GENOMIC FEATURES OF *PSEUDOMONAS PUTIDA* STRAIN KT2440

Vitor A.P. Martins dos Santos¹, Kenneth N. Timmis¹,², Burkhard Tümler³ and Christian Weinel³

¹Division Microbiology, Gesellschaft für Biotechnologische Forschung, Braunschweig, Germany
²Department of Biological Sciences, University of Essex, Colchester, UK
³Klinische Forschergruppe, Medizinische Hochschule Hannover, Germany

1. INTRODUCTION

*Pseudomonas putida* strains are rapidly growing bacteria, frequently isolated from most temperate soils and waters, particularly polluted soils. They are nutritional opportunists *par excellence* and a paradigm of metabolically versatile microorganisms that recycle organic wastes in aerobic and microaerophilic compartments of the environment, and that plays a key role in the maintenance of environmental quality. *P. putida* strain KT2440², ⁵³ is probably the best-characterized saprophytic laboratory Pseudomonad that has retained its ability to survive and function in the environment. The bacterium is a plasmid-free derivative of a toluene-degrading bacterium, originally designated *Pseudomonas arvilla* strain mt-2⁴⁶ and subsequently reclassified as *P. putida* mt-2⁴³, ⁶⁸. It is the first Gram-negative soil bacterium to be certified by the Recombinant DNA Advisory Committee (RAC) of the United States National Institutes of Health as the host strain of a host-vector biosafety (HV1) system for gene cloning in Gram-negative soil bacteria²¹. An extensive spectrum of versatile genetic tools, in particular mini-transposons and tools based on these, have been developed for its analysis, manipulation and use as

*Pseudomonas, Volume 1*, edited by Juan-Luis Ramos
a host for cloned genes from other soil organisms\cite{12,13,35,41}. KT2440 is being exploited in the development of a variety of biotechnological applications, including the design of new catabolic pathways for pollutants\cite{19,51,56}, the production by biocatalysis of intermediates, including chiral synthons for chemical syntheses\cite{72}, and quality improvement of fossil fuels, for example by desulphurization\cite{24}. KT2440 is also able to colonize the rhizosphere of a variety of crop plants, such as corn, wheat, strawberry, sugar cane and spinach\cite{20}, and is being used to develop new biopesticides and plant growth promoters that function in the plant rhizosphere.

The sequencing of the KT2440 genome by a German–American consortium\cite{44}, and comparisons of the genome sequences of KT2440, \textit{P. aeruginosa} strain PAO1 and \textit{P. syringae} strain DC3000, have provided significant new insights into the biology of this paradigm of an important and ubiquitous group of soil bacteria, and the underlying genomic basis of its biosafety features, and have further increased the utility of this model laboratory organism and its biotechnological applications.

2. GLOBAL GENOME FEATURES

The genome of strain KT2440 consists of a single circular chromosome of 6,181,863 base pairs (bp), whose G+C content varies between 43% and 69% (windows of 4 kbp), and has a mean value of 61.6%. The G+C content exhibits a Gaussian-like distribution with a maximum at 63.3% and a skew towards lower values. This skew mostly results from gene islands, phage genome sequence and transposons (see below) and is even more evident in the frequency distribution of tetranucleotide sequences (Figure 1). In this respect, the spatial tetranucleotide composition of the \textit{P. putida} genome is intermediate between the homogeneous \textit{P. aeruginosa} strain PAO1 genome, which contains only a few small islands, and the \textit{P. syringae} strain DC3000 genome, which exhibits a bipartite distribution (Figure 1).

In most prokaryotic genomes, the leading strand for DNA replication is rich in G and the lagging strand is rich in C, such that the origin (ori) and the terminus of replication (ter) are indicated by the change of the GC skew along the chromosome. The ter locus in \textit{P. putida} KT2440 is asymmetrically located with respect to the ori at 3,730,000 ± 10,000 bp. GC skew is similar in \textit{P. aeruginosa} and in \textit{P. putida} (leading strand 0.00322 ± 0.0014; lagging strand − 0.0339 ± 0.0014; mean ± variance). Oligo(C) and oligo(G), which predispose to the A-DNA conformation, are strongly underrepresented in the G+C-rich \textit{P. putida} and \textit{P. aeruginosa} genomes, suggesting that stretches of A-DNA are counterselected. A similar pattern has also been observed in other G+C rich genomes, such as those of the \textit{Actinobacteria}, alpha-, beta- and