



ALICYCLIPHILUS SP. BQ1, BUT NOT *A. DENITRIFICANS* K601 AND BC, IS ABLE TO GROW IN N-METHYL-2-PYRROLIDONE: GENETIC DIFFERENCES THAT MIGHT BE THE ORIGIN OF THIS CAPACITY

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Introduction. We have isolated, from decomposing foam collected at a garbage dump, a bacterial strain BQ1 identified as *Alicyclophilus* sp. This strain is able to grow in minimum medium containing a commercial-polyurethane varnish (Hydroform[®]) as carbon source (MM-PUh). Chemical analysis (IRS and GC-MS) showed that Hydroform also contains N-methyl-2-pyrrolidone (NMP), a cyclic amide (γ -lactame). Because of its high miscibility in water, ethanol and many organic compounds, NMP is used as additive and solvent in many chemical industries. However, evidences indicate that NMP is genotoxic and teratogenic. We have demonstrated that besides attacking PU bonds, BQ1 degrades NMP (1). On the other hand, the strains *A. denitrificans* K601 and BC are able to use diverse cyclic compounds and their genomes have been sequenced.

To determine the genetic bases supporting BQ1 growth on NMP, we compared some microbiological and biochemical features of *Alicyclophilus* strains K601, BC and BQ1, and analyzed their genome organization and the expression of some genes.

Methods. Vitek analyses and growth measurements in MM with NMP, a polyol (a PU precursor), and Hydroform as carbon sources were performed for the three strains. Transposition mutagenesis on BQ1 was performed by using the HiMar transposon (2). Genomic sequencing of BQ1 using the Illumina system, and qRT-PCR from cultures growing on MM-NMP and MM-PUh, to determine gene expression of candidate genes, were performed.

Results. Vitek analyses showed few biochemical differences. Growth in control MM-malate was similar for the three strains and in MM-polyol was slightly better for BQ1. BQ1, but not K601 or BC, was able to grow in MM-NMP and MM-PUh (Fig. 1) more than two-fold and four-fold respectively, than in MM-malate.

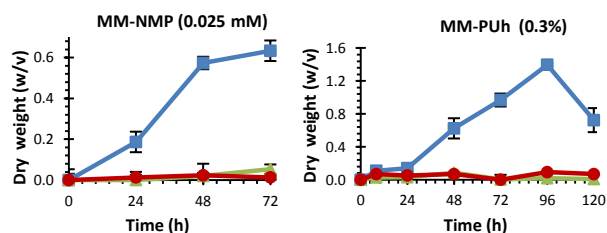


Fig. 1. Growth of BQ1 (squares), K601 (triangles) and BC (circles) strains.

Strains 9 and 38 unable to grow in MM-NMP/MM-PUh were selected from a screening of 800 mutants. The inactivated genes are closely linked and encoded a hypothetical protein and a glyoxalase, respectively. Gene 9 forms part of a four gene cluster (*orfABCD*) not present in the K601 and BC genomes (Fig. 2). qRT-PCR showed that the *orfABCD* is induced when BQ1 is grown in MM-NMP/MM-PUh.

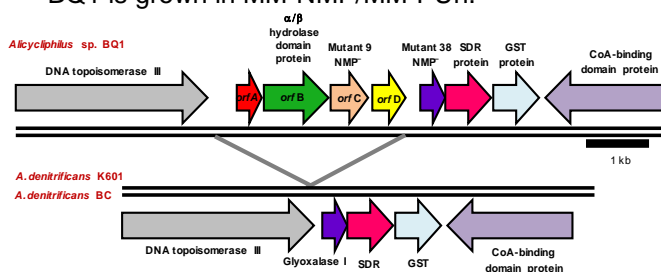


Fig. 2. Genomic organization of BQ1, K601 and BC strains.

Conclusions. *Alicyclophilus* sp. BQ1, but not *Alicyclophilus denitrificans* K601 and BC, is able to grow in NMP. This difference might be based in the presence of a 3 kb DNA insertion in the genome of BQ1 which contains a gene encoding a putative α/β hydrolase protein and three other genes encoding unknown proteins. These genes are up-regulated in MM-NMP/MM-PUh cultures.

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