Surviving within the amoebal exocyst: the *Mycobacterium avium* complex paradigm

Iskandar Ben Salah¹, Michel Drancourt¹,²*

**Abstract**

**Background:** Most of environmental mycobacteria have been previously demonstrated to resist free-living amoeba with subsequent increased virulence and resistance to antibiotics and biocides. The *Mycobacterium avium* complex (MAC) comprises of environmental organisms that inhabit a wide variety of ecological niches and exhibit a significant degree of genetic variability. We herein studied the intra-amoebal location of all members of the MAC as model organisms for environmental mycobacteria.

**Results:** Type strains for *M. avium*, *Mycobacterium intracellulare*, *Mycobacterium chimaera*, *Mycobacterium colombiense*, *Mycobacterium arosiense*, *Mycobacterium marseillense*, *Mycobacterium timonense* and *Mycobacterium bouchedurhonense* were co-cultivated with the free-living amoeba *Acanthamoeba polyphaga* strain Linc-AP1. Microscopic analyses demonstrated the engulfment and replication of mycobacteria into vacuoles of *A. polyphaga* trophozoites. Mycobacteria were further entrapped within amoebal cysts, and survived encystment as demonstrated by subculturing. Electron microscopy observations show that, three days after entrapment into *A. polyphaga* cysts, all MAC members typically resided within the exocyst.

**Conclusions:** Combined with published data, these observations indicate that mycobacteria are unique among amoeba-resistant bacteria, in residing within the exocyst.

**Background**

So-called amoeba-resistant bacteria are characterized by the ability to survive within free-living amoeba (FLA) trophozoites [1,2]. Some amoeba-resistant species have been further demonstrated to survive within the amoebal cyst which may act as a “Trojan horse” protecting the organisms from adverse environmental conditions [1]. The amoebal cyst is comprised of the nucleus and the cytoplasm embedded into three successive layers, *i.e.* the endocyst, the clear region and the outer exocyst. Despite the fact that specific location of amoeba-resistant bacteria into the amoebal cyst could modify the outcome of the organisms, precise location of intracystic organisms has not been systematically studied.

Most of environmental mycobacteria have been demonstrated to be amoeba-resistant organisms also residing into the amoebal cyst [3] (Table 1). The *Mycobacterium avium* complex (MAC) organisms have been used as model organisms for environmental mycobacteria, comprising of mycobacteria that are responsible for opportunistic infections and zoonoses [4-8]. *M. avium* and *Mycobacterium intracellulare* have been recovered from various sources, including fresh water [9-13] and hospital water supplies, in which FLA are frequently isolated [14-17]. Several experimental studies have further demonstrated *M. avium*-FLA interactions, including *Acanthamoeba* spp. [3,18-22] and *Dictyostelium* spp. [23-25]. *M. avium* and *M. intracellulare* have also been grown in the ciliated, unicellular protist *Tetrahymena pyriformis* [26]. It has been demonstrated that *M. avium* subsp. *avium* and *M. avium* subsp. *paratuberculosis* are able to survive within FLA [20-22], which results in their increased virulence [18,19] and protection against adverse situations including exposure to antibiotics [19]. The habitat of the recently described *Mycobacterium chimaera* (formerly sequevar MAC-A), isolated from respiratory tract specimens [27-29]; *Mycobacterium colombiense* (formerly sequevar MAC-X), isolated from the blood of an HIV-positive patient [30] and from enlarged lymph nodes in non-immunocompromised...
children [30-32]; Mycobacterium arosiense isolated from bone lesions [33]; and Mycobacterium marseillense, Mycobacterium timonense and Mycobacterium bouchedurhonense isolated from respiratory tract specimens [34,35], remains however unknown. MAC species exhibit on-going evolutionary divergence as evidenced by the 97.9-98.71% ANI (Average Nucleotide Identity) between the genomes of M. avium subsp. paratuberculosis K10 (NC_000962) and M. avium strain 104 (NC_008595), the 3.7% 16S rRNA gene divergence between M. avium and M. timonense and between M. avium and M. chimaera, and the 7.2% rpoB gene sequence divergence between M. avium and M. colombiense [34].

