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Review

RELATIONSHIPS BETWEEN FREE-LIVING AMOEBA AND THEIR INTRACELLULAR BACTERIA

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An increasing number of bacteria have been described as benefiting from interaction with free-living amoeba. The most common association between free-living amoeba and microorganisms is interaction of various non-pathogenic and pathogenic bacterial species with amoeba. Various pathogenic bacterial species have capacity to resist digestion by free-living amoeba, which has been observed by many researchers. Also, several of these pathogens are able to resist digestion by macrophages. In addition, free-living amoeba have been associated with several diseases in humans. Acanthamniioeba castella *is an important predator of bacteria. It is a ubiquitous organism in water, soil, and air. Attention from a public health perspective is needed by investigation of interaction of foodborne pathogens and free-living amoeba. Bacteria can use free-living amoeba as reservoirs, mediators or vehicles, an infection route, "biological gym" and evolutionary crib or interaction may result in a close endosymbiotic relationship. The purpose of this review is to describe the interaction mechanisms between free-living amoeba and common bacteria species that survive in host cells.*

Key words: Acanthamoeba*, endosymbiosis, endoparasites, resistance, "biological gym".*

INTRODUCTION

Free-living amoebae (FLA) are unicellular, ubiquitous micro-organisms found in diverse habitats like marine water, freshwater, soil, air, and man-made environments (Schmitz-Esser *et al.,* 2010). They are mobile and feed on various microorganisms, such as fungi, protozoa, bacteria, and organic particles (Cateau *et al.,* 2014). Many well-known pathogens of humans are able to infect, survive, and multiply within phagocytosis vacuoles (Greub and Raoult, 2004; Horn and Wagner, 2004). FLA serve as reservoirs and vectors for the transmission of pathogenic bacteria to humans or animals (Lorenzo-Morales *et al.,* 2007; Thomas and Greub 2010; Anacarso *et al.,* 2012; Mella *et al.,* 2016). Several publications appeared in recent years documenting that amoeba can serve as a genetic "melting pot" where gene exchanges occur. Amoeba also may participate in these gene exchanges (Grillot-Courvalin *et al.,* 1998; Moreira and Brochier-Armanet, 2008). Interaction between FLA and bacteria promotes the pathogenicity of bacteria, and amoeba were important for the adaptation of bacteria to higher eukaryotic cells via intracellular growth (Harb *et al.,* 2000; Albert-Weissenberger *et al.,* 2007). An intracellular lifestyle protect bacteria against adverse conditions and disinfectants and some bacteria species are capable to survive in amoeba even during the cyst stage (Borella, 2005; Thomas and Greub, 2010; Melle *et al.,* 2016). However, not all bacteria are ingested or digested by amoeba. Intracellular bacteria have evolved various adaptations to escape ingestion by amoeba and exploit host cell resources (Huws *et al.,* 2008). Intracellular bacteria called also amoeba-resisting bacteria (ARB), use the amoeba as a "training ground" for resistance to destruction by macrophages (Greub and Raoul, 2004; Thomas *et al.,* 2006). An effective tool to detect ARB is co-culture with amoeba (Kebbi-Beghdadi and Greub, 2014). Mostly water (cooling towers, hospital water supplies, rivers, lakes, domestic water supplies and others) and soil have been investigated as possible reservoirs of ARB using the co-culture method described by Pagnier *et al.,* 2008. Aquatic ecosystems around human settlements are recognised as a reservoir for ARB (Pagnier *et al.,* 2008; Xi *et al.,* 2009. There are various studies (Rowbotham, 1998;

