

Insights into the Potential for Microbial Survival and Activity within a Transuranic Waste Drum

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Abstract

The microbial effects of concern to most repository safety case scenarios revolve around the potential activity of repository-indigenous organisms. However, an additional category of microorganisms, those present in the actual waste, may have a significant impact on the waste form, even prior to repository emplacement. Because of obvious logistical difficulties, this group of organisms is rarely considered.

The metagenome of one waste drum intended for placement at the Waste Isolation Pilot Plant (WIPP) has been sequenced and partially analyzed. It provides insight into the types of organisms present in the waste, their strategies for coping with environmental stressors, and their possible degradative capabilities.

Results of this analysis support the findings of the previous identification work performed on this drum and also validate the use of two drum bacterial isolates as representative waste organisms¹. These organisms are under investigation for their interactions with waste components and their tolerance of radionuclides.

Methods

Metagenomic DNA was sequenced at the Biosciences Division at Los Alamos using the Illumina MiSeq platform. Several online computational tools were used to profile the phylogenetic affiliations within the waste community from the metagenomics data, including gottcha, designed at LANL². Feature annotations for the metagenome were obtained through the MG-RAST analysis pipeline (metagenomics.anl.gov; Argonne National Laboratory)³.

Results and Discussion

Breakdown by Phylum

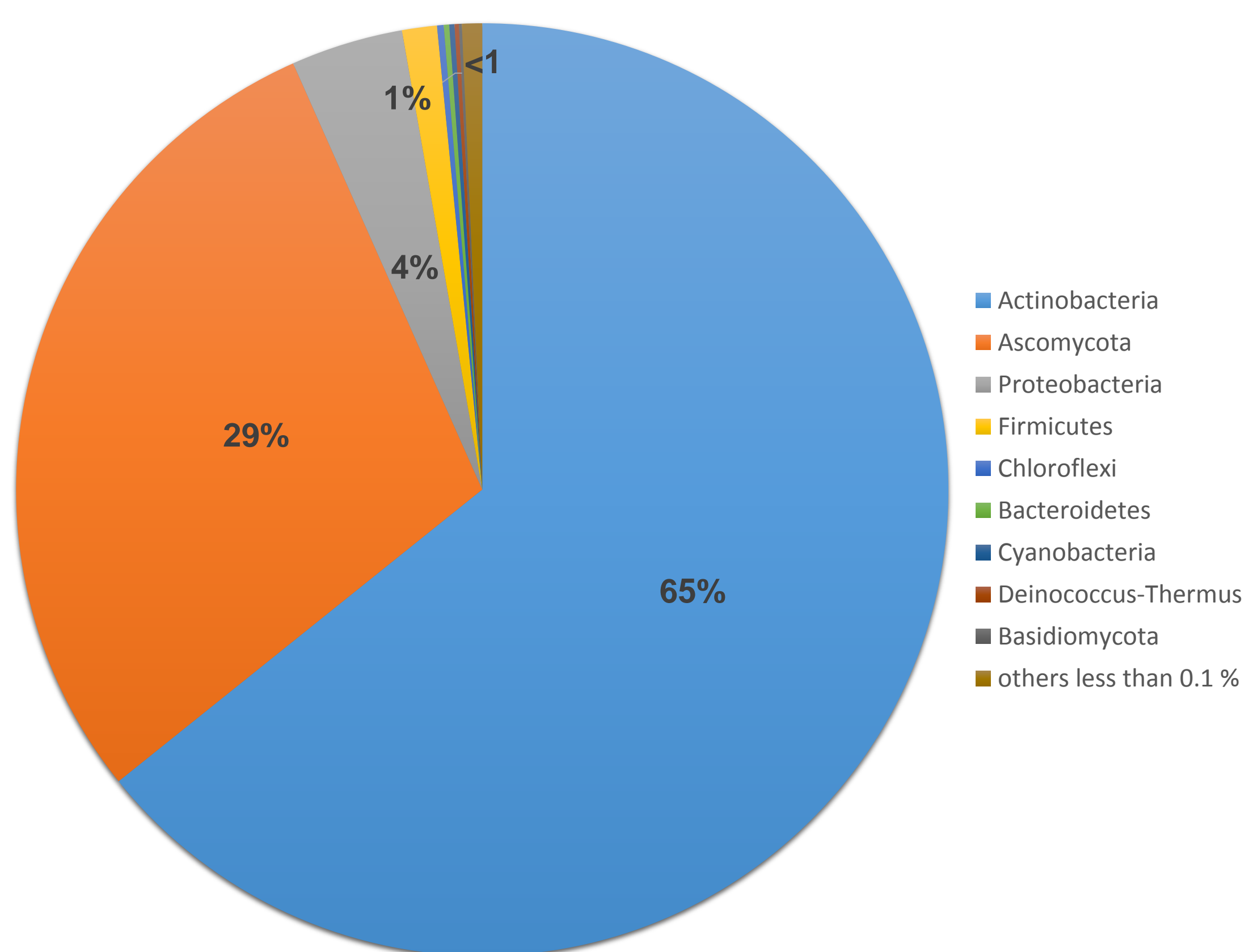


Figure 1: Percentage of sequences belonging to different phyla. Note: ~70% of sequences are bacterial, and ~30% are fungal. Of the bacterial sequences, the majority belong to the *Actinobacteria*.

