). *C. elegans* is killed by many pathogens and many virulence factors produced by *pathogens* that contribute to the pathogenicity in humans have been shown to be important for disease in *C. elegans*, including persistent infection of the intestine, colonisation with biofilm formation on the worm cuticle, and killing by botulinum toxin, hydrogen cyanide or hydrogen peroxide (Kaletta and Hengartner 2006).

The genetically tractable nematode *C. elegans* has been extensively used to study bacterial virulence and offers many advantages (Mahajan-Miklos et al. 1999) as a convenient host for studies of pathogen infections (Ballal and Troemel 2013). This small hermaphroditic animal has been the object of intense study for more than 20 years (Brillard et al. 1999) as a convenient host for studies of pathogen virulence mechanisms (Boyd et al. 2012). Notable research was made in 2014, where worms treated with botulinum toxin A of *Clostridium botulinum* showed slight paralysis and the toxin treatment resulted in the increase of yolk protein concentration in embryos (Kim et al. 2014). *C. elegans* has become an assisting model to probe vital biological and physiological processes and molecular mechanisms involved in many human diseases. It has served as a model for Parkinson’s, Alzheimer’s and Huntington’s disease, diabetes, cancer, immune disorders, and the development and testing of therapeutics agents (Bier and McGinnis 2008, Wilson-Sanders 2011).

*C. elegans* may subsequently be the model of anaerobic microbial processes and toxicity.

Introducing bacteria to worms is quite simple, but laboratory standing conditions differ with the conditions in its natural soil habitat. The nematode absences a variety of mammalian anatomical structures and the reproductive fitness of *C. elegans* strongly depends on the effects of air composition, habitat structure, and bacterial food availability. Owing to these not all diseases and immune responses can be testified (Glavis-Bloom et al. 2012) or have to be carefully interpreted by concern to their natural consequences (Freyth et al. 2010).

**Galleria mellonella as a study model for anaerobic bacteria**

Unique advantages of *G. mellonella* as a model host for studying pathogen virulence mechanisms and the efficiency of potential antimicrobial compounds are its ability to survive at 37°C when studying pathogenic temperature-sensitive virulence and production of microbial toxins. *G. mellonella* can be stored at room temperature,
likewise is straightforwardly and practically obtained in sizes large enough to be inoculated by pathogens (Glavis-Bloom et al. 2012). A reliable and inexpensive experimental infection model is convenient to differentiate between virulent and non-virulent isolates, for the identification of presumed virulence genes through comparative genomics studies and the identification of novel molecular targets for antimicrobial therapy and vaccine development. G. mellonella was recently established as a suitable host model to study the pathogenesis of bacterial and yeast species causing diseases in humans, such as Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus, Cryptococcus neoformans, and Candida albicans (Altincicek et al. 2012). Conversely, G. mellonella cannot replace well-established and more physiological in vivo experimental vertebrate models in the assessment of pathogenic mechanisms associated human diseases (Giannouli et al. 2014, Glavis-Bloom et al. 2012).

Drosophila melanogaster as a study model for anaerobic bacteria

The fruit fly D. melanogaster is an excellent model organism to research intestinal homeostasis, the gut microbiota, pathways that regulate intestinal stem cell signaling, innate immune reactions and disease. About 20 diverse species, including main dominant species of the genus Acetobacter and Lactobacillus and with few anaerobes were found in the intestine of D. melanogaster. No noteworthy anaerobic bacteria had been found in the D. melanogaster gastrointestinal tract (Charroux and Royet 2012) before the anaerobe Clostridium perfringens was isolated. The promoting effect on the growth and development of D. melanogaster were detected (Wei et al. 2016). It is a primary organism used in developing biology (Wilson-Sanders 2011) considering that D. melanogaster indirect-flight-muscle actin was ADP-ribosylated by Clostridium botulinum C2 toxin and Clostridium perfringens iota toxin (Just et al. 1993).

C. perfringens is the most frequently isolated histotoxic clostridia and produces several necrotizing extracellular toxins correlated with tissue necrosis, hemolytic anemia and renal failure and C. botulinum infections can result in intestinal toxemia, food poisoning and wound infections containing a highly potent neurotoxin (Brook 2016). Appropriate management of D. melanogaster for identifying and understanding anaerobes that are presented in human diseases could contribute to biological discoveries.

Drosophila served as a model for host-parasite relationships, is established model for the study of neoplastic diseases (Tipping and Perrimon 2014, Wang et al. 2014, Wilson-Sanders 2011), has been used to study cellular defenses against fungal pathogens (Arvanitis et al. 2013, Fuchs and Mylonakis 2006), it has been proven as excellent system for studying the normal function of human genes and pathways linked to neurodegenerative diseases (Allan et al. 2014, Liang et al. 2013, Mhatre et al. 2013), and is a simple model organism for studying diseases caused by viruses (Bier and Guichard 2012, Panayidou et al. 2014).

Porcellio scaber as a study model for anaerobic bacteria

The terrestrial crustacean, soil dwelling isopod Porcellio scaber is likely to be dependant on microorganisms associated with the gut (Kostanjšek
et al. 2004, Wang et al. 2004, Zimmer 2002). The digestive tract is complete with a well-developed, cuticle-lined, stomodeal foregut and proctodeal hindgut, connected by an entodermally derived midgut. A characteristic feature is a permeable peritrophic membrane to protect the delicate midgut epithelium from abrasion (Bier and McGinnis 2008).