Greub *et al.,* 2004; Collingro *et al.,* 2005; Thomas *et al.,* 2006; Pagnier *et al.,* 2008) that showed that ARB can be a useful tool for recovering potentially pathogenic bacteria. Previous studies indicated that four species of FLA have an association with human disease: *Naegleria fowleri, Sappinia diploidea, Balamuthia mandrillaris* and *Acanthamoeba* spp. These opportunistic pathogens cause fatal human infections involving the central nervous system (*N. fowleri, B. mandrillaris* and *Acanthamoeba* spp.), acute and fulminating meningoencephalitis (*N. fowleri*), encephalitis (*S. diploidea*) and keratitis (*Acanthamoeba* spp.) (Schmitz-Esser *et al.,* 2008; Siddiqui and Khan, 2012a; 2012b). A limited number of studies are available on amoeba biodiversity, because most of them are difficult to recover and a low number of microbiological and molecular methods or specific assays that allow to detect the specific FLA species have been developed. More research into FLA (including species other than *Acanthamoeba* spp.) morphology, physiology and genetic are still necessary. It is important to identify characteristic features for each amoeba species that will facilitate determination of taxa, as amoeba classification requires more research. If we have only analysis of morphology and information on a few genes, then it is not possible to locate amoeba within the eukaryotic tree in an accurate manner. Due to changes in our understanding of phylogenetic lineages of eukaryotes, many of the traditional systematic groups remain no longer valid. Modern morphological approaches and molecular phylogenetic classification of the amoeba differ due to diverese biochemical pathways. According to Cavalier-Smith (2004) and Adl *et al*. (2005; 2012) classification of unicellular eukaryotes, the amoeba belong to the Eukaryota domain Amoebozoa kingdom Amoebozoa phylum. The Phylum was divided into two subphyla's: Conosa and Lobosa, which included typical lobose amoeba. Subphylum are divided in many orders such as Tubulinida, Amoebida and others. Families of naked lobosoans are based on their morphology. Number of families are floating, however; some of these families have remained unchanged, such as Amoebidae, Vannellidae, and Acanthamoebidae and others. Genera of amoeba are not clearly identified, however, one of the commonly isolated is *Acanthamoeba* (Cavalier-Smith, 2004; Adl *et al*., 2005; 2012). Comprehensive FLA classification shown in Table 1.

There are several studies indicating the relevance of the bacterial-amoeba interaction. FLA are still discovered in new habitats, including anthropogenic environments like refrigerators, taps, toothbrush, dishcloths and others. Therefore, from a public health perspective it is important to investigate relationships between FLA and non-pathogenic or pathogenic bacteria, due to frequent contact with human and animals. However, bacteria are developing many new adaptive traits for changing environmental conditions, which creates difficulty in understanding bacteria-amoeba relationships completely.

INTERACTION BETWEEN FLA AND BACTERIA

Amoeba are able to interact with pathogenic bacteria in various ways. There are numerous studies (e.g. Barker and Brown, 1994; Vaerewijck *et al.,* 2008; 2010; 2014; Thomas and Greub, 2010) that one such way is FLA acting as vehicles and fill with living bacteria, in a process called "Trojan horses". Another way is that bacteria are able to resist FLA digestion and live in trophozoites or cysts (Greub and Raoult, 2004; Cateau *et al.,* 2014). Furthermore, it is possible that interactions could occur when bacteria manipulate host gene expression of housekeeping genes and enable themselves to proliferate in amoeba cells. Bacterial interaction with amoeba has been associated with increase in antimicrobial resistance of bacterial pathogens (Cirillo *et al.,* 1997). Therefore, FLA may be viewed as a "biological gym" where bacterial pathogens are continuously trained to

Table 1

CLASSIFICATION OF FREE LIVING AMOEBA*

Domain	Kingdom	Family	Genus	Examples
Eukaryota	Amoebozoa	Acanthamoebidae	*Acanthamoeba*	*A. astronyxis* *A. castellani* *A. culbertsoni* *A. mauritaniensis* *A. polyphaga* *A. rhysodes*
		Balamuthiidae	*Balamuthia*	*B. mandrillaris*
		Dictyosteliidae	*Dictyostelium*	*D. discoideum*
		Endamoebidae	*Entamoeba*	*E. histolytica*
		N/A	*Ehinamoeba*	*Ehinamoeba* spp.
		Hartmannellidae	*Hartmanella*	*H. vermiformis*
			Vermamoeba	*V. vermiformis*
		Vahlkamphiidae	*Vahlkamphia*	*V. avara*
			Naegleria	*N. gruberi*
		Thecamoebidae	*Thecamoeba*	*T. quadrilineata*

