The glycan alphabet is not universal: a hypothesis

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**MS-EXCEL file provided separately**: Supplementary Data.xlsx

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| Worksheet 1    | Details of HMM profiles                                                      |
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Figure S1 The number of species for which different number of strains are sequenced. Six or fewer strains are sequenced for most of the species. On the other hand, more than 50 strains are sequenced for 29 species. *Escherichia coli* and *Salmonella enterica* have the highest number of sequenced strains (714 and 602, respectively). Genus and species names are not known for 45 endosymbionts; only their host name is known e.g., *Legionella* endosymbiont. Each such case is considered as a distinct species.
Figure S2a TDP-/dTDP-linked monosaccharides derived from glucose-1-phosphate. Abbreviated names are used for some of the monosaccharides. Full names of these are given in Supplementary_data.xlsx:Worksheet4.
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Figure S2c UDP-linked monosaccharides derived from glucose-1-phosphate. Abbreviated names are used for some of the monosaccharides. Full names of these are given in Supplementary_data.xlsx:Worksheet4.
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Figure S2f CMP- and UDP-linked monosaccharides derived from UDP-Glc2NAc (2 of 2). CMP-Leg5Ac7Ac may be biosynthesized through GDP-linked or UDP-linked intermediates. Abbreviated names are used for some of the monosaccharides. Full names of these are given in Supplementary_data.xlsx:Worksheet4.
Figure S2g  ADP- and GDP-linked heptoses derived from sedoheptulose-7-phosphate.
Abbreviated names are used for some of the monosaccharides. Full names of these are given in Supplementary_data.xlsx:Worksheet4.
Figure S3 Setting bit score thresholds for HMM profiles with varying substrate specificities. TrEMBL database was scanned using the profiles shown along the X- and Y-axes in the above scatter plots; for these scans, default values set by HMMer were used for all the parameters. Hits that are common to a pair of profiles (shown along X- and Y-axes) were chosen and bit scores of such hits were plotted against each other. Bit score thresholds (indicated by red lines) were chosen such that a protein is a hit for only one of the two profiles. Threshold was revised for GPE05331 set to exclude PdeG.
**Figure S4** Variations in the proteome size of organisms which encode the same number of monosaccharides. Only the smallest and largest proteome sizes are shown. As can be seen, the number of monosaccharides used by an organism is independent of the proteome size. For instance, *Helicobacter pylori* PNG84A (proteome size = 1353) uses the same number of monosaccharides (7) as *Sorangium cellulosum* So0157-2 (proteome size = 10480).
Figure S5 (A) The prevalence of each monosaccharide as percentages of the genomes analyzed in this study (viz., 12939) and the number of species covered by these genomes (viz., 3384; Figure S1(a)). The diagonal line is manually drawn to facilitate visualization of deviations. (B) Zoomed in view of the region near the origin in (A). Data for most of the monosaccharides lie on the diagonal suggesting that the sequencing of a large number of strains for a few species has not biased the outcome, with the exception of TDP-dTDP-Fuc4NAc and UDP-L-Qui2NAc. TDP-dTDP-Fuc4NAc (a point below the diagonal line) is present in fewer species but represents a larger fraction of genomes since 679 strains of E. coli contain this monosaccharide. Conversely, presence of UDP-L-Qui2NAc (a point above the diagonal line) is highly strain specific. Abbreviated names are used for some of the monosaccharides. Full names of these are given in Supplementary_data.xlsx:Worksheet4.
| Tool / Database | Version / Release | URL                                      | Reference |
|-----------------|------------------|------------------------------------------|-----------|
| BLASTp          | 2.2.31+          | ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/2.2.31/ | (1)       |
| HMMER           | 3.1b2            | http://hmmer.org/download.html           | (2)       |
| MUSCLE          | 3.8.31           | https://www.ebi.ac.uk/Tools/msa/muscle/  | (3)       |
| CD-Hit          | 4.6              | http://weizhongli-lab.org/cd-hit/        | (4)       |
| UniProt         | 2018_07          | https://www.uniprot.org/                 | (5)       |
| Genome          | 2019_03          | https://www.ncbi.nlm.nih.gov/genome/     | (6)       |
| Pubmed          | Not applicable   | https://www.ncbi.nlm.nih.gov/pubmed/     | (6)       |
| CATH-Plus       | 4.2              | http://www.cathdb.info/                 | (7)       |
| PDB             | Not applicable   | https://www.rcsb.org/                   | (8)       |
| UniRule         | Not applicable   | https://www.uniprot.org/help/unirule     | (5)       |
| SAAS            | Not applicable   | https://www.uniprot.org/help/saas        | (5)       |
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Table S2 Monosaccharides whose both enantiomers are considered in the present study: comparison of the nucleotide to which the enantiomer is linked and the precursor for its biosynthesis