Using optic microscopy, electron microscopy and culturing methods, we herein used the MAC species as model organisms to study the location of environmental mycobacteria into the amoebal cyst and we further compared these observations with previously published data to find out that residing into the exocyst is a unique characteristic of environmental mycobacteria among amoeba-resistant organisms.

### Results and Discussion

The 11 MAC strains (8 species) studied survived, but did not grow, after a 24-hour incubation in Page’s modified Neff’s Amoeba Saline (PAS) at 32°C. Microscopic examination of infected amoeba demonstrated that all MAC organisms were entrapped in A. polyphaga trophozoites and were visible in 3- to 5-μm large “Mycobacterium containing vacuoles” as early as 24 hours post-infection; 1 to 12 such vacuoles were observed per infected amoeba (Figure 1). The mean number of “Mycobacterium containing vacuoles” was not statistically different between the various MAC species. Electron microscopy observations revealed that, in the “Mycobacterium containing vacuoles” containing only one organism, there was a close apposition of the vacuole membrane all over the mycobacterial cell surface (Figure 2A, B), which was tightly apposed all over the organism cell wall, in contrast to organisms in vacuoles that contained several to many mycobacteria (Figure 2C, D, E). In this study, we did not resolved whether the presence of several mycobacteria within one vacuole resulted from the uptake of clumped mycobacteria, the replication of mycobacteria or the coalescence of several, single-organism vacuoles remains undetermined. In any case, our observations agree with previous studies that M. avium is initially entrapped in the vacuoles of amoebal trophozoites [18,23,24,21,22] and macrophages [36] (Table 1). In Dictyostelium,  

---

### Table 1: Studies of interactions between MAC species and amoeba.

| Mycobacterium avium Species | Strains           | Amoeba species | Survival in A. polyphaga | Reference |
|-----------------------------|------------------|----------------|--------------------------|-----------|
| M. avium subsp. avium       | M. avium 109     | A. castellanii | +                        | [47]      |
| M. avium subsp. avium       | CIP104244T       | A. polyphaga Lnc-AP1 | +               | [3]       |
| M. intracellulare           | CIP104243T       | A. polyphaga Lnc-AP1 | +               | [3]       |
| M. avium subsp. paratuberculosis | ?               | A. castellanii CCAP1501 | +           | [22]      |
| M. avium subsp. paratuberculosis | ?               | A. castellanii | +                        | [20]      |
| M. avium subsp. avium       | ?                | D. discoidium AX2 | +               | [24]      |
| M. avium subsp. avium       | ?                | A. castellanii | +                        | [48]      |
| M. avium subsp. hominissuis | M. avium 104     | A. castellanii ATCC30234 | +           | [49]      |
| M. avium                    | Serotype 4       | A. castellanii ATCC30872 | +           | [21]      |
| M. avium                    | ?                | A. castellanii ATCC30234 | +           | [18]      |
| M. avium subsp. avium       | ATCC 25291T      | A. polyphaga Lnc-AP1 | +           | Present study |
| M. avium subsp. paratuberculosis | ATCC 19698T     | -              | +                        | -         |
| M. avium subsp. hominissuis | IWGMT 49        | -              | +                        | -         |
| M. avium subsp. silvaticum   | ATCC 49884T      | -              | +                        | -         |
| M. intracellulare            | ATCC 15985       | -              | +                        | -         |
| M. chimaera                  | DSM 44623T       | -              | +                        | -         |
| M. colombiense               | CIP 108962T      | -              | +                        | -         |
| M. marseillense              | CSUR P30T        | -              | +                        | -         |
| M. timonense                 | CSUR P32T        | -              | +                        | -         |
| M. bouchedurhonense          | CSUR P34T        | -              | +                        | -         |
| M. arosiense                 | DSM 45069T       | -              | +                        | -         |

---
M. avium accumulated within vacuoles decorated with vacuolin, the Dictyostelium flotilin homologue, but it did not break the vacuole membrane, in contrast to Mycobacterium tuberculosis and Mycobacterium marinum. This result was linked to the absence of a particular region of difference (RD1), which in M. tuberculosis and M. marinum, encodes a type seven secretion system along with secreted effectors [23].

