**S3 Text  Comparison of orphan kinase catalytic domain content**

The differences between the orphan kinases associated with the two pathway architectures extend beyond specificity residues: the histidine kinase catalytic domains found in the two sets also differ. Histidine kinase catalytic domains have been categorized into 23 subfamilies based on HMM models of multiple sequence alignments [Alexander and Zhulin, 2007]. While many of these catalytic domain subtypes are present in kinases of the Firmicutes, only two, HK_Ca:2 and HK_Ca:3, are commonly observed in orphan kinases. In genomes with a predicted direct phosphorylation Spo0 pathway, 51 out of 53 orphan kinases (96%) possess a HK_Ca:2 type catalytic domain. In genomes with a predicted Spo0 phosphorelay, 130 out of 155 orphan kinases (86%) possess a HK_Ca:3 type catalytic domain. The same dichotomy is observed for experimentally verified kinases: direct phosphorylation architecture Spo0 kinases in *R. thermocellum*, *C. difficile*, and, with one exception, *C. acetobutylicum* encode an HK_Ca:2 type catalytic domain [Mearls and Lynd, 2014; Steiner et al., 2011; Underwood et al., 2009]. The phosphorelay sporulation kinases in *B. subtilis*, *D. acetoxidans*, and other experimentally verified Spo0 phosphorelays harbor a HK_Ca:3 type catalytic domain (see Table S1 for a complete list). The one exception in the experimentally verified set is Ca_C3319 in *C. acetobutylicum*, which encodes an HK_Ca:3 domain.
Bibliography