| Monosaccharide | D enantiomer | L enantiomer |
|----------------|--------------|--------------|
|                | Nucleotide   | Precursor    | Nucleotide | Precursor    |
| Rhamnose       | GDP          | Glc-1-P      | TDP, dTDP, UDP | Glc-1-P      |
| 6-Deoxytalose  | GDP          | Glc-1-P      | TDP, dTDP  | Glc-1-P      |
| Galactose      | UDP          | Glc-1-P      | GDP        | Glc-1-P      |
| Fucose         | TDP, dTDP    | Glc-1-P      | GDP        | Glc-1-P      |
| Fuc2NAc        | UDP          | UDP-Glc2NAc  | UDP        | UDP-Glc2NAc  |
| Qui2NAc        | UDP          | UDP-Glc2NAc  | UDP        | UDP-Glc2NAc  |
Flowchart S1 Procedure used to generate HMM profiles

| 1. Generation of Exp dataset and Exp profile, and setting $T_{exp}$ |
|---|
| **Step 1a** | Consider only those enzymes which are characterized by direct enzyme activity assay |
| **Step 1b** | Remove redundancy (80% sequence identity cutoff) and obtain a multiple sequence alignment (MSA) |
| **Step 1c** | Use the MSA as input to generate an HMM profile |
| **Step 1d** | Score Exp dataset sequences against this HMM profile |
| **Step 1e** | Set the bit score of the lowest scoring sequence as the bit score threshold for Exp dataset, $T_{exp}$ |

| 2. Generation of Extend dataset and Extend profile, and setting $T_{extend}$ |
|---|
| **Step 2a** | Add sequences that meet any of the following criteria to the Exp dataset |
| (i) | SwissProt entries satisfying the threshold $T_{exp}$ |
| (ii) | SwissProt entries scoring $< T_{exp}$ provided they show conservation of active site residues. Active site residues were collated based on site directed mutagenesis studies or ligand-bound 3D structures |
| (iii) | TrEMBL entries for which molecular function has been inferred from experiments other than direct enzyme assays viz., complementation assays, phenotypic studies, etc. |
| (iv) | TrEMBL entries with solved 3D structure |
| (v) | FunFam members (CATH database) but only in the case of CDP-glucose 4,6-dehydratase (FunFam 20603) and phosphomannoisomerase family 3 (FunFam 54112) |
| **Step 2b** | Remove redundancy (80% sequence identity cutoff) and obtain a multiple sequence alignment (MSA) |
| **Step 2c** | Use the MSA as input to generate an HMM profile |
| **Step 2d** | Score Extend dataset sequences against this HMM profile |
| **Step 2e** | Set the bit score of the lowest scoring sequence as the bit score threshold for Extend dataset, $T_{extend}$ |
**Flowchart S2** Precedence rules for assigning annotation to proteins that are hits to two or more profiles and/or BLASTp queries

| Case 1 of 14 |
|--------------|
| 
| # specific_aminoTs = [GPE01710, GPE01430, GPE01530]  
| # C3_C4_aminoTs = [GPE01910, GPE01830]  
| # EXPECTED: for a protein which is a hit for one of the specific_aminoTs is expected to be a hit in C3_C4_aminoTs as well as GPE01230  
| IF (hit for any one of specific_aminoTs) THEN  
| IF (hit for any one of C3_C4_aminoTs) THEN  
| IF (hit for GPE01230) THEN  
| Pass (i.e., this is as expected)  
| ELSE  
| Alert: Hit for one of C3_C4_aminoTs but not GPE01230  
| ENDIF  
| ELSE  
| Alert: Hit for one of specific_aminoTs but not C3_C4_aminoTs  
| ENDIF  
| ENDIF  
| IF (hit for any one of C3_C4_aminoTs) THEN  
| IF (hit for GPE01230) THEN  
| Pass (as expected)  
| ELSE  
| Alert: Hit for one of C3_C4_aminoTs but not GPE01230  
| ENDIF  
| ENDIF |

| Case 2 of 14 |
|--------------|
| IF a protein is a hit for any one of specific_aminoTs, it should be assigned that annotation  
| IF (hit for any one of specific_aminoTs) THEN  
| Assign annotation  
| ENDIF  
| ENDIF |

| Case 3 of 14 |
|--------------|
| For a protein which is a hit for one of C3_C4_aminoTs but not any of specific_aminoTs, it should be assigned the former  
| IF (hit for one of C3_C4_aminoTs and not for any of specific_aminoTs) THEN  
| Assign GPE01830/GPE01910 annotation  
| ENDIF |
# Case 4 of 14
# For a protein which is a hit for GPE00210
# GPE00210 is used only in combination with GPE00231
# A protein which is a hit for GPE00210 is expected to be a hit for GPE00231 also
IF (hit for GPE00210) THEN
    IF (hit for GPE00231) THEN
        Assign GPE00210 annotation
    ELSE
        Alert: Hit for GPE00210 but not GPE00231
    ENDIF
ENDIF