* According to Cavalier-Smith, 2004; Adl *et al.,* 2005; 2012

be more resistant on impact with more developed host cells. Since ARB can exchange genetic material with other intracellular bacteria and develop virulence traits, FLA are used as an evolutionary crib, which explains their adaptation for survival within macrophages (Harb *et al.,* 2000; Huws *et al.,* 2008; Kebbi-Beghdadi and Greub, 2014). A selective agent that determines features that are relevant for bacterial survival and evolution is predation (Matz and Kjelleberg, 2005). Interaction between FLA and bacteria promotes survival of bacteria under grazing pressure, and therefore bacteria develop new adaptive features such as cell-to-cell communication, microcolony formation, swimming speed and bioactive metabolites (Matz *et al.,* 2004). Due to morphological traits of bacteria, such as motility, high abundance and small size, they easily interact with various FLA. Bacteria needs to develop pre- (extracellular) and post- (intracellular) ingestional adaptions to survive in food vacuoles (Jürgens and Matz, 2002). Huws *et al.* (2008) noted that in 1% amoeba, intracellular replication of *Salmonella* occurred only in the contractile vacuole. One of the extracellular features of amoeba-bacterial interaction is a massive and oversized morphology. Bacteria such as *Comamonas acidovorans* develop non-digestible filamentous cells. A second adaptive trait is increased bacterial motility. Bacteria can achieve a high swimming speed and a "run and reversal" swimming pattern if they contact a predator. This mechanism acts as a resistance strategy to escape from FLA grazing (Jürgens and Matz, 2002). Another adaptive feature is cell-cell contact by membrane-bound receptors. The bacterial cell surface develops specific biochemical structures and unspecific interaction forces, and form grazing-resistant morphological structures, such as filaments and aggregates (Matz and Jürgens, 2003). For example, long pili mediate *Legionella* adhesion to the host cell (Koval, 1993). Surface properties are essential for bacteria because feeding is based on interaction at the cell-cell interface, e.g., *Acanthamoeba* has carbohydrate-sensitive sites on the cell surface. These sites help in the phagocytosis process, because some bacteria are coated with specific biochemical components such as polysaccharides or proteins (Jürgens and Matz, 2002). This means that food selection is based on the recognition of specific bacterial biochemical surface compounds (Matz and Jürgens, 2003).

Intracellular adaptive features developed in response to acid and enzymatic degradation. One of the mechanisms is enzymatic resistance to avoid digestion. The protective function of microbial S-layers allows *Synechococcus* cells to resist chemical degradation and digestion in *Tetrahymen*a. The bacteria *Janthinobacterium lividum* and *Chromobacterium violaceum* have developed more effective resistance mechanisms than for *Synechococcus* cells. They release bioactive metabolites upon digestion, which cause rapid death and lysis (Koval, 1993; Matz and Kjelleberg, 2005). In amoeba and human macrophages, bacteria such as *Listeria*, *Rickettsia*, *Mycobacterium*, *Legionella* and *Chlamydia* use related mechanisms to survive in cells. Amoeba and macrophages have similar phagocytic mechanisms, such as prey recognition by cell surface receptors and killing of prey by oxygen radicals (Barker and Brown, 1994).

M. avium enters in amoeba by a complement-independent mechanism of uptake. Intracellular growth causes various physiological changes. These bacteria promote entry into amoeba by upregulation of a factors that is directly involved in entry, like as-yet-unidentified entry factors, or downregulation or modification of a gene product(s). During the first 15 min after *Acanthamoeba* infection, *M. avium* bacteria is admitted into individual vacuoles, which are later fused into one large vacuole. Alteration occurs with expression of the gene involved in entry while bacteria grow in the host cell. Due to the prevention of lysosomal fusion in a similar manner to that observed in macrophages, *M. avium* is able to survive and replicate in amoeba cells. Cirrilo *et al.* (1997) found that replication of *M. avium* occurs at temperatures as low as 24 °C and that growth and interaction of *M. avium* with FLA can increase virulence. One of the interaction mechanisms by which *M. avium* spreads in the environment is the killing of infected amoeba (Koval, 1993; Cirillo *et al.,* 1997; Matz and Jürgens, 2003). Salah *et al*. (2009) provided a list of *Mycobacterium* species that were isolated from amoebal co-cultures. Amoeba models allowed to investigate bactericidal mechanisms, phagocytosis and surface receptors. All tested *Mycobacterium* species were phagocytosed and were able to penetrate into amoebal vacuoles, including some species like *M. tuberculosis* that could survive in cysts under aerobic conditions (Salah *et al.,* 2009). Endoparasites of FLA *Legionella* enter macrophages or amoeba by coiling phagocytosis after which it enters the phagosome. Proteins encoded by the Dot/Icm type IV secretion system genes are secreted by *Legionella* and they inhibit lysosome adhesion and phagosome maturation. Components from rough endoplasmic reticulum prevent lysosome adhesion and acidification of vesicles by the phagosome, protecting bacteria from attack. *Legionella* replicates and when amino acid deficiency occurs the FLA release the bacteria (Taylor *et al.,* 2009). Pathogenic chlamydiae enter and replicate in protozoa supported by the type III secretion system associated with pathogenicity, but *L. pneumophila* has receptors such as putative galactose/*N*-acetylgalactosamine (Gal/GalNAc) lectin that mediate the attachment to the host cell. The amoeba has cup-shaped invaginations (zipper phagocytosis) on the cell surface that uptake various microorganisms. After ingestion, bacteria release the Dot/Icm type IV secretion system, which transfers macromolecules into the host cell to evade endocytic fusion (Molmeret *et al.,* 2005). This secretion system is required for intracellular growth (Christie and Volgel, 2000). Also, the amoeba form of *Dictyostelium discoideum* supports intracellular replication of *L. pneumophila*. *D. discoideum* cells infected by *Legionella*-containing vacuole cause transformation of rough endoplasmic reticulum, which prevents *Legionella*-containing vacuole fusion with lysosomes. The Dot/Icm type IVb secretion supports modulation of host cell functions and mediates transfer of bacterial proteins into eukaryotic host cells (Molmeret *et al.,* 2005).

