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THE TERMINAL GLYCAN MOTIF OF BURKHOLDERIA PSEUDOMALLEI CAPSULAR POLYSACCHARIDE IS UNIQUE AMONG THE BACTERIAL SPECIES: A BIOINFORMATICS APPROACH

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MOTIVATION

- ✓ *Burkholderia pseudomallei,* a Gram-negative bacillus, is the causative agent of ***melioidosis*** [1], a potential fatal infection of humans and animals.
- ✓ Listed as a select agent of potential bioterrorism by the CDC and as a category B Priority Pathogen by the NIAID.
- ✓ No prophylaxis/vaccine is available.
- ✓ Intrinsically resistant to penicillin and gentamycin. Treatment is very costly.
- ✓ Needs better diagnostic reagent/s.

OBJECTIVE

- ✓ Bioinformatics analysis of the Capsular Polysaccharides from *Burkholderial* species to identify the glycan motif/**glycocode** that can be utilized for developing a potential diagnostic reagent and, perhaps, a vaccine.

BACKGROUND

- ✓ Datta Consulting Group is engaged in the Bioinformatics analysis of the Glycome, particularly to identify Glycocode/s involved in various pathophysiological processes [2-4].
- ✓ All the pathogenic species of *Burkholderia* express capsular polysaccharides (CPS), which are both a virulent factor and a protective (to the bacteria) antigen.
- ✓ Among the CPSs, CPS-1 consisting of 1,3-linked unbranched homopolymer of 2-O-acetylated 6-deoxy-β-D-manno-heptopyranosyl residues, is present in all the strains of this pathogenic species [5]. This may serve as a potential diagnostic marker for *B.pseudomallei*.
- ✓ A monoclonal antibody (mAB, 4C4) has been shown to be specific for this structure [6] raising the possibility that this 4C4 can be used for developing a diagnostic reagent for *B.pseudomallei*.

BIOINFORMATICS ANALYSIS OF CPS

- ✓ CSDB is the largest database focusing on the structures of glycans and glycoconjugates in prokaryotes, plants, and fungi [7, 8].

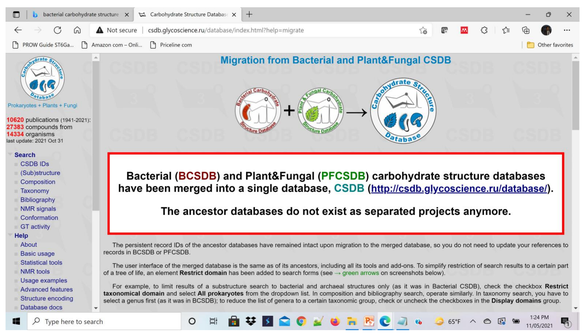

Figure 1. CSDB, available at http://csdb.glycoscience.ru/database is the result of the merge of previously developed bacterial (BCSDB) and plant & fungal (PFCSDB) carbohydrate structure databases.

- ✓ To begin with, *B.pseudomallei* strain 824a, which has PS-1 but not PS-II [9], is first used to check the search results and the whole database.

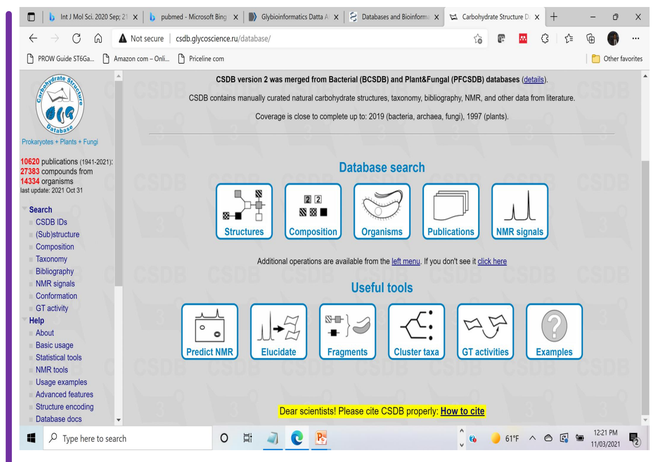

Figure 2. CSDB provides various search options. It also provides useful tools for statistical analysis and tools for comparative studies of carbohydrate contents [10].

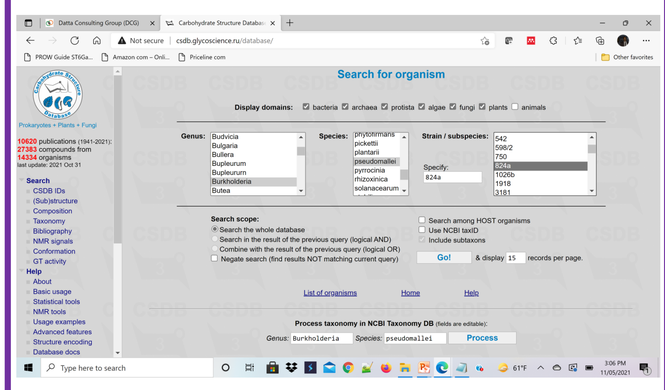

Figure 3. CSDB interface for searching the *B.pseudomallei* strain 824a.