Electron microscopy further disclosed that the 11 MAC strains under study were entrapped inside of the A. polyphaga cysts (Figure 2C, D). In all cases, the intracystic organisms were localized within the exocyst. In addition, M. marseillense could be observed in the clear region between the exocyst and the endocyst and in the inner side of the endocyst, and this was also the situation for M. intracellulare (Figures 2C, D) (Table 2). We further observed that a 36-hour exposure of the cysts to HCl did not affect the viability of the cysts, as new trophozoites emerged after 7-day incubation in peptone yeast extract-glucose (PYG) media at 32°C as determined by light microscopy. Sub-culturing such trophozoites on Middlebrook 7H10 agar yielded mycobacteria for all of the 8 MAC species (11 strains) under study after a 15-day incubation, whereas the cyst washing fluid remained sterile. Interestingly, we observed that these mycobacteria occupied a preferential location within the amoebal exocyst, where they were found in-between the two layers of the exocyst. Among the several Mycobacterium species reported to survive within amoebal cysts, such a particular feature has been previously illustrated only for M. avium in A. polyphaga cysts [21]; M. smegmatis [37]; M. abscessus, M. chelonae and M. septicum [3]; and M. xenopi [38]. Among intra-amoeobal bacteria, location within the exocyst has also been reported for Simkania negevensis [39], despite the fact that S. negevensis organisms could also be observed within the cytoplasm of the cyst, depending on the strain under study [40]. Location within exocyst wall contrasts with the...
observation of *Legionella pneumophila*, which was found within the cytoplasm of pre-cysts and mature cysts of *A. polyphaga* [41] or non-entrapped within amoebal cysts [42]. Reviewing published data regarding amoebal-resistant bacterial species [1,2] found that 11/32 (34.37%) *Mycobacterium* species versus 1/28 (3.57%) non-*mycobacterium* amoebal-resistant bacterial species have been reported to survive within *A. polyphaga* exocyst (*P* = 0.003) (Figure 3). As both *L. pneumophila* and mycobacteria are pathogens, the intracystic location of organisms may not influence their virulence. The mechanisms and biological significance of this particular location remain to be studied. It has been established that *A. polyphaga* exocyst is composed of cellulose [43] and the authors have observed that mycobacteria encode one cellulose-binding protein and one or two cellulases which are

Table 2 Abundance of mycobacteria in *A. polyphaga* strain Linc-AP1 and their preferential location in amoebal cyst wall.

| MAC species                  | No. of vacuoles that contain mycobacteria | Location in amoebal cyst wall       |
|-----------------------------|-----------------------------------------|-------------------------------------|
| *M. timonense*              | 1.3 ± 0.5 vacuoles                      | Exocyst                             |
| *M. bouchedurhonense*       | 2.1 ± 1.7 vacuoles                      | Exocyst                             |
| *M. marseillense*           | 2.4 ± 1.4 vacuoles                      | Exocyst, clear region, cytoplasm    |
| *M. avium* (*M. avium* subsp. *avium*) | 2.6 ± 2.2 vacuoles | Exocyst                             |
| *M. chimaera*               | 3.6 ± 2.6 vacuoles                      | Exocyst, cytoplasm                  |
| *M. intracellulare*         | 4.6 ± 4.8 vacuoles                      | Exocyst, Endocyst                    |
| *M. colombiense*            | 5.7 ± 6.2 vacuoles                      | Exocyst, cytoplasm                   |
| *M. arosiense*              | 9.4 ± 15.2 vacuoles                     | Exocyst                             |
indeed transcribed [44]. Cellulase encoded by mycobacteria may play a role in their unique exocyst location.

Moreover, we observed that all MAC species can survive within such *A. polyphaga* cyst. This occurrence did not merely result from the potential contamination of the amoeba by extra-amoebal mycobacteria, since we destroyed any MAC organism left on the surface of cysts by incubating the cysts in HCl, a method previously demonstrated to kill remaining trophozoites, immature cysts and extra-amoebal *M. avium* [21]. We checked the efficacy of this process by incubating the rinsing buffer on Middlebrook and found no growth of mycobacteria, which indicated that the HCl had indeed destroyed any extracystic MAC organisms. The fact that all of the MAC species survived in the exocyst may be relevant to the persistence of these organisms in the environment despite adverse conditions. Non-tuberculous mycobacteria, including *M. avium*, have been shown to persist up to 26 months in drinking water systems despite filtration and ozonation [45]. Also, *M. intracellulare* and other non-tuberculous mycobacteria have been shown to be protected against 15 mg/liter of free-chlorine for 24 hours by entrapment within *A. polyphaga* cysts [3]. Therefore, free-living amoeba cysts may be a “Trojan horse” for MAC organisms and protect them from adverse environmental conditions, including high concentrations of chlorine, as previously reported for other environmental mycobacteria.

