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Review

Understanding of Interactions Between *Acanthamoeba* and *Escherichia coli* on Cell-Based System

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Free-living *Acanthamoeba* are eukaryotic protozoan organisms that are widely distributed in the air, water, etc such as environment. *Acanthamoeba* ingest the *Escherichia coli* which will replicate in cytoplasm of *Acanthamoeba*. Bacterial pathogenicity or virulence is one of important determinant factors to survive in free-living *Acanthamoeba* and otherwise Acanthamoebic pathogenicity is also an important factor for their interactions. Bacterial association with pathogenic strain of *Acanthamoeba* T1 and T4 was lower about two times than non-pathogenic T7. Bacterial invasion percentages into T1 were higher about three times than T7 but bacterial survival in T7 was increased as T1. The capsule-deletion mutant exhibited limited ability for invasion/uptake by and survival inside pathogenic *Acanthamoeba* T4. *E. coli*-outer membrane protein A (OmpA) decreased bacterial association with *A. castellanii* by about three times and it had higher effects than lipopolysaccharides (LPS). Under favorable conditions, the mutants were not survived in *Acanthamoeba* up to 24 h incubation. Therefore, this review will report pathogenic and non-pathogenic *Acanthamoeba* strains interactions with *E. coli* and its several mutants, i.e., capsule, OmpA and LPS.

Key Words: *Acanthamoeba*, *Escherichia coli*, Capsule, Outer membrane protein A, Lipopolysaccharide

Acanthamoeba encephalitis, keratitis and taxonomy

Free-living *Acanthamoeba* are eukaryotic protozoan organisms that are widely distributed in the air, water, etc such as environment (Jung et al., 2007; Khan, 2007; Visvesvara, 2010). Given the opportunity and dependence on the status of the host immune system, *Acanthamoeba* can cause chronic granulomatous amoebic meningoencephalitis (GAE) and amoebic keratitis (AK), which is associated with contact lens user. The clinical course of GAE tends to be subacute or chronic and is usually associated with trauma or underlying disease, not swimming. In the later case, AK infections have been seen in both hard- and soft-lens wearers, and particular attention has been paid to soft-lens disinfection systems, including home-made saline solutions (Kilvington et al., 1990). The life cycle of *Acanthamoeba* consists of two stages: a vegetative trophozoite stage and a dormant cyst stage (Fig. 1). The trophozoite stage is normally in the size of 12~35 μm in diameter. Cysts are airborne, which may help *Acanthamoeba* spread in the environment and/or help it reach susceptible hosts. One reason that *Acanthamoeba* infections are notoriously difficult to treat is the rapid propensity of the trophozoites to transform into cysts, which are highly resistant to antimicrobial compounds (Khan, 2008). To differentiate *Acanthamoeba* by a molecular taxonomy, 18s rDNA sequence analysis was applied (Gast et al., 1996; Kong et al., 1996). Twelve of T1 to T12 types include the morphologically assigned 18 isolates and 35 new isolates. Among them, three species in the morphological group I were identified as T7 for *A. astronyxis*, T8 for *A. tubiashi*, and T9 for *A. comandoni*. In group 3, *A. culbertsoni* was identified as T10 and *A. healyi* as T12, and

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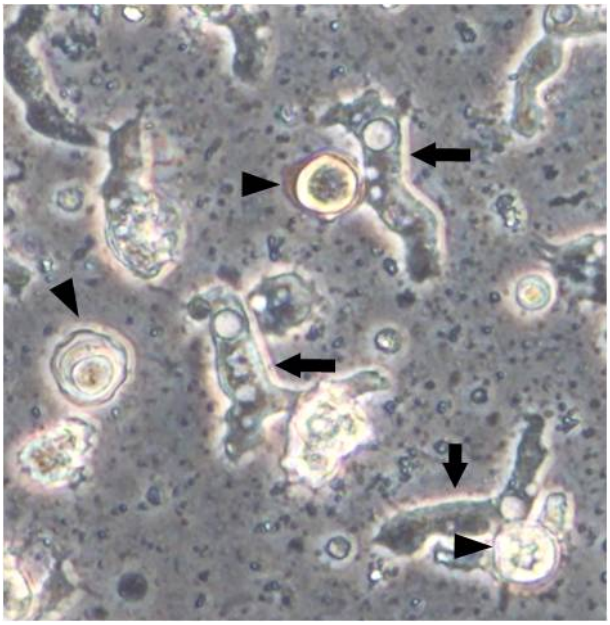