For genetic exchange and the supply of effector molecules to eukaryotic cells, bacteria use type IV secretion systems, such as VirB/VirD4 (pTi) or Trw, cagPAI, Ptl or Dot/Icm (Cascales and Christie, 2003). One of the most important adaptive mechanisms is by obtaining a gene, as it promotes intracellular growth, induces the synthesis of nutrients and prevents defence mechanisms. To export substrate molecules to a wide range of target cells during infection, pathogens such as *Bordetella pertussis*, *L. pneumophila*, *Brucella* spp., *Bartonella* spp., *Coxiella burnetii* and *Helicobacter pylori* use type IV machines (Christie and Volgel, 2000).

FLA are used by bacteria as transmission reservoirs, mediators, vectors or vehicles (Table 2) (Siddiqui and Khan, 2012b; Cateau *et al.,* 2014; Vezzulli *et al.,* 2014). Declerck *et al.* (2009) confirmed that in anthropogenic aquatic systems, *A. castellanii* play a decisive role in the increase and spread of *L. pneumophila*. There are several known interaction mechanisms, such as actin rearrangement, amoeba cell surface adhesion to microorganisms, coding of sugar-manipulating enzymes, amoeba genotype virulence and host cell morphogenetic changes. These interaction mechanisms provide disease (such as Q fever, preventable blindness, sexually transmitted diseases, bronchiolitis) dispersal routes, which are common in man-made and food-related environments (Cavalier-Smith *et al.,* 2004; Thomas and Greub, 2010).

CONCLUSIONS

Among the discovered FLA species, *S. diploidea*, *B. mandrillaris, Acanthamoeba* and *N. fowleri* are opportunistic pathogens. Several studies indicated that amoeba may play roles as mediator, vector, "Trojan horse" or "biological gymnasia". Many bacteria are able to penetrate into amoeba cell, although research results are still few and conflicting. Future studies are required to study the genes regulated by bacteria growth, which may provide better understanding of the factors responsible for bacteria entry, intracellular survival, replication and release. However, not all interaction mechanisms between FLA and bacteria are fully described, due to both technical limitation and relative low number of samples tested. Therefore, in future studies a higher number of samples should be used. Also, environmental and clinical studies are required to estimate conditions that allow bacteria to penetrate, survive and proliferate in host cell. Thus, co-culture should be used to discover new pathogenic bacterial species, due to amoeba's major role on the composition of microbiota.

