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SINGLE-MOLECULE FORCE SPECTROSCOPY TO COMPARE THE SURFACE PROPERTIES OF FOOD PROPIONIBACTERIA STRAINS WITH DISTINCT ABILITIES FOR EXOPOLYSACCHARIDE PRODUCTION

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P. freudenreichii to simultaneously improve texture and healthiness of cheese

Propionibacterium freudenreichii (PF) is a food grade bacterium with “generally recognized as safe” status, most commonly used as a starter for the manufacture of fermented dairy products such as Emmental and Leerdammer cheeses (Fig. 1). It is also known for its production of vitamin B12 and propionic acid, and for its remarkable anti-inflammatory properties^[1,2]. Exopolysaccharide (EPS) production by food bacteria such as *PF*^[3,4] is receiving increasing attention for their potential applications in simultaneously improving texture and the health properties of common foodstuff in the western diet. In this work, we explored the genetic and phenotypic bases for the surface properties of the EPS-producing *PF* strain P52.

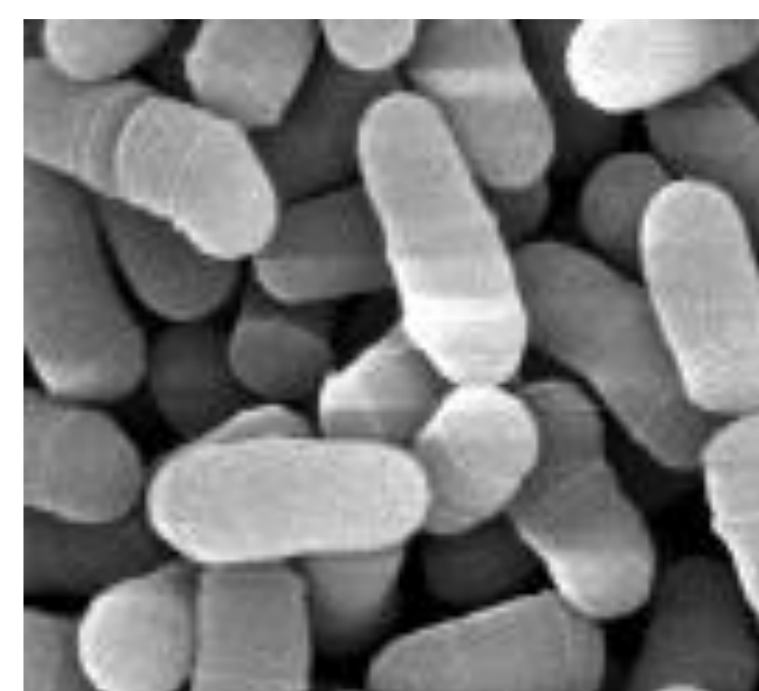


Fig.1 *Propionibacterium freudenreichii* (left image^[3]) is a usual starter for the manufacture of hard-cheeses such as Emmental or Leerdammer.