Fig. 1. *Acanthamoeba* trophozoites and cysts. *Acanthamoeba* were cultured under peptone-yeast extract-glucose (PYG) media for 20 days at 27℃. Vegetative trophozoites and harsh cysts were observed on a slide at the same time. Arrows indicated trophozoites of surface spines and arrowheads indicated cysts of wrinkled double wall. ×200.

A. palestinensis, *A. pustulosa*, and *A. lenticulata* represented T1, T2, and T5, respectively. T4 was classified as *A. castellanii* of morphological group II.

Interaction of *Acanthamoeba* and *Escherichia coli* K1

Due to living at air, water and soil, it has been reported that *Acanthameoba* interact with bacteria, viruses, etc (Ghedin and Fraser, 2005; Jung et al., 2008; La Scola and Raoult, 2001). In particular, *Acanthamoeba* ingest the bacteria which will replicate in cytoplasm of *Acanthamoeba*. For example, bacterial pathogens including *Legionella pneumophila* (causative agent of Legionnaire's disease) (Bozue and Johnson, 1996; Horwitz, 1983; Rowbotham, 1980), *Coxiella burneti* (agent of Q fever) (La Scola et al., 2003), *Vibrio cholerae* (cholera) (Thom et al., 1992), *Pseudomonas aeruginosa* (agent of nosocomial pneumonia and keratitis) (Garau and Gomez 2003; Kreger, 1983; Obritsch et al., 2005), *Helicobacter pylori* (gastric ulcers) (Winiecka-Krusnell et al., 2002), *Listeria monocytogenes*

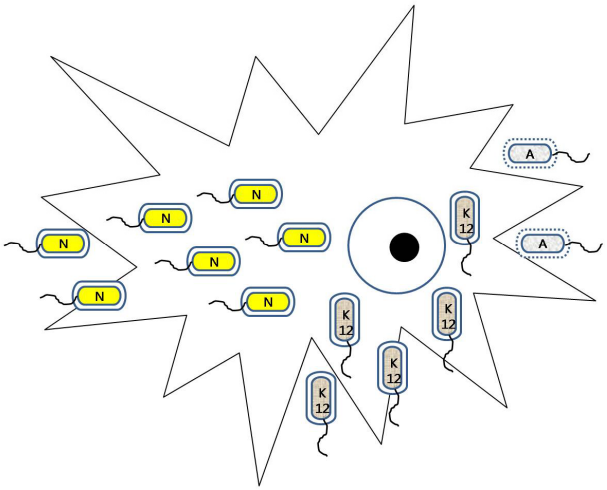

Fig. 2. Schematic review of *Acanthamoeba* interactions with *E. coli*. N, A and K12 indicated normal *E. coli* K1, capsule mutant and non-pathogenic *E. coli* K12, respectively.

