POSTER - PHYLOGENY AND EVOLUTION

NOVEL PILZN-PILZ FUSION FAMILY AND SPIROCHAETAL PILZ CENSUS

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Leptospirales family has a major expansion in PilZ family members, in comparison to other Spirochetes, which contain up to 3 orthologs of PilZ fusions, whilst Leptospirales such as Leptospira, Turneriella and Leptonema have up to 7 orthologs of PilZ fusions and maintain the high of other PilZ family members as all Spirochetes have. DUF1577 is a member of PilZ fusion member, that includes PilZN+GAF+PilZ architecture, in which the GAF portion is interrupted by the first beta-sheet of PilZ, what could indicate a insertion from known PilZN-PilZ(flagellar regulator, MrkH), which controls motility of the flagella modulated by cytosolic c-di-GMP concentration. Spirochetales present several PilZ fusions such as MrkH-like, NpzN (N-terminal helical region PilZ transmembrane protein), and PilZ+PilZ proteins. In Leptospira interrogans there are at least 6 paralogs of DUF1577, which is found only in Spirochete phylum. In order to retrieve every PilZ family member, a search containing pfam PilZ models and our group PilZ models were used as seed, also considering the whole DUF1577 proteins from Leptospira as seed to jackhmmer iterative searches against a 1256 Spirochetal genome database. The resulting sequences were clustered with MMseqs2 to remove redundancy, and groups of

80 % coverage and 10e-3 evalue were aligned to contain their 80 % coverage 70 % identity representative proteins.

Analysing those clusters, we were able to expand the detection of DUF1577, by splitting into its subdomains, characterized as PilZN, PilZ and GAF also building a wider model for PilZ including the divergent GAF insertion. Some MrkH fusions presented a unknown N-terminal extension, here named NpzN, that is predicted structurally to interact with the membrane. Dali searches were made in order to establish the fold of FMN-binding from DUF1577 model, showing that searches for the whole protein only gather proteins similar to MrkH, that have a conserved PilZN adjacent to a PilZ domain, but with no matches for the GAF insertion, except when only the GAF insertion was the search query, although the N-terminal of the insertion is not detected, only the last 2 a-helices were overall retrieved from the GAF family. Further iterative searches showed a connection between PilZN and PilZ, but none retrieved the GAF(PAS-like) insertion. DUF1577 orthologs were thoroughly annotated ranging only from 3 to 8 paralogs in Spirochetes, presuming it’s origin occurred in the root of spirochetal lineages, and recent duplications are shown in most Leptospirales, mainly on pathologic strains, which accumulate up to 3 fold PilZ family member proteins as of Spirochetales other lineages.