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Evidence of horizontal gene transfer between amoeba and bacteria

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Free-living amoebae are protozoa that feed by phagocytosing energy-rich particles present in their environment. Some bacteria have evolved to survive amoebal internalisation and multiply within phagocytosis vacuoles [1]. Studies have suggested that these amoebae serve as a genetic 'melting pot' where amoeba-resistant microorganisms exchange genes. These horizontal gene transfers (HGTs) may occur between intracellular pathogens, as shown for *Rickettsia bellii* [2]. Indeed, this bacterium was not found in amoebae but its genome possesses genes closely related to genes from the amoeba-parasites *Legionella pneumophila* and *Protochlamydia amoebophila*, suggesting that the rickettsiae ancestor and other intracellular bacteria exchanged genes. Some of these HGTs have also been described between bacteria and viruses, particularly with the giant virus *Acanthamoeba polyphaga* Mimivirus whose genome presents genes apparently acquired from several bacteria [3].

Amoebae not only represent a place of gene exchange for these microorganisms, but can also participate in these exchanges. Indeed, studies have shown that amoebae can transmit genes to *A. polyphaga* Mimivirus, which possesses several genes likely to have been acquired from amoebae [3].

Recently, it was shown that some bacteria can also transfer parts of their genome to eukaryotic cells. *In vitro*, it was demonstrated that an *Escherichia coli* strain, made invasive by cloning the invasion gene from *Yersinia pseudotuberculosis*, can transfer functional DNA to mammalian cells [4]. *In vivo*, it was shown that *Wolbachia* endosymbiont can transfer large parts of its genome into the genome of their eukaryotic hosts, as nematodes and insects [5].

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Here, we present a new example of HGT between bacteria and eukaryotes, utilising amoebae. A gene was exchanged between amoebae and an obligate intra-amoebal bacterium recently described belonging to the *Legionella* genus, which includes facultative intra-amoebal bacterium, *Legionella drancourtii* (formerly named LLAP12) [6].

A three-fold genome sequencing of *L. drancourtii* was performed with the GS20 sequencer (454 Life Sciences, Branford, CT, USA). Sequence reads were assembled into consensus sequences (contigs) using the GS20 Newbler Assembler Software. Resulting contigs were submitted to Open Reading Frame (ORF) prediction using the GeneMark.hmm program for prokaryotes (http://exon.gatech.edu/geneMark). ORFs were then compared with GenBank using the Blastp program from the National Center for Biotechnology Information. Significant alignments (E-value $<1.10^{-10}$) with *Acanthamoeba* or *Dictyostelium* species were selected and kept if the orthologous proteins had approximately the same length, and confirmation of similarity was obtained by reciprocal Blast match. A 528-amino acid protein exhibited a 54% amino acid identity rate with a 543-amino acid protein from the amoeba *Dictyostelium discoideum*, the malate synthase, which is a key enzyme of the glyoxylate cycle and essential for growth on acetate as carbon source. This protein is present within some bacteria, fungi and plants.

A phylogenetic analysis of this 528-amino acid protein was performed. For this, proteic sequences of homologous were collected from GenBank. These sequences were aligned with MUSCLE and their phylogenetic relationships were inferred using the PHYML (PHYlogenetic inferences using Maximum Likelihood) program. The phylogenetic tree (Fig. 1a) showed that the *L. drancourtii* ORF and the *D. discoideum* malate synthase were clustered with a bootstrap value of 88%. Its presence within *D. discoideum* with a clear phylogenetic grouping with bacterial malate

