



Focus Article: Recent advances in *Rhodococcus equi*

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The genus *Rhodococcus* belongs to one of the largest microbial groups on earth, the Actinobacteria. The rhodococci include "friendly" organisms that produce antibiotics and other clinically useful secondary metabolites, but also major pathogens, such as the causative agents of tuberculosis (TB), leprosy or diphtheria. They are widely distributed in the environment and are important in biotechnology due to their extraordinary metabolic versatility and biodegradative properties. The genus also includes an animal pathogen, *Rhodococcus equi*, a soil inhabitant that uses herbivore manure as growth substrate and is ubiquitous in the farm environment.

Horse pathogen

R. equi is the causative agent of a severe respiratory disease of horses that is a leading cause of mortality in foals. Rhodococcal pneumonia typically affects foals between one and six months of age, follows an insidious course with sudden onset of overt symptoms, and is generally fatal if antibiotic treatment is not rapidly administered. Secondary enterocolitis and mesenteric lymphadenitis are other common manifestations. Less frequent forms of presentation include synovitis, uveitis, osteomyelitis and septic arthritis. The lung infection is contracted through inhalation of contaminated soil dust or the breath of infected animals. *R. equi* can also multiply in the intestine, contributing to its dissemination via an oral-fecal cycle that enriches the farm environment with virulent (plasmid-bearing) strains. Transmission is more likely to occur in conditions that favour aerosolisation of contaminated soil particles, i.e. dry weather and crowded paddocks. The organism becomes endemic on stud farms, where it represents a real challenge as there is no effective vaccine available. This is compounded by the intrinsic resistance of *R. equi* to many antibiotics (e.g. penicillins, cephalosporins, sulfamides, quinolones, tetracyclines, clindamycin, and chloramphenicol) and the recent emergence of acquired resistance to currently used drugs. The intracellular localization of the pathogen complicates its therapeutic management, making it necessary to administer lengthy treatments, of up to three months or more, with no guaranteed success [1,2,3].

Due to its high fatality rate, the lack of effective early diagnosis and preventative measures, and the costs of the prolonged antibiotic treatments (often administered prophylactically in endemic studs), *R. equi* has a major economic impact and is recognized as one of the most important infectious problems that afflict the equine industry worldwide.

A multihost pathogenic actinomycete

R. equi research has been traditionally "equinocentric" and the appreciation of the organism as a multihost bacterial pathogen has been largely neglected. *R. equi* is often isolated from submaxillary pyogranulomatous adenitis in pigs and TB-like abscesses in retropharyngeal and pulmonary lymph nodes in cattle [2]. Data from abattoir surveys in Ireland indicate that up to 4% of suspected bovine TB cases may in fact be *R. equi* infections [4]. Since the emergence of the AIDS pandemic, *R. equi* has also gained prominence as a human opportunistic pathogen. Human cases are generally associated with immunosuppressive conditions, and in HIV patients they usually present as TB-like purulent cavitary pneumonia with a high mortality (50-55%) [5]. *R. equi* infections have been reported in a variety of other animal species, including dogs and cats [6].



The number of reports of non-equine infections is on the rise, probably due to increased awareness about this pathogen and the application of improved detection techniques. However, *R. equi* is still frequently misidentified in the laboratory. A careful differential diagnosis must always be carried out in any suspected case of mycobacterial (TB) infection to exclude *R. equi*.

Pathogenesis and molecular virulence determinants - before the genome

R. equi is a facultative intracellular parasite of macrophages that replicates within a modified endosomal compartment, the *R. equi*-containing vacuole (RCV). *R. equi* virulence depends on the presence of a circular plasmid of 80 to 90 kb. This plasmid confers the property of arresting RCV acidification and maturation and its loss results in an inability to cause disease in foals and to replicate in macrophages *in vitro* and in mouse tissues *in vivo*. A surface lipoprotein antigen, VapA encoded in the plasmid, is a key mediator of these effects [2,3,7]. The vapA gene is located in a 21-Kb pathogenicity island (PAI) together with several other vap genes [8]. Recent findings from our laboratory indicate that specific virulence plasmid types are associated with specific animal hosts (horse, cattle, pigs). The host-associated plasmids differ in vap gene complement within the PAI, suggesting an involvement of the Vap proteins in the determination of *R. equi* host tropism [9]. This unique plasmid-determined host specificity constitutes a new paradigm in bacterial pathogenicity. The precise role and mechanism of the Vap proteins remain unknown.

Chromosomal factors are also likely to participate in *R. equi* pathogenesis. The *R. equi* glyoxylate shunt enzyme isocitrate lyase is required for full virulence in macrophages and mice, consistent with lipids being a major carbon source for the organism *in vivo*. Cell wall mycolic acid-containing glycolipids may promote survival within phagocytes and granuloma formation. Mannose-capped lipoarabinomannans may inhibit phagosome maturation or trigger the release of interferon- and IL-12 from infected cells, as shown in *Mycobacterium tuberculosis* (Mtb). Other possible chromosomal factors include cholesterol oxidase [45, 46], the capsular polysaccharide, iron uptake systems [43, 44], and homologues of the HtrA chaperone, NarG nitrate reductase and PepD peptidase [47]. However, their roles in virulence, if any, remain largely speculative [2,3].

