Micro Biotec'13

PORTUGUESE CONGRESS OF MICROBIOLOGY AND BIOTECHNOLOGY

6th – 8th December | Aveiro Portugal

Abstracts Book





Environmental Microbiology and Biotechnology

P105

BACILLUS INVICTUS SP. NOV., A NEW SPECIES ISOLATED FROM MEDICINAL PRODUCTS IN PORTUGAL

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Three Gram-positive, rod-shaped endospore-forming *Bacillus* isolates Bi.FFUP1, Bi.FFUP2, and Bi.FFUP3 recovered in Portugal from medicines' contaminants were subjected to a polyphasic study. Acid production from mannose and its absence when inositol, mannitol, methyl α -D-glucopyranoside, maltose, turanose and L-tryptophan is tested, discriminate these new isolates from its closest relatives, Bacillus pumilus and Bacillus safensis. Additionally, a significant different protein and carbohydrate signature was evidenced by spectroscopic techniques. PCA (principal component analysis) of this spectral data clearly delineated the novel species isolates. 16S rDNA analysis placed isolates within the genus *Bacillus* with highest similarities (\geq 99.7%) with *B. safensis* and B. pumilus. Nevertheless, only 49-50% DNA relatedness (DDH studies) was observed between Bi.FEUP1 and B. safensis FO-036bT. Variable DDH values were obtained when Bi._{FEUP1} was compared with *B. pumilus*ATCC 14884 ($39.75\% \pm 0.35$) and ATCC 7061⁺ (69.40% $\pm \alpha 2$), despite the genotypic and phenotypic similarity of these two *B. pumilus* strains, highlighting the ambiguities in the taxonomic grouping based on a strict DDH cutoff. However, novel species isolates share 93.0% gyrB similarity with B. pumilus ATCC 14884 and 7061^T and 91.3% with *B. safensis* FO-036b^T. Further, *rpoB* similarity of 96.4% with close reference strains, together with gyrB and rpoB phylogenetic tree topology supported the delineation of a new species. On the basis of phenotypic characteristics (metabolic profile, protein and carbohydrate content) and phylogenetic analyses of rpoB and gyrB sequences, the three isolates represent a novel species of Bacillus genus, for which the name Bacillus invictus sp. nov. is proposed, with strain Bi.FEUP1 as the type strain.