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because most of the tools could capture ec numbers that had not yet been incorporated into the other tools, most of the ec numbers from the first analysis did not have metabolic annotations yet. so we investigated what fraction of the ec numbers are annotated on a genome-wide level by examining the annotations of bacterial genomes that have been sequenced as a part of the human microbiome project [2], for which we also have some annotations of metabolic genes. the fraction of ec numbers without metabolic annotations for the hmp genomes was 6%, much lower than the 25% observed for the randomly selected genomes from biocyc (see table 2). our analysis demonstrated the limitations in the coverage of metabolic annotations of the different tools. we found that while all the tools perform better than the broad level annotations from the broad institute annotations, and cover more ec numbers than the more in depth rast annotations, none of the tools are complete. in particular, there is a problem of too few annotations in certain groups, such as ribosomal proteins, and too many in others, such as miscellaneous cellular functions. the comparison also suggests that the data from annotations by different tools can be complementary, which highlights the difficulty in combining the annotations from different tools. the annotations from different tools in the same project can also be complementary, as seen in the hmp genomes, but there is also a lack of annotations in proteins involved in ribosomal processes.

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the combination of the kegg and brenda databases produced the most complete set of unique ec numbers and gene-ec associations. with a third of the unique ec numbers from the kegg database and a quarter of the ec numbers from brenda, the coverage of this combination of databases was very similar to that of the kegg database alone. note, however, that because brenda only provides annotations for those enzymes that are assigned to ec numbers, the coverage of the brenda annotations are generally lower than the kegg annotations, but they are still much better than the other methods. in terms of gene-ec associations, the coverage of the brenda annotations was not as good as the coverage of the kegg annotations, but the number of gene-ec associations were nearly equal. for this analysis, we created a curated set of genomes containing only genomes that have been manually annotated using at least two of the above annotation tools. while the majority of the genomes in the curated genome collection are listed as species in the ncbi genome database, many genomes that are placed in the species category have not been manually annotated by any of the databases or tools, and therefore were not included in this curated set of genomes. the curated genomes were downloaded on oct 5, 2011 and included 131 species. note that although we only included genomes that were manually annotated with at least two tools, we did not perform any validation of the automatic annotations on the curated genomes. thus, we cannot say that the curated genomes are completely free of errors in automated annotations. in addition, while we used only bacterial genomes in our curated genome collection, we did not exclude any genomes based on what level of data quality we expected for them (e.g. genomes with only ec numbers or ec numbers with no gene-ec annotations were included). 5ec8ef588b

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