

Phylogenomics of the genus *Alcaligenes*: proposal of *Alloalcaligenes* gen. nov.

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The genus *Alcaligenes* (sensu lato) is comprised by 17 species validly published under the ICNP, with 7 effectively (not validly) published species, albeit many of them reclassified into other or novel genera. In contrast, *Alcaligenes* (sensu stricto) is currently comprised of four validly published species (viz. *A. faecalis*, *A. aquatilis*, *A. pakistanensis* and *A. endophyticus*) predominantly represented by *A. faecalis* in public genome sequence databases (> 70%). Strains identified as species of *Alcaligenes* (sensu stricto) have been isolated from human clinical samples, insect gut, agricultural soil, phyllosphere, rhizosphere, marine sediment, marine and fresh water, polluted ecosystems, and industrial sites, showing a ubiquitous presence. *Alcaligenes* (sensu stricto) spp. strains are halotolerant (NaCl tolerance up to 8% w/v), and others able to degrade aromatic compounds (e.g. phenol, etc.). Direct ammonia oxidation (Dirammox) discovery under aerobic conditions in strains of *Alcaligenes* spp. suggests its implication and relevance for the global nitrogen cycle. In this study, a detailed analysis of the *Alcaligenes* genus based on phylogenomics was conducted to understand the genus and species boundaries within *Alcaligenes*, with a special focus on osmoprotective capabilities, aromatic catabolism and Dirammox metabolism. *Alcaligenes* (sensu stricto) genome sequences (n = 73) were retrieved from the NCBI database, including an additional 29 genome sequences from the species type strains of *Alcaligenes* (sensu lato) and *Alcaligenaceae* genera, as outgroups. Three missing genome sequences of type strains of *Alcaligenes* were determined and assembled. Quality assessment of the assemblies were evaluated, using CheckM, admitting a total of 94 genomes for further analysis. Based on whole-genome comparison, using a core genome tree, obtained by PhyloPhlan3.0, average nucleotide identity (ANIb) and digital DNA-DNA hybridization (dDDH), using the TYGS server, we showed that eight species-clusters comprise the diversity of *Alcaligenes* (sensu stricto), supporting the reclassification of *Alcaligenes faecalis* subsp. *parafaecalis* and *Alcaligenes faecalis* subsp. *phenolicus* to the species level. Genomic context analysis showed that the *dnfABCD* gene cluster associated with Dirammox, the ectoine-hydroxyectoine biosynthetic gene cluster (*ectABCD*) and the phenol hydroxylase encoding gene cluster *dmpKLMNOP*, were conserved in the *Alcaligenes* (sensu stricto). Average amino acid identity (AAI), GC content, and the lack of distinctive metabolic traits for *Alcaligenes* (sensu stricto) confirmed that *Alcaligenes endophyticus* represents a novel genus of the *Alcaligenes* (sensu lato) complex, proposed as *Alloalcaligenes* gen. nov.

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