The *R. equi* genome

To gain insight into the biology and virulence mechanisms of *R. equi*, in 2004 we determined with the Sanger Institute and an international collaborative consortium the complete genome sequence of a prototypic foal isolate, strain 103S (accessible at NCBI http://www.ncbi.nlm.nih.gov/nuccore/NC_014659). An overview of the genome was presented at a Havemeyer Foundation-sponsored international workshop we organised in 2008 in Edinburgh [10], and a paper with a detailed analysis has been recently published [11] (<http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1001145>). *R. equi* 103S has a genome of just above 5 millions base pairs, with a circular chromosome of 5,043 Kb and a virulence plasmid of 80.6 Kb. The G+C content is 68.8%. Whole genome comparisons showed it is highly similar to that of the soil-restricted versatile biodegrader *Rhodococcus jostii* RHA1 (9.7 Mb) [50] and of two recently sequenced environmental rhodococci, *Rhodococcus erythropolis* PR4 (6.89 Mb) and *Rhodococcus opacus* B4 (8.17 Mb) (<http://www.nite.go.jp/index-e.html>); it is, however, significantly smaller in size, due to genome expansion in environmental rhodococci, not reductive evolution in *R. equi*. Next in overall genome similarity was *Nocardia farcinica* IFM 10152 followed by Mtb (Fig. 1).

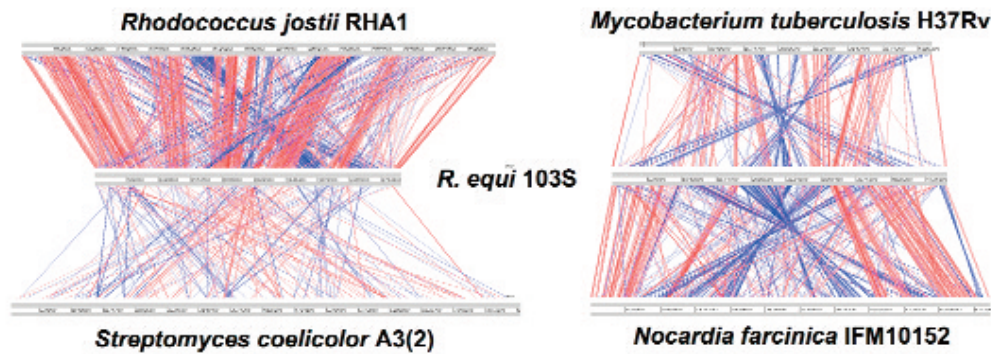


Fig. 1. Pairwise chromosome alignments of *R. equi* and selected Actinobacteria. Red and blue lines connect homologous regions (tBLASTx) in direct and reverse orientation, respectively

Niche adaptive features

The 103S genome possesses a large complement of genes involved in lipid metabolism but lacks sugar transporters and PTS components, consistent with the inability of *R. equi* to use carbohydrates. *R. equi* is capable of synthesizing all amino acids from inorganic nitrogen and has the potential for anaerobic respiration via denitrification and nitrogen assimilation from nitrate/nitrite (Fig. 2). This metabolic profile probably represents a specialization vis-à-vis two key *R. equi* habitats: the volatile fatty acid-dominated environment of herbivores' distal intestine and feces; and the intramacrophage and granulome environments, presumably rich in membrane-derived lipids and poor in amino acids, sugars and oxygen. The inability to use sugars is unique among related actinomycetes and possibly confers a competitive advantage against carbohydrate-fermenting intestinal microbiota (which generate large quantities of short-chain fatty acids, a primary carbon source for *R. equi*). It lacks the extensive metabolic network and catabolic abilities of environmental rhodococci, as well as the extensive secondary metabolism found in many other Actinobacteria. In contrast, it has a larger than average secretome and many surface proteins (406 or 8.9% of genes) and regulators (464, 10.26% of genes), consistent with its dual lifestyle as soil saprotroph and intracellular parasite. *R. equi* is also well endowed to survive desiccation, important for its dustborne transmission during hot, dry weather. As typical in soil bacteria, the 103S genome is heavily shielded with an array of antibiotic resistance determinants, including 10 β -lactamases, five aminoglycoside phosphotransferases and four multidrug efflux pumps. This illustrates how naturally selected resistance traits may have a significant impact on the clinical management of bacterial infections [11].

Cooptive virulence

The *R. equi* genome has provided important clues to understand how virulence was shaped in this pathogen and in the actinomycetes. Our findings suggest a mode of virulence evolution in which a few decisive niche (host)-adaptive HGT events in a direct ancestor of *R. equi*, such as the acquisition of the plasmid vap "intramacrophage survival" PAI, triggered the rapid conversion of a "preparasitic" commensal organism into a pathogen via the appropriation or cooption of pre-existing bacterial functions. Gene cooption is a key evolutionary mechanism by which traits that evolved for one purpose serve new functions in different circumstances, thus allowing rapid adaptive changes. A way in which gene cooption operates is through critical modifications in the expression of the appropriated genes to adapt their function to the new needs.



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