Breakdown by Subsystems

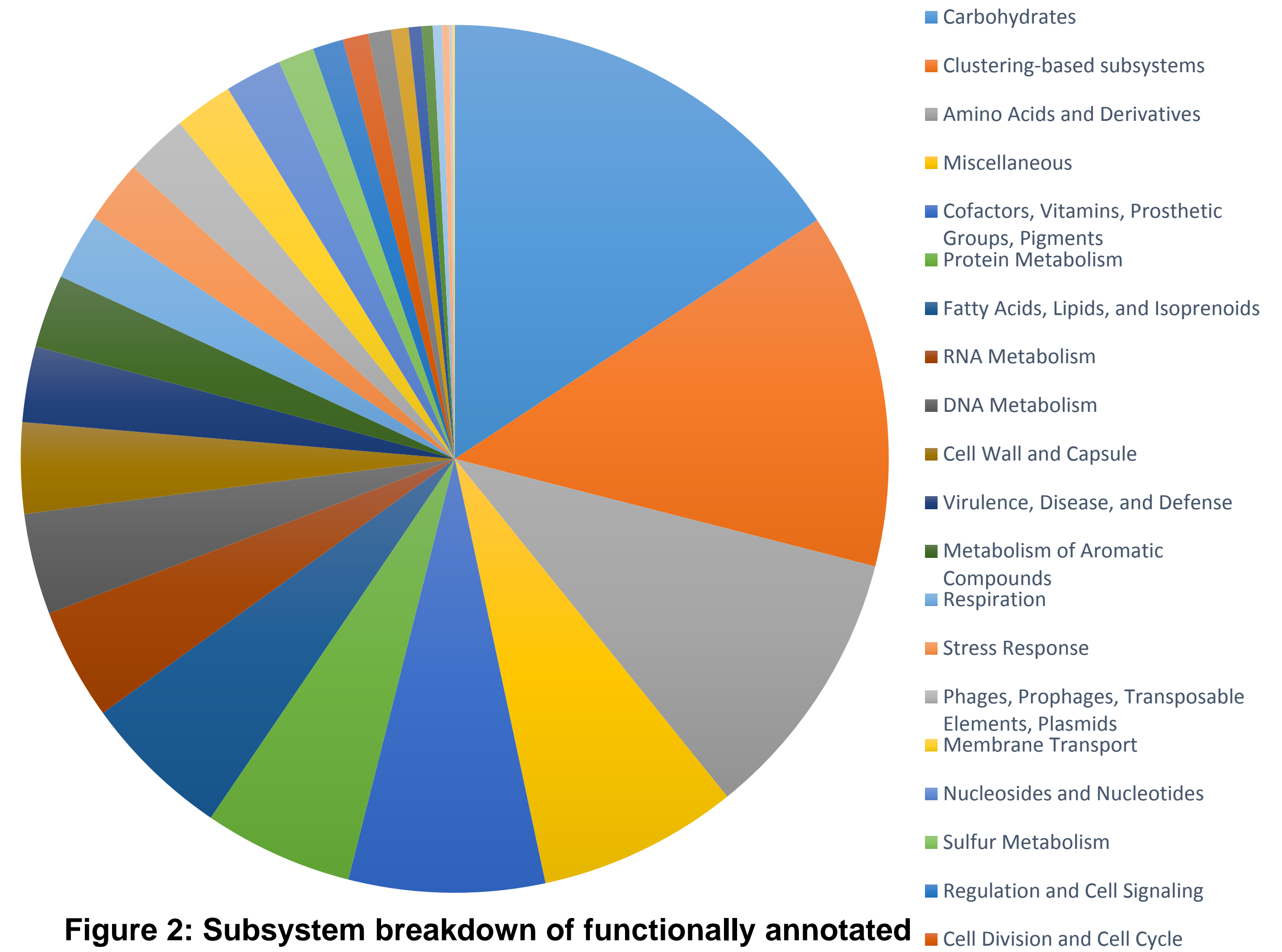


Figure 2: Subsystem breakdown of functionally annotated genes. Note: majority of genes encode for carbohydrates, amino acids, unknown related clusters of genes, and miscellaneous functions.