# Case 5 of 14
# For a protein which is a hit for GPE02430
# GPE02430 is used only in combination with GPE02530
# A protein which is a hit for GPE02430 is expected to be a hit for GPE02530 also
IF (hit for GPE02430) THEN
    IF (hit for GPE02530) THEN
        Assign GPE02430 annotation
    ELSE
        Alert: Hit for GPE02430 but not GPE02530
    ENDIF
ENDIF

# Case 6 of 14
# For a protein that is a hit for GPE03130
# GPE03130 is used only in combination with GPE03430
# A protein which is a hit for GPE03130 is expected to be a hit for GPE03430 also
IF (hit for GPE03130) THEN
    IF (hit for GPE03430) THEN
        Assign GPE03130 annotation
    ELSE
        Alert: Hit for GPE03130 but not GPE03430
    ENDIF
ENDIF
# Case 7 of 14
# For a protein that is a hit for GPE03210
# GPE03210 is used only in combination with GPE03430
# A protein which is a hit for GPE03210 is expected to be a hit for GPE03430 also
  IF (hit for GPE03210) THEN
    IF (hit for GPE03430) THEN
      Assign GPE03210 annotation
    ELSE
      Alert: Hit for GPE03210 but not GPE03430
    ENDIF
  ENDIF

# Case 8 of 14
# For a protein that is a hit for GPE09130
# GPE09130 is used only in combination with GPE09630
# A protein which is a hit for GPE09130 is expected to be a hit for GPE09630 also
  IF (hit for GPE09130) THEN
    IF (hit for GPE09630) THEN
      Assign GPE09130 annotation
    ELSE
      Alert: Hit for GPE09130 but not GPE09630
    ENDIF
  ENDIF

# Case 9 of 14
# For a protein that is a hit for GPE09230
# GPE09230 is used only in combination with GPE09330 and GPE09630
# A protein which is a hit for GPE09230 is expected to be a hit for GPE09630 also
  IF (hit for GPE09230) THEN
    IF (hit for GPE09630) THEN
      Assign GPE09230 annotation
    ELSE
      Alert: Hit for GPE09230 but not GPE09630
    ENDIF
  ENDIF
# Case 10 of 14
# For a protein that is a hit for GPE09330 and GPE09630
# GPE09330 is used only in combination with GPE09630
# A protein can be a hit for GPE09330 or GPE09630, but not for both (non-orthologous)
IF (hit for GPE09330 AND hit for GPE09630) THEN
    Alert: Hit for GPE09330 and GPE09630
ENDIF

# Case 11 of 14
# For a protein that is a hit for GPE00620 and GPE00720
# GPE00620 is used only in combination with GPE00720
# A protein can be a hit for GPE00620 or GPE00720, but not for both (non-orthologous)
IF (hit for GPE00620 AND hit for GPE00720) THEN
    Alert: Hit for GPE00620 and GPE00720
ENDIF

# Case 12 of 14
# Isomerases: GPE07030, GPE07130, GPE07230, and GPE07330
# A protein can be a hit for any one of the above four profiles (non-orthologous)
    For GPE07030, GPE07130, GPE07230 and GPE07330
    IF (hit for more than one)
        Alert: Hit for (list all profiles which appear as hits from above list)
ENDIF

# Case 13 of 14
# For a protein that is a hit for GPE00430 and GPE00530
# GPE00430 is used only in combination with GPE00530
# A protein can be a hit for GPE00430 or GPE00530, but not for both (non-orthologous)
IF (hit for GPE00430 AND hit for GPE00530) THEN
    Alert: Hit for GPE00430 and GPE00530
ENDIF

# Case 14 of 14
# For a protein that is a hit for GPE05332 and Q81A42:1-328
# GPE05332 is used only in combination with Q81A42:1-328
# A protein can be a hit for GPE05332 or Q81A42:1-328, but not for both (non-orthologous)
IF (hit for GPE05332 AND hit for Q81A42:1-328) THEN
    Alert: Hit for GPE05332 and Q81A42:1-328
ENDIF
Research articles which report the characterization of enzymes involved in the biosynthesis of monosaccharides are listed below. Amino acid sequences of these enzymes were either used to generate HMM profiles or used as BLASTp queries. The PubMed IDs of these research articles are included in the GlycoPathDB (www.bio.iitb.ac.in/glycopathdb/) against respective sequence entry. These PubMed IDs are hyperlinked to the corresponding PubMed webpage.

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