- ✓ Such analysis was repeated for other strains and other bacterial species to check the current status of the database for carbohydrate structures.
- ✓ CPS-1 structure, ->3)-β-D-6dManHepp2OAc-(1->, was then used to search the whole CSDB database.
- ✓ This specific structure and its variations were used to search other carbohydrate structural databases [2] including GlyTouCan [11].

RESULTS AND DISCUSSIONS

- ✓ Searching the whole database for *B.pseudomallei* strain 824a (Organism ID: 5728) showed the following results:

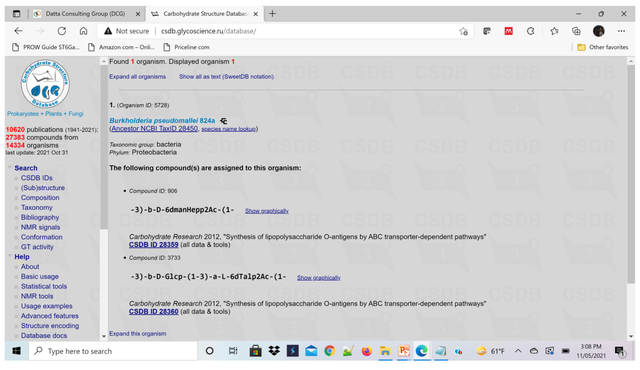

Figure 4. CSDB interface for searching the *B.pseudomallei* strain 824a.

- ✓ The results were available both in the text and graphics (SNFG or **S**ymbol **N**omenclature **F**or **G**lycans) for the carbohydrate structures. Two structures were shown:

-3)-b-D-6dmanHepp2Ac-(1-, Compound ID: 906 representing PS-I, and -3)-b-D-Glcp-(1-3)-a-L-6dTalp2Ac-(1-, Compound ID: 3733, along with the publication (CSDB ID 28359 [7]); this result showed the current status of the CSDB database. As of today, these published carbohydrate structures are made available in the CSDB. The search results also identified two other pathogenic strains of *Burkholderia pseudomallei*: 304b and 57576 . All these three strains belong to the NCBI Taxonomy ID 28450.

- ✓ We then used compound ID 906, which is shown as -3)-b-D-6dmanHepp2Ac-(1- in the CSDB, for the successive analysis.
- ✓ There are couple of options for inserting the structure for searching. One can input the structure using 'Structure Wizard', selecting from' library', drawing in 'SNFG editor', 'Convert from GlycoCT', or using 'expert form'. All these options were tried.
- ✓ Searching using 'expert form' may show parsing error depending on the text term used in the form. For example, using the text term '-3)-b-D-6dmanHepp2Ac-(1-' showed parsing error. It is because, in this mode, open linkages are treated as linkage limitation than polymer indication. The term ' -3)-b-D-6dmanHepp2Ac-(1-' is parsed as ANY (?-3)bD6dmanHepp(%)Ac-(1-?)ANY but not as a repeating unit.
- ✓ For successful parsing, searching was done using CSDB ID: 906.
- ✓ The search results showed the presence of this PS-I structure only in *Burkholderia pseudomallei* 304b, *Burkholderia pseudomallei* 824a and some other pathogenic strains of Burkholderia, but not in other bacterial species.
- ✓ Reviewing the published literature [12] on *melioidosis*, this author could not find any other prokaryotic species that has this unique PS-I carbohydrate structure.
- ✓ Moreover, this unique glycan structure could not be found in any other species by searching other glycan databases [2] including GlyTouCan [11]. GlyTouCan database is designed to contain all the known published carbohydrate structures in the SNFG format [13]. However, when searched, no results for this structure could be found. It is possible that GlyTouCan database is yet to be updated with this PS-I structure from *Burkholderial* species. This work is continued to eliminate such possibility.

CONCLUSION

- ✓ The PS-I or 1,3-linked unbranched homopolymer of 2-O-acetylated 6-deoxy-β-D-manno-heptopyranosyl residues (CSDB compound id 906) of *Burkholderia pseudomallei* is unique among the bacterial, fungal and plant species. This unique glycan structure is also absent in the eukaryotes, thereby, raising the possibility of its use for developing a vaccine. Moreover, this unique structure can serve as a diagnostic marker and reagent, such as, mAB 4C4 has the potential to be developed as the diagnostic reagent.

FUTURE WORK

- ✓ This homopolymer carbohydrate structure needs further analysis to identify the optimal number of carbohydrate units that would provide higher binding efficiency for this monoclonal antibody.

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