Table 2

SUMMARY OF INTERACTION BETWEEN FREE LIVING AMOEBA AND BACTERIA

Species of FLA	Bacteria	Interaction mechanism and references
Acanthamoeba astronyxis	*Helicobacter pylori*	Reservoir (Siddiqui and Khan, 2012a)
A. castellanii	*Shigella dysenteriae, S. flexneri, S. sonnei*	Reservoir (transmission reservoir) (Saeed *et al.,* 2009; 2012)
	Acinetobacter baumannii, Bosea sp., *Campylobacter jejuni, C. coli, Coxiella burnetii, Francisella tularensis, Kocuria kristinae, Roseomonas gilardii*	Reservoir (Baron *et al.,* 1980; Hackstadt and Williams, 1983; Snelling *et al.,* 2006; Thomas *et al.,* 2010; Saeed *et al.,* 2012; Cateau *et al.,* 2014)
	Yersinia enterocolitica	Increased disinfection resistance when internalized (Snelling *et al.,* 2006)
A. culbertsoni	*Acinetobacter baumannii, Mobiluncus curtisii*	Reservoir (Thomas *et al.,* 2010; Cateau *et al.,* 2014)
A. polyphaga	*Mycobacterium abscessus, M. avium* subsp. *avium, M. aurum, M. bohemicum, M. chelonae, M. fortuitum, M. gastri, M. goodii, M. gordonae, M. immunogenum, M. intracellulare, M. kansasii, M. lentiflavum, M. mageritense, M. malmoense, M. marinum, M. massiliense, M. mucogenicum, M. peregrinum, M. porcinum, M. septicum, M. simiae, M. smegmatis, M. szulgai, M. terrae, M. tusciae, Shigella dysenteriae, S. flexneri, S. sonnei*	Reservoir (transmission reservoir) (Adekambi *et al.,* 2006; Saeed *et al.,* 2009; 2012)
	Aeromonas eucrenophila, A. salmonicida, Agrobacterium tumefaciens, Bosea spp., *Bradyrhizobium* spp., *Burkholderia cepacia, Chryseobacterium indologenes, Klebsiella variicola, Pseudomonas mendocina, Rasobacterium* spp., *Simkania negevensis, Sphingobacterium multivorum, Staphylococcus pasteuri*	Reservoir (Snelling *et al.,* 2006; Thomas *et al.,* 2010)
A. rhysodes	*Salmonella* spp.	Mediator (Vaerewijck *et al.,* 2014)
	Salmonella enterica	Reservoir (Tezcan-Merdol *et al.,* 2004)
Acanthamoeba spp.	*Legionella pneumophila*	Reservoir, vector and vehicle (Cirrillo *et al.,* 1997; Harb *et al.,* 2000; Schuster *et al.,* 2002; 2004; Dalebroux *et al.,* 2009; Iseberg *et al.,* 2009; Dupuy *et al.,* 2011)

Table 2 (continued)