Spontaneous mutation of P52 eliminates the EPS production

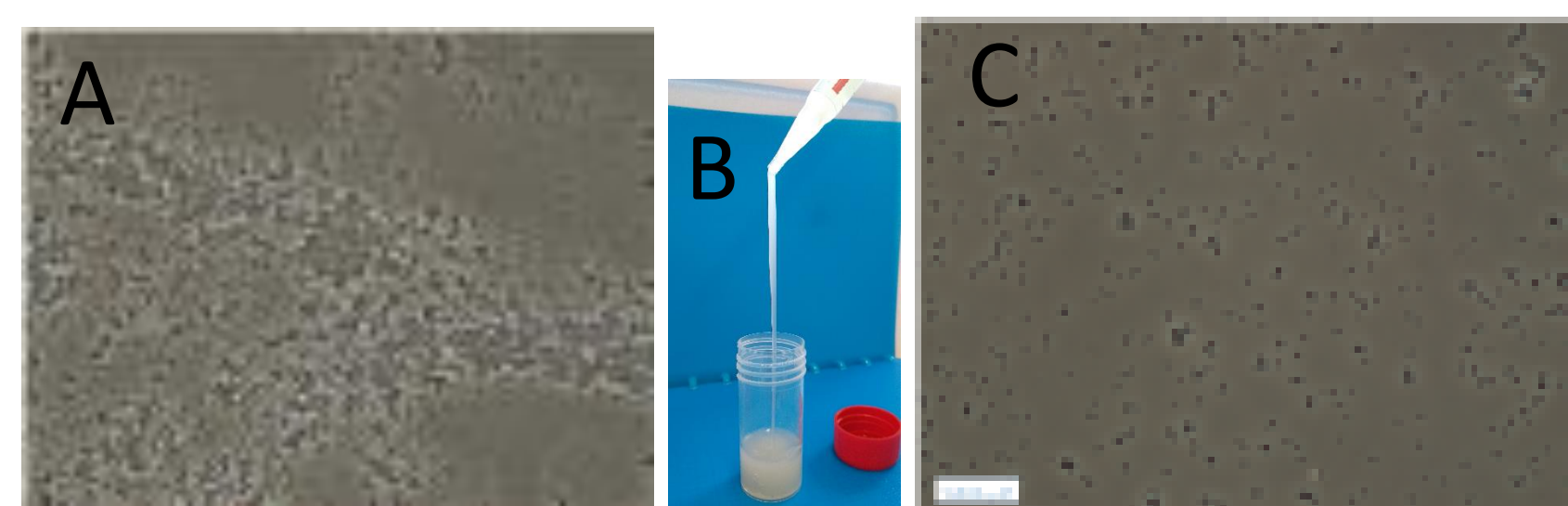


Fig.2 Microscopic observation of cells of *Propionibacterium freudenreichii* P52 (A) and its mutant (C).

Cells of *PF* P52 are strongly aggregative when grown in liquid culture medium (A) and the culture presents a rosy phenotype (B). After consecutive subcultures, a mutant was spontaneously obtained, which lost the aggregative and rosy phenotypes (C).

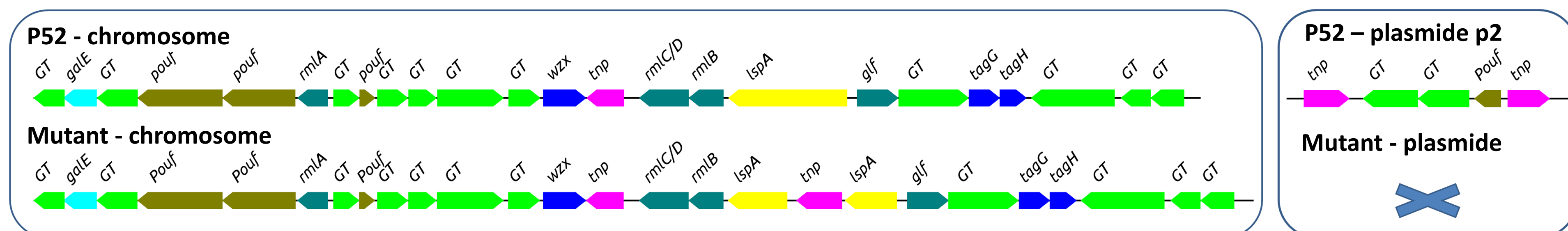


Fig.3 Physical map of the *eps*-genes cluster in the rosy strain *PF* P52 and in its non rosy spontaneous mutant. Genes are categorised according to their potential function: ■ Precursor biosynthesis, ■ Glycosyltransferases, ■ transport/polymerization, ■ surface protein, ■ transposase, ■ unknown function.

The genome of *PF* P52 was sequenced. It is constituted of one chromosome and 2 plasmids (p1 and p2). The EPS encoding genes are distributed at two locations: on the chromosome (23 genes) and on the plasmid p2 (3 genes). In the mutant, the EPS encoding genes located on the chromosome are 100% conserved compared to *PF* P52, excepted *lspA*, a gene not described as implicated in the EPS biosynthesis which is disrupted by a transposase. The 3 plasmidic EPS encoding genes are missing, as the plasmid p2 is lost in the mutant, making impossible the production of EPS.

The mutant exhibits softer surface and significantly less and shorter EPS polymers

Cells of *PF* P52 or its mutant in stationary phase were washed in HEPES-Na buffer at pH 6.8 then immobilized in presence of Ca and Mn ions onto silanized glass slides pre-coated with the Concanavalin A lectin (Con-A) using carbodiimide chemistry.