**Conclusion**

The data presented herein on MAC species illustrate that survival within the amoebal exocyst is a significant feature of environmental mycobacteria. This particular location, preserving mycobacteria from adverse environment, nevertheless allow them to rapidly escape from the amoebal cyst. The mechanisms for such unique location remain to be established in environmental mycobacteria.

**Methods**

**Mycobacterium strains**

*M. avium* subsp. *avium* ATCC 25291T, *M. chimaera* DSM 446232T, *M. colombiense* CIP 108962T, *M. arosiense* DSM45069T [33], *M. marseillense* CSURP30T, *M. timonense* CSURP32T and *M. bouchedurhonense* CSURP34T [35] reference strains that were previously identified by 16S rRNA and *rpoB* gene sequencing [34] were subcultured on Middlebrook 7H10 agar (Becton Dickinson, Le Pont de Claix, France) for 7 days at 30°C under a 5% CO₂ atmosphere. Cells were washed in 1.5 ml phosphate buffered saline (PBS), pH 7.3, by centrifugation at 8,600 g, and the inoculum was adjusted to 10⁶ bacteria/ml in PBS.

**Infection of amoeba**

The *A. polyphaga* strain Linc-AP1 was obtained from T. J. Rowbotham, Public Health Laboratory, Leeds, United Kingdom and cultured at 28°C for 3 days in 150 cm² culture flasks (Corning, New York USA) that contained 30 ml PYG broth [46]. Amoebal cells were harvested by centrifugation at 500 g for 10 min. The pellet was suspended twice in PAS to obtain 5 × 10⁵ cells/ml. One milliliter of this suspension was dropped into each well of a 12-well microplate (Corning) and incubated at 33°C.
for 7 days. The microplate, prepared as described above, was used for culturing the mycobacteria. Each well of the microplate was inoculated with a final concentration of $10^6$ mycobacteria/ml (MOI = 10). The inoculum was sonicated for 5 min at 234 watts (BRANSON 2210; Branson Ultrasonics Corporation, Danbury, CT, USA) in order to limit mycobacteria cell clumping. The microplate was centrifuged at 1,000 $g$ for 30 min and incubated at 33°C under a humidified, 5% CO$_2$ atmosphere. This microplate was examined daily for 15 days for cytopathic effects and the presence of intra-amoebal organisms by shaking, cytocentrifugation at 200 $g$ for 10 min and Ziehl-Neelsen staining.

**Encystment and excystment of infected amoeba**

In 25 cm$^3$ culture flasks (Corning), 10 ml of amoeba that had been infected for 48 hours were rinsed once with encystment buffer adapted from [21] (0.1 M KCl, 0.02 M Tris, 8 mM MgSO$_4$, 0.4 mM CaCl$_2$, 1 mM NaHCO$_3$). After centrifugation at 500 $g$, the pellet was resuspended in 10 ml of fresh encystment buffer and incubated for 3 days at 32°C. The excystment of the cysts was examined by light microscopy. Amoebal cysts were pelleted by centrifugation at 1,000 $g$ for 10 min and treated with 3% (vol/vol) HCl as previously described [21]. Treated cysts were then washed three times with PAS buffer. Half of the sample was processed for electron microscopy (see above), and the other part was incubated for 7 days in PYG medium at 33°C. Intra-amoebal mycobacteria were released by lysing the monolayer with 1 ml of 0.5% sodium dodecyl sulfate, followed by two successive passages through a 27-gauge needle [3]. The presence of viable mycobacteria was documented by detecting colonies on Middlebrook 7H10 agar inoculated with 200 $μ$l of the cell lysate and incubated at 30°C for 15 days. The identities of the mycobacteria were confirmed by Ziehl-Neelsen staining and partial rpoB gene sequencing using primers Myco-F (5’-GGCAAGGTCACCCCGAAGGG-3’) and Myco-R (5’-AGCGGCTGCTGGGTGATCATC-3’) [34]. All experiments were repeated three times.

