## Supplementary Figure 1



## **S1 Fig: Overexpression, gene replacement mutant generation and characterization of** *rodA* and *pbpA*.

(a) Fresh cultures of *Msm::pN*, *Msm::pN-r*, *Msm::pN-p* were seeded at an initial A<sub>600</sub> of 0.1 in 7H9 medium and the cells were cultured in presence of 5 μM isovaleronitrile for 12 h. at 37°C, 100 rpm followed by fixation. Morphology of the cells was observed through scanning electron microscopy at 10,000X. Scale bar, 1.0 μm. Mean cell lengths obtained are *Msm::pN*-5.178 μm; *Msm::pN-r*-10.23 μm and *Msm::pN-p*-6.706 μm.

(b) Cell lengths were measured independently using Smart Tiff software and plotted as scattered dot plot with mean values using GraphPad Prism6. Similar results were obtained in two independent experiments performed in duplicates. Statistical analysis was performed with the help of One way ANOVA test. \*\*\*\*, P<.0001.

(c) Schematic depiction of strategy used for the generation of gene deletion mutants. Primers used for the PCR confirmation are indicated

**d-e**. Genomic DNA was isolated from log cultures of wild type and mutants and PCR reactions were performed with defined set of primers (as indicated). Lane1; Mr=1 kb ladder; lane 2: *Msm*; lane3: *Msm* $\Delta r$ ; lane 4: *Msm* $\Delta p$  and lane 5: *Msm* $\Delta rp$ .

Expected size for the F1 and R1 pair (d) are Msm-1416 bp;  $Msm\Delta r$ -1300 bp;  $Msm\Delta p$ -1416 bp and  $Mtb\Delta rp$ -nil. Expected size for the F2 and R2 pair (e) are Msm-1476 bp;  $Msm\Delta r$ -1476 bp;  $Msm\Delta p$ -1300bp and  $Mtb\Delta rp$ -nil. (f) Western blots analysis to check polarity effects: WCLs prepared from  $Msm\Delta r$ ,  $Msm\Delta p$  and  $Msm\Delta rp$ strains were resolved and probed with  $\alpha$ -PstP,  $\alpha$ -PknA, and  $\alpha$ -GroEL-I antibodies.