Terrestrial isopods have an inherent and multiplicity gut microflora (Drobne 1995). Therefore, slight changes in the animal fitness might have effects on the microbial community in the intestinal tract (Guarnier and Malagelada 2003; Loker et al. 2004). Due to their significant environmental aspect and their complete digestive tract a considerable amount of experimentation was focused on *P. scaber*. Anaerobic bacteria from *P. scaber* hindgut were identified. Further, obligate anaerobic bacteria of genus *Bacteroides* and *Enterococcus* species were isolated. Additionally, bacteria from the genus *Desulfotomaculum* were isolated from gut wall and cultivated under anaerobic conditions (Kostanjšek et al. 2004). Bacteria from the genus *Desulfotomaculum* were isolated from gut wall and cultivated under anaerobic conditions (Kostanjšek et al. 2004). Nothing can be concluded about the changed structure or function of the entire gut bacterial community. Gut microflora toxicity studies are a promising way to get applicable facts on terrestrial environments.

**Conclusion**

Importance of invertebrates as reservoirs of multihost pathogens often plays a crucial role of various infections. The invertebrates appeared for a long time to be an unsuitable environment for growth of anaerobic bacteria. The finding of anaerobic bacteria reveals that a unique microbial environment remains an interesting field for further microbiological research (König 2006) focusing on genotoxicity and other virulence factors, inflammation, host defences modulation, and bacterial derived metabolism. A better understanding of the interactions between the invertebrate host and anaerobe pathogenicity depends on further functional studies and findings in almost every area of biology and medicine (Gagniere et al. 2016).

A very few anaerobic bacteria have been isolated in only two invertebrate models, *D. melanogaster* and *P. scaber*. Although anaerobic bacteria are unimpressive found in gastrointestinal tract of invertebrates, inflammatory diseases of the intestine arise from imbalanced interactions between the host gut epithelia and resident or ingested anaerobic microbes. Developing *in vivo* disease models with well characterized development and simple immunity (Chamilos et al. 2007) can help to explicate the basic mechanism underlying disease (Mhatre et al. 2013) start with a similary important pathogenic role and life-threatenig infections in invertebrates. The intestinal niche is also challenged continuously by numerous environmentally derived bacteria because of its exposed anatomy that is accessible to the external environment (Lee and Lee 2014).

Invertebrate model hosts represent valuable tools for the study of host-pathogen interactions because they facilitate the identification of bacterial virulence factors and allow the discovery of novel components involved in host innate immune responses (Miyata et al. 2003, König 2006). As well as facilitating the identification and study of virulence mechanisms (Mahajan-Miklos et al. 2000), simple model system may also permit direct genetic approaches for the study of host defenses (Ewbank 2002). The finding that diverse bacteria are pathogenic to invertebrate models opens the prospect of using this experimentally simple model to identify genes that are necessary not only for pathogenesis in study model but also for virulence or symbiosis in other hosts (Aballay et al. 2000, König 2006).

To study the pathogenesis in mammalian models is complicated by difficulties of handling, long reproductive cycles, small brood sizes, physiological and anatomical complexity, regulatory requirements, high cost, and ethical considerations. Workers in the field of pathogenesis have the opportunity to select from several invertebrate animal model systems in their studies. An understanding of the unique strengths and limitations associated with each model host is necessary, as particular virulence characteristics are not equally important in all systems and genetic tractability is not available in all model hosts (Mylonakis et al. 2007) and especially when a presence of the anaerobic pathogens is looking for. A better model...
systems may be identified and fully characterized in the future, like feeding-based infections, to use pathogens that invade the luminal side of epithelial cells (Balla and Troemel 2013) or to study systemic infections by microbial injection into the hemolymph (Panayidou et al. 2014). By using and trying different experimental techniques and protocol details, observed the progression of infection in real-time by light microscopy, by fluorescence microscopy and by electron microscopy (Shu et al. 2011) and with well-established genetic, molecular and biochemical analyses of invertebrate animal models, a research model to facilitate maintenance of virulence by anaerobic bacteria could be created.

Genetic screening, the RNAi technique in the genetically tractable invertebrate model organisms have been proved to be a powerful and valuable tool for understanding of fundamental principles of bacterial resistance to infection and may be useful in screening for potential neurotoxicity (Abnave et al. 2015, Altincicek et al. 2007). Inflammatory diseases of the intestine, gastrointestinal cancer and gut-associated pathologies arise from imbalanced interactions between the host gut epithelia and resident or ingested microbes, interactions that are still poorly understood at the molecular level. *D. melanogaster* has been a very powerful model to study development and diseases (Charroux and Royet 2012).

The models reviewed are relatively inexpensive, easy to work with, have short lifespans, and often have very well characterized and stereotypical development and behaviour. Invertebrate models could serve as references for scientists concerned in alternatives to vertebrate animals (Lehner and Lee 2008) and could be a challenge for studying the pathogenesis of infections caused by anaerobic bacteria.

**Povzetek**

Preučevanje interakcij med nevretenčarji in anaerobnimi patogenimi bakterijami je na vseh področjih biologije in medicine pomembno, saj lahko vsaka odkrita medsebojna odvisnost pomembno vpliva na nastanek in razvoj bolezni (Gagniere in sod. 2016).


Nevretenčarji so bili dolgo časa neprimerni za rast in razmoževanje anaerobnih bakterij, saj organizem ne ustvarja pogojev zanje. Znanstveniki pa so z izolacijo anaerobnih bakterij pri nevretenčarjih potrdili, da se lahko zaradi prilagodljivosti v okolju ustvarijo tudi rastni pogoji za pritritev in razmoževanje anaerobnih bakterij. Prav ta ugotovitev je vzbudila raziskovalce za nadaljevanje mikrobioloških raziskav v tem edinstvenem mikrobnem okolju nevretečarjev (König 2006).

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.


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