**Electron microscopy**

Non-ingested mycobacteria were eliminated by rinsing the amoebal monolayer twice with sterile PBS. The amoeba monolayer that was previously infected by MAC species was then fixed in 2% glutaraldehyde and 0.1 M cacodylate buffer overnight. After this first fixation, the bacteria were fixed in 2% glutaraldehyde and 0.33% acroleine in a 0.07 M cacodylate buffer for 1 hour. After washing in 0.2 M cacodylate buffer, the bacteria were post-fixed in 1% osmium boxide in 0.1 M potassium ferricyanure for 1 hour and dehydrated in an ascending series of ethanol concentrations, and after 100% ethanol, the dehydration was finished in propylene oxide, and the samples were embedded in an Epon 812 resin. Sections (70 nm) were stained with 5% uranyl acetate and lead citrate before examination with a transmission electron microscope (Philips Morgagni 268D, Eindhoven, the Netherlands). For the determination of mycobacterial abundance, we made observations on a total of 30 A. polyphaga trophozoites for each of the 8 MAC species. In order to determine the total number of mycobacteria per trophozoite, we recorded the total number of vacuoles with one Mycobacterium organism and the total number of vacuoles with $> 1$ Mycobacterium organism. We also made observations on a total of 30 A. polyphaga organisms for each of the 8 MAC species in order to determine their intracystic location, which was considered as intracystic when apposed to the cyst wall and reaching into the cyst wall (between the endo- and the exocyst). These observations were performed in triplicate.

**Statistical tests**

Comparison among amoeba-resistant bacterial species [2] as for their survival within exocyst was done using the $χ^2$ test and corrected by Mantel Haeenszel method. Comparisons of mean ± standard deviation of the number of infected vacuoles were done using the ANOVA test. A $P$ value < 0.05 was considered to be significant.

**Acknowledgements**

The authors acknowledge Bernard Campagna for his help with the electron microscopy observations.

**Author details**

1. Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, UMR CNRS-6236, IRD 189, IFR 48 Faculté de Médecine, Université de la Méditerranée, Marseille France. 2. Assistance Publique des Hôpitaux de Marseille, Fédération de Microbiologie clinique Hôpital la Timone Marseille-France.

**Authors’ contributions**

IBS performed the experiments, he interpreted data and wrote the manuscript. MD designed the experiment, he provided support, interpreted data and wrote the manuscript. Both authors have read and approved the final version of the manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Received:** 4 December 2009  **Accepted:** 1 April 2010  **Published:** 1 April 2010

