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Background The host-dependent (H-D) *Bdellovibrio bacteriovorus*, bacterium can spontaneously shift to axenic, host-independent (H-I) growth under laboratory conditions. H-I strains were shown to be able to form biofilms. The genetic elements involved in this shift are less understood. **Methods** In this work, 350 spontaneous H-I strains of *B. bacteriovorus* H-D100Sm were assessed based on their biofilm-formation ability, defining low (LBF), medium (MBF) and high (HBF) biofilm formers. **Results** H-I isolates displayed predatory abilities, and enhanced biofilm-formation under low temperature (18°C), in pH ranging from 6 to 8, and with divalent cations (Mg²⁺ and Ca²⁺). Enzymatic assays and specific staining revealed that the biofilm-matrix comprises extracellular proteins, carbohydrates and little DNA. The genomes of H-D100Sm and of seven H-I strains from three biofilm-forming classes were sequenced. The LBFs MHI154 and MHI167 had only a single mutation in the flagellum machinery gene *fliL* (*Bd1076*). FliL is associated with the stator proteins MotAB. Moreover, MHI153, one of the two MBFs, had a single mutation in *motA*. Both LBFs had an intact flagellum machinery but slower motility. Expression analysis revealed that flagellar motility, chemotaxis, biofilm formation, and prey-invasion-related genes were significantly deregulated in both LBFs. Furthermore, *fliL* deletion in HBF backgrounds led to reduced biofilm-formation. Lastly, *fliL* complementation in MHI154 restored a wild-type like H-D phenotype, while, *fliL* deletion from the H-D wild-type resulted in MHI154-like phenotypes. **Conclusions** Taken together, these results support a central role for FliL as necessary for maintaining the H-D phenotype, and affecting initial steps of H-I biofilm formation.

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