PF P52

mutant

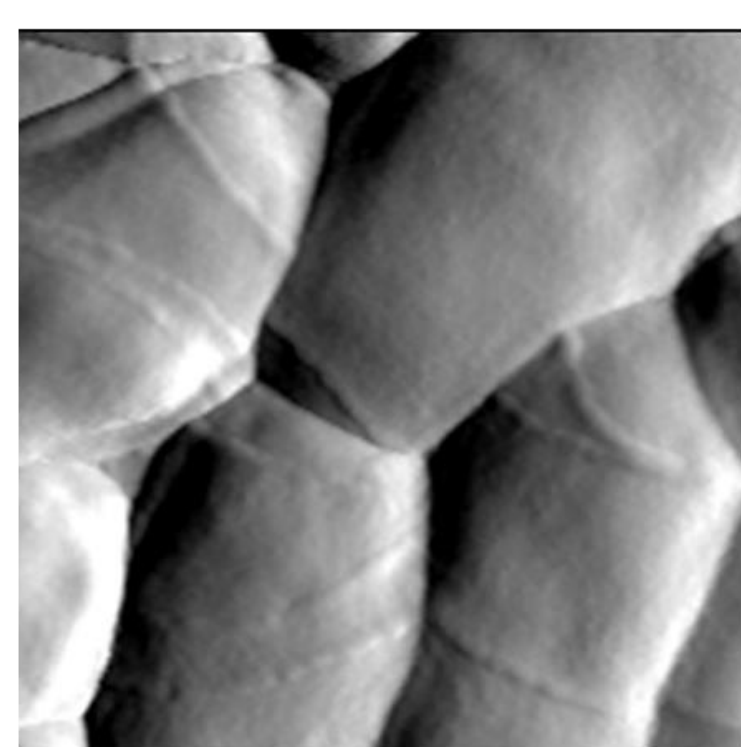
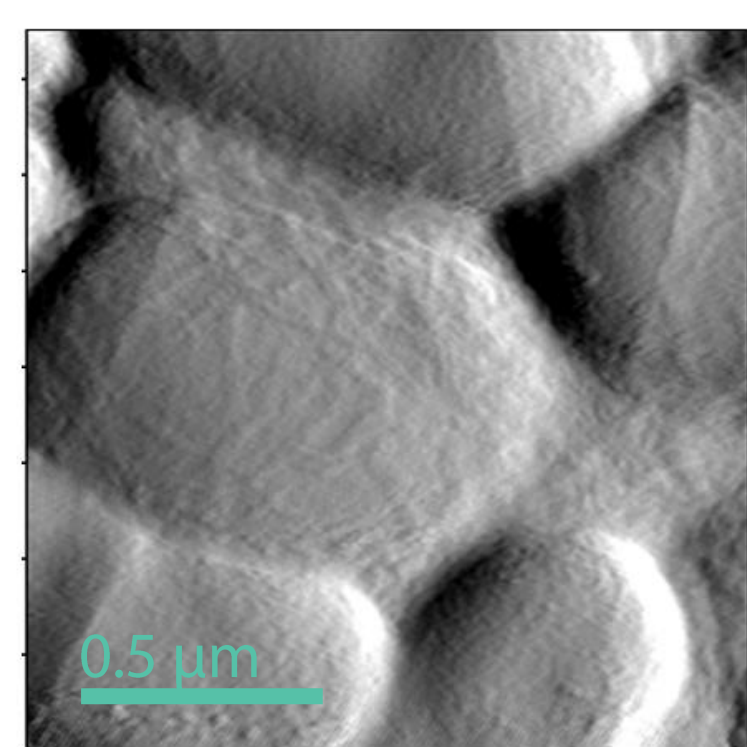