**References**

1. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. in Microbiol Rev 2004, 17:413-433.
2. Thomas V, McDonnell G, Denyer SP, Maillard JY. Free-living amoeba and their intracellular pathogenic microorganisms: risks for water quality. FEMS Microbiol Rev.
3. Adekambi T, Ben Salah S, Khifi M, Raoult D, Drancourt M. Survival of environmental mycobacteria in Acanthamoeba polyphaga. Appl Environ Microbiol 2006, 25974-5981.
4. Tortoli E, Cichero P, Persimon C, Simonetti MT, Gesu G, Nista D: Use of BACTEC MGIT 960 for recovery of mycobacteria from clinical specimens: multicenter study. J Clin Microbiol 1999, 37:3578-3582.
5. Tuereen CY, Wallace R, Behr MA: Mycobacterium avium in the postgenomic era. Clin Microbiol Rev 2007, 20:205-229.
6. Yajko DM, Chin DP, Gonzalez PC, Nassos PS, Hopewell PC, Reingold AL, Horsburgh CR Jr, Yaksu MA, Ostrom SM, Hadley WK: Mycobacterium avium complex in water, food, and soil samples collected from the environment of HIV-infected individuals. J Acquir Immune Defic Syndr Hum Retrov 1995, 9:176-182.
7. Kavakousis PC, Moore RD, Chaisson RE: Mycobacterium avium complex in patients with HIV infection in the era of highly active antiretroviral therapy. Lancet Infect Dis 2004, 4:457-465.
8. Lauzi S, Pasotto D, Armadori M, Archetti IL, Poli G, Bonizzi L: Evaluation of the specificity of the gamma-interferon test in Italian bovine tuberculosis-free herds. Vet J 2000, 160:17-24.
9. Falkinham JO, Norton CD, LeChevallier MW: Factors influencing numbers of Mycobacterium avium, Mycobacterium intracellularis, and other mycobacteria in drinking water distribution systems. Appl Environ Microbiol 2001, 67:1225-1231.
10. Falkinham JO, Isemann DE, de Haas P, van Soolingen D: Mycobacterium avium in a shower linked to pulmonary disease. Lancet Infect Dis 2004, 4:371-373.
11. Hilborn ED, Yakrus MA, Covert TC, Harris SI, Donnelly SF, Schmitt MT, Falkinham JO, Iseman MD, de Haas P, van Soolingen D: Mycobacterium avium complex in the water treatment plant influent of a hospital. J Water Health 2009, 7:470-477.
12. Ben Salah I, Adekambi T, Raoult D, Drancourt M: Comparison of large restriction fragments of Mycobacterium avium to resolves that of Mycobacterium avium complex species. J Appl Bacteriol 2000, 89:285-289.
13. Santos R, Oliveira F, Goncalves S, Macieira F, Cadete M: Detection and identification of mycobacteria in the Lisbon water distribution system. Water Sci Technol 2002, 45:255-260.
14. Le Dantec C, Duguet JP, Montiel A, Dumoutier N, Dubrou S, Vincent V: Occurrence of mycobacteria in water treatment lines and in water distribution systems. Appl Environ Microbiol 2002, 68:3716-3723.
15. Santos R, Oliveira F, Júnior J, Gonçalves S, Macêre F, Cadete M: Detection and identification of mycobacteria in the Lisbon water distribution system. Water Sci Technol 2002, 45:255-260.
16. van den Hill ED, Yakrus MA, Covert TC, Harris SI, Donnelly SF, Schmitt MT, Toney S, Bailey SA, Stella GM Jr: Molecular comparison of Mycobacterium avium isolates from clinical and environmental sources. Appl Environ Microbiol 2008, 74:4964-4968.
17. de St럴er M, Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
18. Suliman O, Torkko P, Andersen AB, van den Hill ED, Yakrus MA, Covert TC, Harris SI, Donnelly SF, Schmitt MT, Toney S, Bailey SA, Stella GM Jr: Comparison of another restriction-fragment fragments of Mycobacterium avium isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J Clin Microbiol 1999, 37:1008-1012.
19. du Moll GC, Strotmeier KD, Pelletter PA, Tsang AY, Hedley-White J: Concentration of Mycobacterium avium by hospital hot water systems. JAMA 1988, 260:1559-1601.
20. Goslee S, Wolinsky E: Water as a source of potentially pathogenic mycobacteria. Am Rev Respir Dis 1976, 113:287-292.
21. van den Hill ED, Yakrus MA, Covert TC, Harris SI, Donnelly SF, Schmitt MT, Toney S, Bailey SA, Stella GM Jr: Molecular comparison of Mycobacterium avium isolates from clinical and environmental sources. Appl Environ Microbiol 2008, 74:4964-4968.
22. de St嵘ler M, Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
23. Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
24. de St嵘ler M, Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
25. Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
26. Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
27. Ben Salah I, Adekambi T, Raoult D, Drancourt M: Mycobacterium avium complex from water in the United States, Finland, Zaire, and France. J Appl Microbiol 2000, 89:285-289.
47. Danelishvili L, Wu M, Stang B, Harriff M, Cirillo SI, Cirillo JD, Bildfell R, Arbogast B, Bermudez LE: Identification of *Mycobacterium avium* pathogenicity island important for macrophage and amoeba infection. *Proc Natl Acad Sci USA* 2007, 104:11038-11043.

48. Krishna-Prasad BN, GSK: Preliminary report on engulfment and retention of mycobacteria by trophozoites of axenically grown *Acanthamoeba castellanii* Douglas 1930. *Curr Sci* 1978, 45:245-247.

49. Tenant R, Bermudez LE: *Mycobacterium avium* genes upregulated upon infection of *Acanthamoeba castellanii* demonstrate a common response to the intracellular environment. *Curr Microbiol* 2006, 52:128-133.

doi:10.1186/1471-2180-10-99

Cite this article as: Ben Salah and Drancourt: Surviving within the amoebal exocyst: the *Mycobacterium avium* complex paradigm. *BMC Microbiology* 2010 10:99.