Toxigenic *Bacillus* species induce a distinct cytokine release signature in macrophages, revealing inflammasome activation

This heatmap visualises the cytokine landscape induced by 14 *Bacillus* species, uncovering a distinct inflammasome activation signature associated with bacterial toxicity. Each row represents a *Bacillus* species, and each column corresponds to one of 32 cytokines released by wild-type bone marrow–derived macrophages following 3 hours of stimulation. Cytokine values were normalised to the mean of each cytokine across all species, allowing for direct comparison of relative induction patterns independent of baseline variability.

A prominent red cluster emerges for toxigenic species such as *B. cereus*, *B. idriensis, B. mycoides, B. pseudomycoides* and *B. thuringiensis*, reflecting robust induction of canonical inflammasome-associated cytokines such as the IL-1β cytokine. In contrast, non-toxigenic strains such as *B. subtilis* and *B. megaterium* form a muted grey cluster, indicating a limited inflammatory response. Hierarchical organization of the data reveals a clear separation between inflammatory and low-response phenotypes without the need for manual grouping, highlighting the discriminatory power of the pattern itself.

This visualization encapsulates a fundamental principle of host–pathogen interaction**:** toxin-mediated inflammasome activation imprints a distinctive cytokine signature that separates pathogenic from environmental *Bacillus* strains. The use of a single colour gradient focuses attention on biologically meaningful contrasts while maintaining interpretability for non-specialists. By condensing high-dimensional multiplex data into a structured, visually intuitive format, the heatmap transforms a complex dataset into a clear mechanistic narrative.

Beyond its immunological insight, this figure demonstrates how deliberate visualization design can reveal pathogen-specific inflammatory fingerprints at a glance, bridging experimental data with conceptual understanding. It underscores how data visualization is not merely descriptive but an analytical tool in itself.