(Listeriosis) (Ly and Muller 1990), *E. coli* O157 (haemolytic uraemic syndrome) (Barker et al., 1999), *E. coli* K1 (meningitis) (Jung et al., 2008), and *Mycobacterium avium* (respiratory infections) (Krishna-Prasad and Gupta, 1978). Bacterial pathogenicity or virulence is one of important determinant factors to survive in free-living *Acanthamoeba*. For example, a non-pathogenic bacterium, e.g., *E. coli* K12 of a laboratory strain, can act as a nutrient to *Acanthamoeba* and then some are removed in *Acanthamoeba* cytoplasm or a few are survived (Fig. 2). On the other hand, Acanthamoebic pathogenicity may be an important factor to determine interactions between reservoir-amoeba and bacteria. According to our group's previous report (Jung et al., 2008), bacterial association with pathogenic strain of *Acanthamoeba* T1 and T4 was lower about two times than non-pathogenic T7 but bacterial invasion percentages into T1 were higher about three times than T7. This suggested that *Acanthamoebic* pathogenicity would affect bacterial invasion. Otherwise, very interestingly, bacterial survival in T7 was increased as T1. Once bacteria enter into *Acanthamoeba* cytoplasm and they can replicate, irrespective of bacterial and Acanthamoebic pathogenicity. Avirulent strain, *E. coli* K12, was associated with *Acanthamoeba* but was not invaded and survived in *Acanthamoeba*. However, under favorable conditions such as growth media, even though the number of bacteria was very low, the bacteria

were survived in *Acanthamoeba*. In fact, *Acanthamoeba* and *E. coli* possessed the ability of killing target cells, which could become themselves in their interactions. *Acanthamoeba* have broad area of cell surface to contact, interact with and ingest the bacteria, pathogenic *E. coli* K1 and non-pathogenic *E. coli* K12. Most *E. coli* K12 were destroyed and killed by *Acanthamoeba* but *E. coli* K1 could also invade the membrane of *Acanthamoeba*, which then could multiply inside cytoplasm of *Acanthamoeba*.

Bacteria mutants interactions with *Acanthamoeba*

Gram-negative bacterium, *E. coli* possesses the capsular polysaccharide antigen K1 capsule (Xie et al., 2004). Previous studies have shown that the K1 capsule is a critical determinant that enhances *E. coli* serum resistance and anti-phagocytic properties (Allen et al., 1987; Kim et al., 1992). Capsules are composed of homopolymers of a (2,8)-linked sialic acid (Kim et al., 2003; Read et al., 1996; Silver et al., 1988) and are known to be involved in a number of functions including prevention of desiccation, adherence for colonization, resistance to complement, resistance to host immune system due to poor antibody response and protection from engulfment by predatory phagocytes and/or antimicrobial agents (Allen et al., 1987; Kim et al., 1992; also reviewed in Roberts, 1996). This mutant lacks the neuDB genes cluster that is necessary for production of cytoplasmic precursors to the exopolysaccharide capsule (Kim et al., 2003). The capsule-deletion mutant exhibited limited ability for invasion/uptake by and survival inside pathogenic *Acanthamoeba* T4 (Jung et al., 2007). This result suggested that the capsule of *E. coli* outer surfaces enhanced *E. coli* association, invasion and survival of *Acanthamoeba* trophozoites (Fig. 2). On the other hand, Alsam et al. (2006) reported that bacteria mutant factors e.g., outer membrane protein A (OmpA) and lipopolysaccharide (LPS) were important for *E. coli* K1 interactions with *A. castellanii*. OmpA decreased bacterial association with *A. castellanii* by about three times and it had higher effects than LPS. Under favorable conditions, the mutants were not survived in *Acanthamoeba* up to 24 h incubation. T1 and T4 above are pathogenic *Acanthamoeba* but T7 is non-pathogenic strain. In our studies used bacterial mutants, non-pathogenic T7 exhibited increased susceptibility to *E. coli* and bacterial mutants. The precise mechanisms remain unclear, the larger size of the environmental isolate may contribute to increased interaction with *E. coli*. In support, the size of the T4 tested here is approximately 12 μm, the size of the T1 is approx. 15 μm and the T7 being the largest, at approx. 22 μm (Jung et al., 2008). An alternative explanation may be that the higher yield of *E. coli* from environmental amoebae is due to their reduced bactericidal activities, i.e., clinical isolates of *Acanthamoeba* show similar levels of association but exhibit increased bactericidal activities (Jung et al., 2008).

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