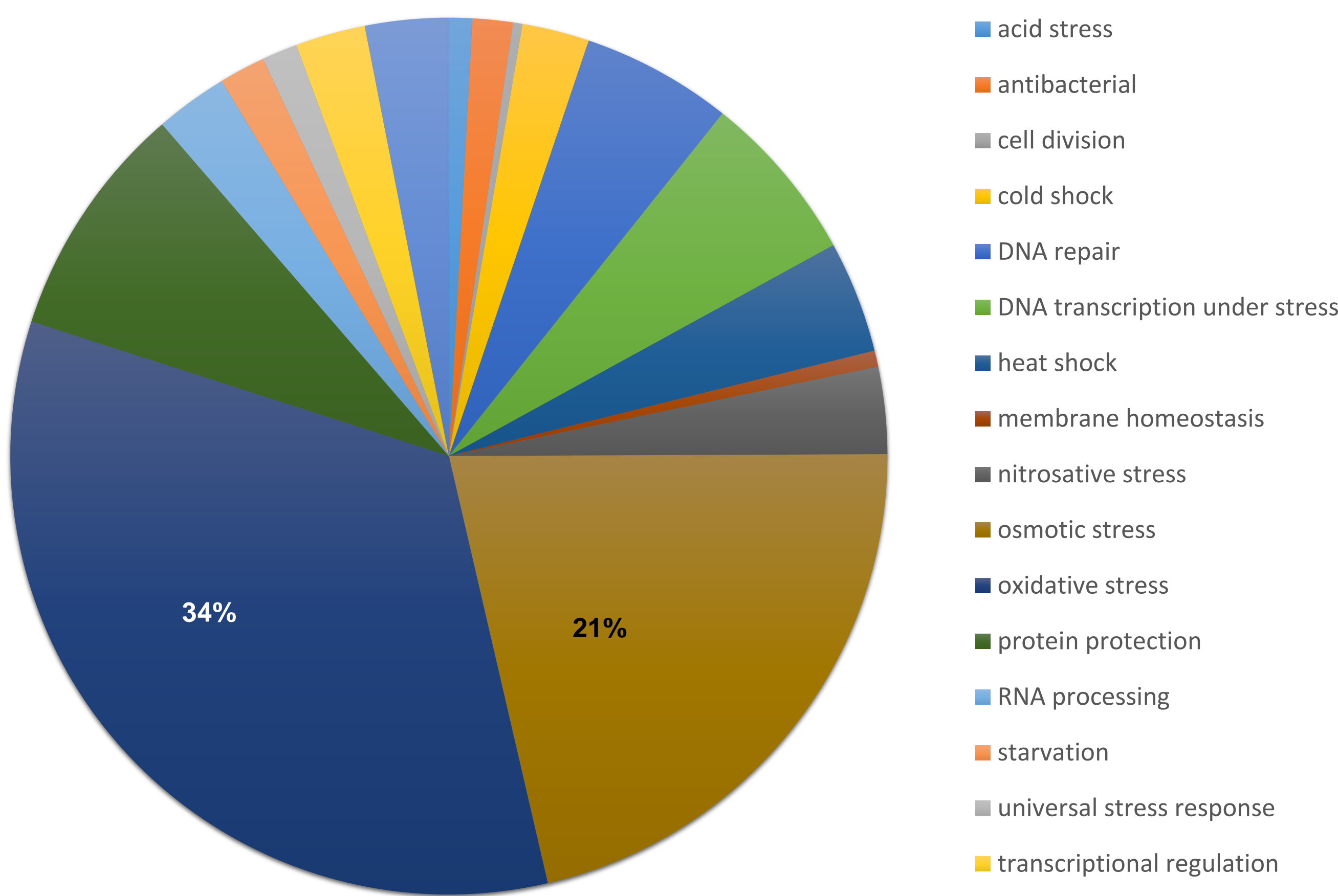


Figure 3: Stress response genes. Note: over 50% of the stress response genes comprise those encoding for osmotic stress and oxidative stress. Genes for osmotic stress can also be upregulated upon exposure to salt.