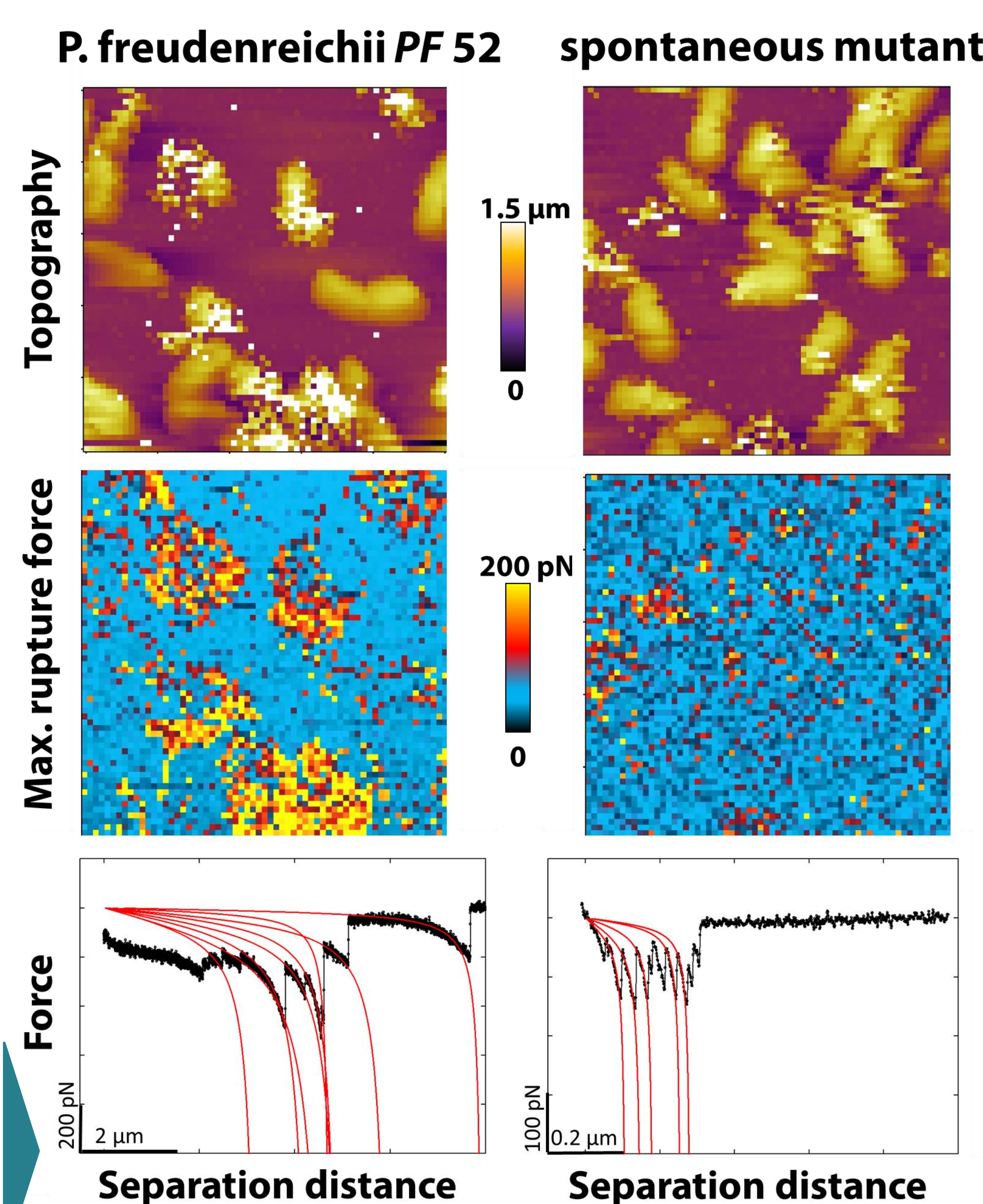
Fig.4 Deflection images of *P. freudenreichii* P52 (left) and its spontaneous mutant (right) obtained in air with an MFP-3D AFM and MSNL probes ($k \sim 0.03 \text{ N.m}^{-1}$)

Contact mode atomic force microscopy (AFM) images obtained in air showed that *PF* P52 cells exhibited a thick capsule of entangled filaments that had disappeared in the spontaneous mutant (Fig.4).

Using Con-A-grafted MSNL probes, single-molecule force spectroscopy was used to stretch EPS polymers over the immobilized individual bacteria^[5,6] in HEPES-Na buffer in presence of Ca and Mn.

Correlated topography and adhesion force maps confirmed that the EPS capsule, containing glucose and/or mannose, was significantly lesser around the mutant than around *PF* P52 bacteria. Where present, retraction force curves evidenced that spontaneous mutation could lead to 10-fold reduction of the length of the EPS polymers in the mutant. Post-treatment using the extended Freely Jointed Chain model (FJC+) indicated up to 30×10^3 Kuhn segments for the EPS-producing mutant, vs 5×10^3 for the mutant.

Fig.5 Topography force maps, adhesion force maps and typical retraction force curves with applied Freely Jointed Chain + model (in red) obtained using Con-A-grafted AFM probes over immobilized *P. freudenreichii* P52 (left) and its spontaneous mutant (right) in HEPES-Na-Ca-Mn buffer at 21°C and pH 6.8.



References

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