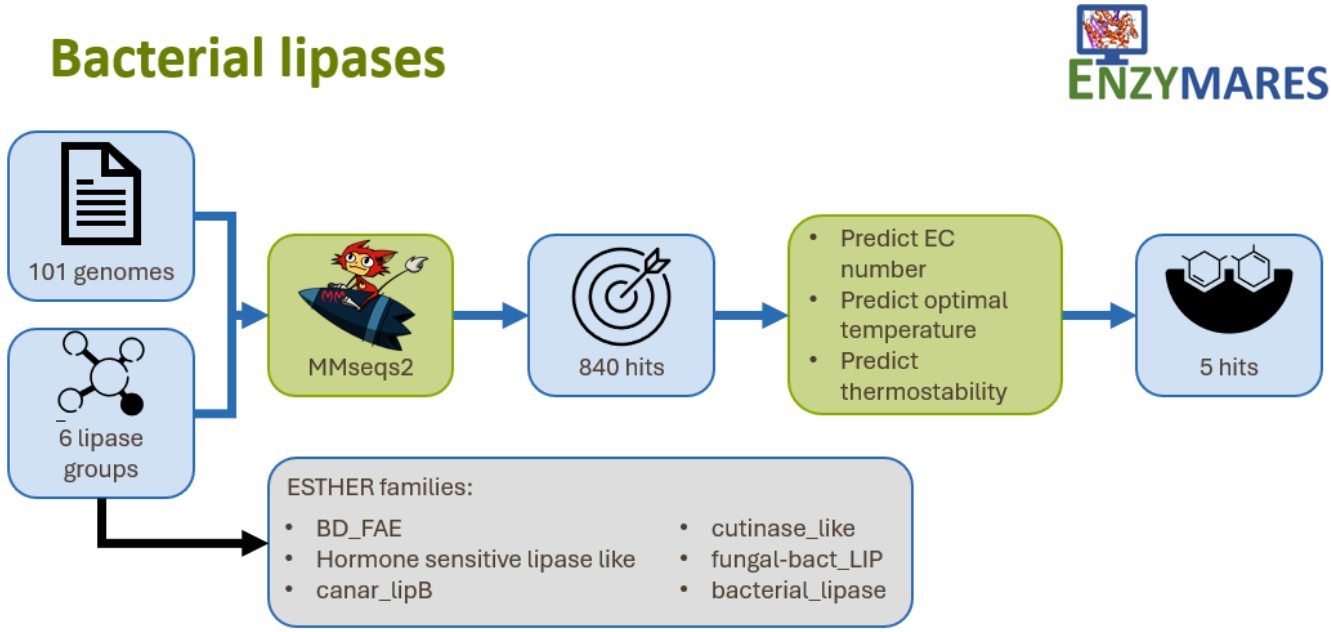
Use case: novel lipases

To illustrate the potential of the Enzymares toolbox, we present a case study focused on lipases—enzymes with broad industrial relevance, particularly in the synthesis of specialty esters. This example demonstrates how the Enzymares pipeline can support the identification, characterization, and application testing of novel lipases derived from marine bacteria.

The journey began with a large-scale enzymatic screening of marine bacterial isolates curated in the UGent biobank. Out of 206 strains tested, 83 (46.1%) showed lipase activity. These promising candidates were further analyzed through whole-genome sequencing and annotation, providing the necessary input for the Enzymares toolbox. The toolbox was then used to identify putative lipases based on sequence similarity with reference enzymes of 6 lipase/esterase families from the ESTHER database. In twelve genomes, lipases with predicted thermostability were found. Further investigation of the data resulted in five potential candidate enzymes of which two stood out. The first one, belonging to the hormone sensitive lipase like family is a predicted thermotolerant lipase from an isolate identified as a gram-positive strain belonging to the order of *Bacillales*, with optimal temperatures predicted up to 55 °C. The second enzyme belongs to the same family as the *Candida antarctica* lipase B, a reference enzyme widely used in industry and commercialized as Novozym®435. This enzyme originates from a marine isolate belonging to the class of Gammaproteobacteria.



These candidates were selected for recombinant production using a pET-22b(+) expression vector in *E. coli* BL21(DE3), which proved to be an effective host system. After successful cultivation and purification, preliminary

activity assays confirmed lipase functionality. The best-performing candidate—originating from the *Bacillus* isolate—was prioritized for further testing.

To evaluate its industrial potential, Bio Base Europe Pilot Plant (BBEPP) scaled up production using a 4L fed-batch fermentation protocol. A lipase activity assay, developed earlier in the project and based on a hydrolysis reaction, was used to monitor enzyme performance during fermentation and downstream processing. Freeze-dried enzyme samples were then transferred to VITO for application testing.

VITO focused on comparing the new lipase candidate with the benchmark enzyme Novozym®435 (CalB) in solvent-free esterification reactions. First, a preliminary esterification assay was applied. The esterification activity of the new enzyme was near equal at 40 °C and 60 °C, revealing an enzyme that may be active at a broad temperature range. A kinetic model was applied to assess substrate preferences and identify potential inhibition phenomena. The new lipase was tested in the esterification of geraniol with acetic acid at 40 °C, under various substrate ratios and enzyme doses. The new candidate showed limited conversion (max. 10%) compared to the benchmark, which achieved full conversion under similar conditions (i.e. temperature, substrate ratio and applied units).

In conclusion, the Enzymares toolbox successfully supported the identification and characterization of novel lipases from marine bacteria. Although the selected candidate would require further optimization and development towards industrial performance criteria, its potential as a lipase with an extended temperature operating window could be shown and, overall, the workflow demonstrated the value of integrating genomics, bioinformatics, and process engineering to accelerate enzyme discovery and testing. This approach significantly reduces the time and resources typically required for enzyme screening, offering a streamlined path from discovery to application.