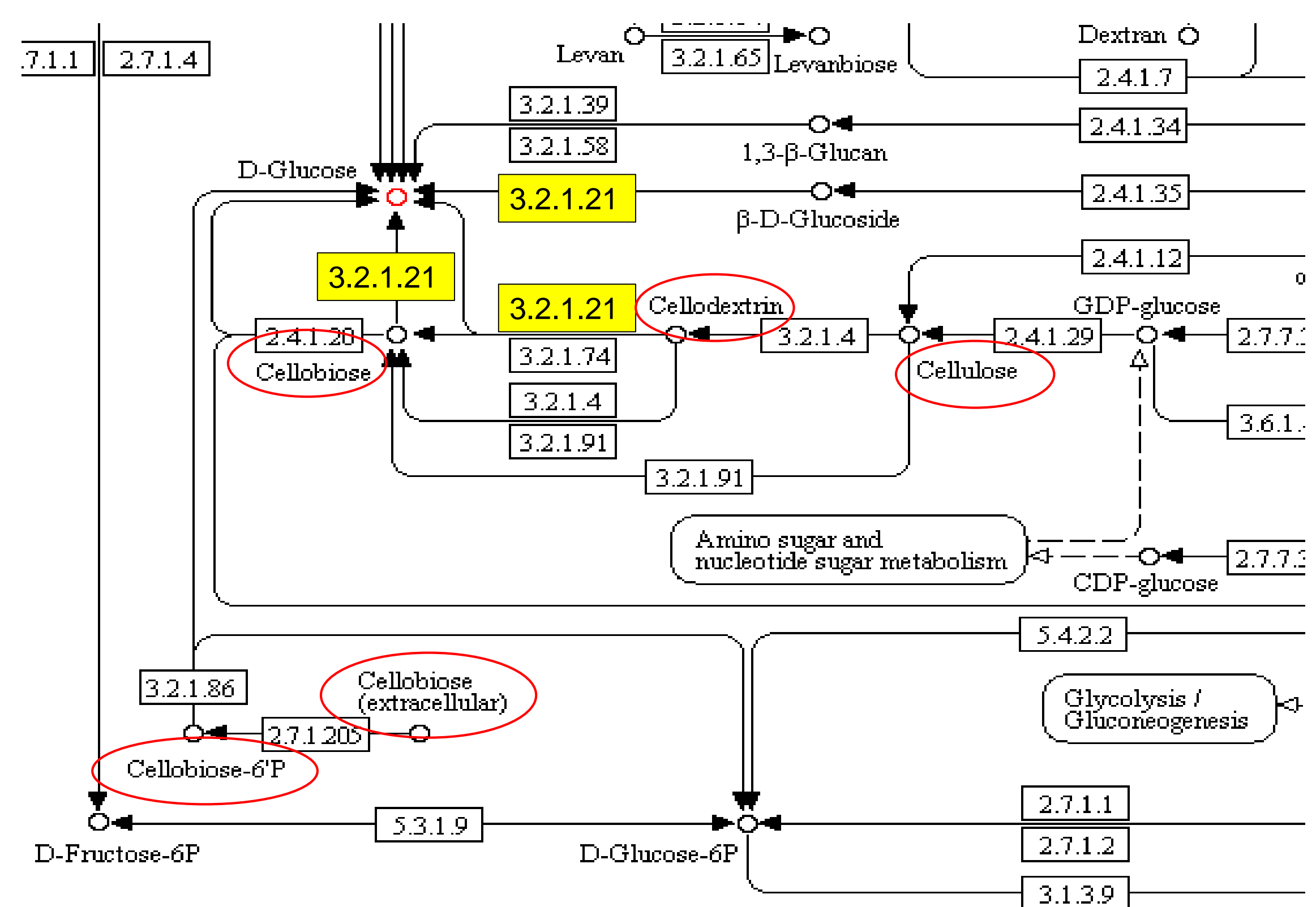


Figure 4: Biochemical pathways for the degradation of sugars and starches; cellulose-degradation genes found in this sample are highlighted (KEGG pathway 00500; Kinehasa Laboratories)

Conclusions

It has been suggested that the organisms capable of survival in waste drums must be both desiccation and radiation tolerant¹. Thus, it is not surprising that work on this drum should result in the detection and isolation of members of the *Actinobacteria* and *Firmicutes*. Fungi were not tested for in the previous work, but their presence in the waste drum is also not surprising given their xerotolerance. Metagenomic analysis of the stress response genes in this sample drum also supports the tolerance hypothesis. Approximately half of these genes encode for osmotic and oxidative stress, and another 25% encode for DNA and protein protective and repair functions, which can also be viewed as responses to oxidative stress.

The potential for within-drum, pre-emplacement degradation of waste components can be inferred from the data, to some degree. Although this drum was not known to contain large amounts of cellulosic waste, some of the genes involved in the cellulose degradation pathway were found. It is likely that fungi play the larger role in initial cellulose hydrolysis, and known cellulolytic fungi comprise a significant percentage of the fungal genes present in the sample. Still, the cellulose degradation genes make up only a small percentage (~2%) of the total carbohydrate utilization genes and are far fewer than those encoding general starch and sugar metabolism. This may be due to the fact that MG-RAST does not support eukaryotic genomes sequence analysis. Other organisms known to be capable of cellulose utilization were also detected.

Genes encoding the transformation of several xenobiotic compounds were also found. These include NTA, chlorinated hydrocarbons, and aromatics. The presence of arsenate reductase genes (~3%) could suggest oxyanion respiration or detoxification. Otherwise, the majority of respiratory genes suggest glycerolipid metabolism, CO metabolism, and carboxylic acid metabolism.

This analysis only points to the genetic potential for various activities; proof of function must be empirically shown.

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