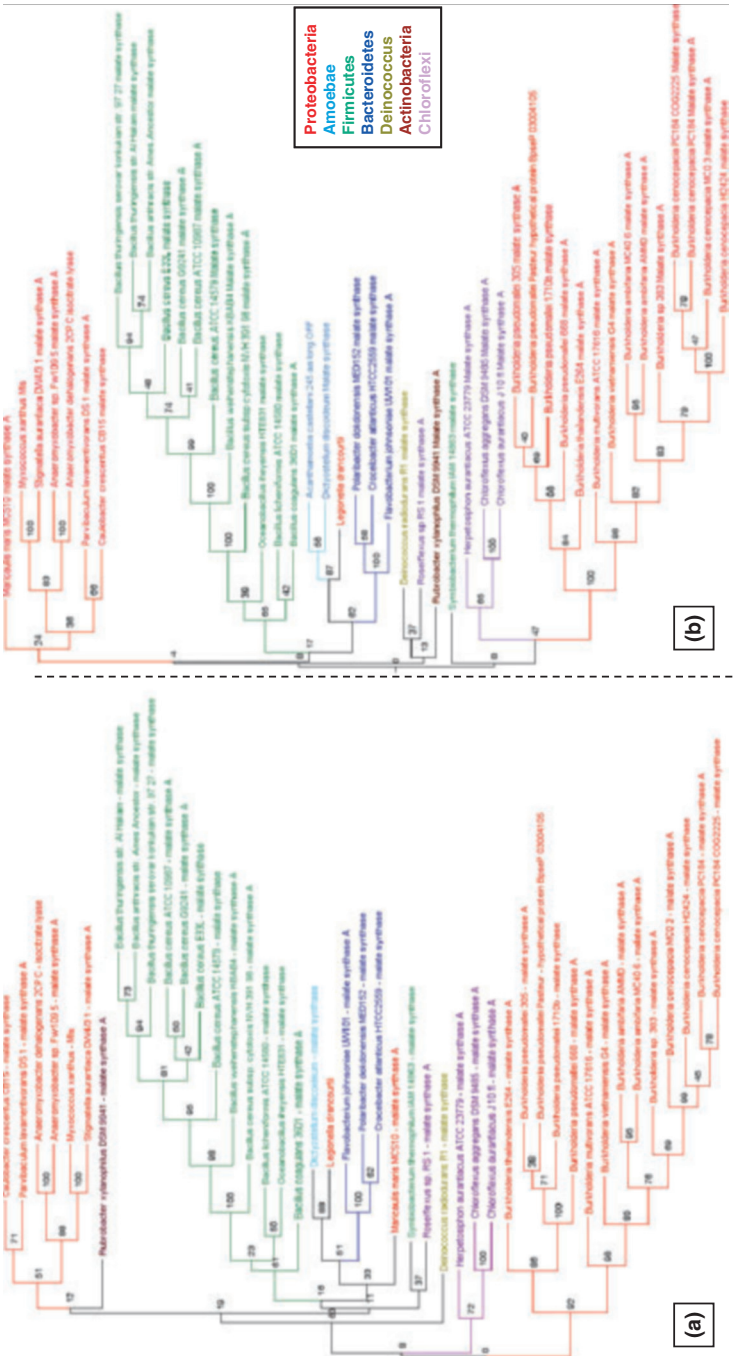

Fig. 1. Phylogenetic analysis of the malate synthase protein. (a) Phylogenetic tree of selected protein sequences matching the *Legionella drancourtii* malate synthase. The analysis was performed using the maximum likelihood method. The tree was rooted on the midpoint. Bootstrap values are indicated at the nodes. (b) The 245 amino acid-long ORF of *Acanthamoeba castellani* was integrated to the previous tree.

synthase proteins suggests that amoebae and *L. drancourtii* recently exchanged this gene.

To strengthen this hypothesis, we searched for this protein in other amoebae. *D. discoideum* and *L. drancourtii* malate synthase proteins were aligned with the linear scaffolds database of the *Acanthamoeba castellani* Neff genome project using the tblastn program on the BCM-HGSC web site (http://www.hgsc.bcm.tmc.edu/projects/microbial/microbial-detail.xsp?project_id=163). A nucleotidic sequence was found to present an amino acid identity rate of 54% with the two malate synthases. It was submitted to an ORF prediction using the GeneMark program for eukaryotes and presented two ORFs, 245 and 331 amino acid-long, corresponding to a split malate synthase encoding gene. The resulting ORFs were integrated in the phylogenetic analysis of the malate synthase, which showed that the 331 amino acid-long ORF is not closely related to *L. drancourtii* and *D. discoideum* (data not shown). On the other hand, the 245 amino acid-long ORF is clustered with the *D. discoideum* malate synthase with 56% support, and the *L. drancourtii* protein is clustered with this group of amoebal malate synthases with 87% support (Fig. 1b). This result is consistent with the hypothesis of an HGT between *L. drancourtii* and amoebae. However, the source of this transfer is difficult to determine because neither the bacterium nor the amoebae are clustered with their relatives. According to the phylogenetic tree, the most parsimonious hypothesis is that this gene was transferred from aquatic Bacteroidetes to *L. drancourtii*, either in the same aquatic ecological niche or within amoebae, and was transmitted recently from *L. drancourtii* to amoebae.

This new example of HGT illustrates the role of amoebae as a place of gene exchanges, not only between intra-amoebal bacteria but also between these bacteria and amoebae.

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