Species of FLA	Bacteria	Interaction mechanism and references
Acanthamoeba spp.	*L. anisa, L. lytica*	Facultative intracellular (Greub and Raoult, 2004)
	Chlamydia spp.	Vehicle (Collingro *et al.,* 2005)
	Aeromonas spp., *Amoebophilus asiaticus, Bacillus* spp., *Bartonella henselae, Burkholderia cepacia, B. pseudomallei, Campylobacter coli, C. jejuni, C. lari, Coxiella* spp., *Flavobacterium* spp., *Helicobacter* spp., *Listeria monocytogenes, Mezorhizobium amorphae, Mobiluncus curtisii, Mycobacterium avium, M. fortuitum, M. marinum, M. leprae, M. phlei, M. simiae, M. smegmatis, M. ulcerans, Parachlamydia acanthamoebae, Pasteurella* spp., *Porphyromonas* spp., *Prevotella* spp., *Procabacter acanthamoebae, Protochlamydia amoebophila, P. naegleriophila, Pseudomonas aeruginosa, Pseudomonas* spp., *Ralstonia pickettii, Rickettsia* spp., *Salmonella* spp., *Simkania negevensis, Shigella* spp., *Staphylococcus aureus, Waddlia chondrophilia*	Reservoir (Greub and Raoult, 2004; Axelsson-Olsson *et al.,* 2005; Snelling *et al.,* 2006; Huws *et al.,* 2008; Thomas *et al.,* 2010; Bui *et al.,* 2012; Siddiqui and Khan, 2012a; Cateau *et al.,* 2014)
	Vibrio cholerae, V. mimicus, V. parahaemolyticus	Intra-amoebal habitat and reservoir (Thom *et al.,* 1992; Laskowski-Arce and Orth, 2008; Matz *et al.,* 2008; 2011; Abd *et al.,* 2010; Vezzulli *et al.,* 2010)
	Yersinia enterocolitica	Reservoir, vector, infection route, biological gym, and evolutionary crib (Huws *et al.,* 2008)
	E. coli, Salmonella spp.	Mediator (Vaerewijck *et al.,* 2014)
	Afipia birgiae, A. broomae, A. felis, A. massiliensis	Facultative intracellular (Greub and Raoult, 2004)
Balamuthia spp.	*Legionella pneumophila*	Reservoir, vector and vehicle (Cirrillo *et al.,* 1997; Harb *et al.,* 2000; Schuster *et al.,* 2003; Schuster and Visvesvara, 2004; Dalebroux *et al.,* 2009; Iseberg *et al.,* 2009; Dupuy *et al.,* 2011)
	Simkania negevensis	Reservoir (Thomas *et al.,* 2010)
Dictyostelium discoideum	*Mycobacterium avium, M. marinum*	Reservoir (Cateau *et al.,* 2014)
	Vibrio cholerae, V. mimicus, V. parahaemolyticus	Intra-amoebal habitat and reservoir (Thom *et al.,* 1992; Laskowski-Arce and Orth, 2008; Abd *et al.,* 2010; Vezzulli *et al.,* 2010; Matz *et al.,* 2011)
	Pseudomonas aeruginosa	Reservoir (Snelling *et al.,* 2006)
Entamoeba histolytica	*Shigella dysenteriae, S. flexneri, S. sonnei*	Reservoir (transmission reservoir) (Diamond *et al.* 1972; Saeed *et al.,* 2009)
Ehinamoeba spp., *Hartmanella* spp., *Vahlkamphia* spp.	*Legionella pneumophila*	Reservoir, vector and vehicle (Cirrillo *et al.,* 1997; Harb *et al.,* 2000; Schuster 2002; Schuster and Visvesvara, 2004; Snelling *et al.,* 2006; Dalebroux *et al.,* 2009; Iseberg *et al.,* 2009; Dupuy *et al.,* 2011)
Naegleria gruberi	*Vibrio cholerae, V. mimicus, V. parahaemolyticus*	Intra-amoebal habitat and reservoir (Thom *et al.,* 1992; Matz *et al.,* 2005; 2011; Laskowski-Arce and Orth, 2008; Abd *et al.,* 2010; Vezzulli *et al.,* 2010)
Rhynochomonas nasuta	*V. cholerae, V. mimicus, V. parahaemolyticus*	Intra-amoebal habitat and reservoir (Thom *et al.,* 1992; Matz *et al.,* 2008; 2011; Laskowski-Arce and Orth, 2008; Abd *et al.,* 2010; Vezzulli *et al.,* 2010)
Vermamoeba vermiformis	*Staphylococcus aureus*	Reservoir (Huws *et al.,* 2008)
Thecamoeba quadrilineata	*Yersinia enterocolitica*	Reservoir, vector, infection route, biological gym, and evolutionary crib (Lambrecht *et al.,* 2013)
	Campylobacter jejuni, C. coli, C. lari	Reservoir (Axelsson-Olsson *et al.,* 2005; Bui *et al.,* 2012)

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MIJIEDARBĪBA STARP BRĪVI DZĪVOJOŠĀM AMĒBĀM UN TO IEKŠŠŪNU BAKTĒRIJĀM

Vairākas patogēno baktēriju sugas ir rezistentas pret brīvi dzīvojošām amēbām un pat pret makrofāgu sagremošanu. Baktērijas turpina adaptēties saimniekorganisma vidē un attīsta jaunus izdzīvošanas mehānismus. Baktērijas var izmantot brīvi dzīvojošās amēbas kā rezervuārus, mediatorus, infekcijas ceļus, "bioloģiskās treniņvietas" un evolucionāro šūpuli, vai mijiedarbība var kļūt par ciešām endosimbiozes attiecībām. Šobrīd nav aprakstīti un nav zināmi visi mijiedarbības mehānismi, daļa no tiem ir tikai pieminēti un trūkst sīkāka apraksta. Turklāt pētījumos par brīvi dzīvojošo amēbu un to iekšŠūnu baktēriju mijiedarbību ir nepieciešams izmantot lielāku paraugu skaitu un amēbu kopkultūras nekā līdz šim, lai iegūtu precīzākus un ticamākus datus par patogēnajām baktērijām un to mijiedarbības