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Esterase Resistant to Inactivation by Heavy Metals

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Technology description

The Red Sea brine pools are one of the extreme environments that allow the survival of diverse extremophiles. The Atlantis II brine pool is one of these extreme environments that possesses multiple harsh conditions such as; high temperature, salinity, pH and high concentration of multiple, including toxic heavy metals. Therefore the survival of microbial life in such environment utilizing their resistant enzymes makes them an excellent source for tolerant enzymes. We constructed a fosmid metagenomic library using DNA isolated from the deepest and most secluded layer of this pool and identified a novel esterase. We report the isolation and biochemical characterization of this unique esterase; EstATII from the lower convective layer of the Atlantis II brine pool. EstATII is a halotolerant, thermophilic and resistant to a spectrum of heavy metals including toxic concentration of metals. To the best of our knowledge, this is the first study to identify and characterize a novel enzyme from the Atlantis II brine pool in the Red Sea.

Challenge

Currently, only a dozen thermo-stable lipases/esterases have been isolated, of which only 7 esterases of thermophilic origin had been sequenced. Newly isolated candidates like the acetylesterase, isolated from Fervidobacterium nodosum strain is considered highly thermo-stable, retaining 50% of its activity at 60°C. However, just like all other esterases it starts to rapidly lose activity when exposed to high temperatures above 65°C or 70°C. Another issue is showing resistance to heavy metal ions, most enzymes usually show resistance to only one or two heavy metals. In order to fulfill unique niches in industrial applications, there are many growing efforts to obtain esters, from extreme environments that can withstand higher temperatures and still retain full activity.
In 2012, the global market for lipases was $100 million. Unfortunately the existing market is described as “stagnant”, as it’s in desperate need for novel, multi-stress resistant enzymes that can fulfill over 300 industrial processes that rely heavily on biocatalysts.

Solution

This esterase was cloned from a pool of DNA, collected from an extreme environment that displays multiple harsh conditions; A brine pool at the bottom of the Red Sea, that is characterized by high temperatures (68°C), hyper-salinity, the presence of dissolved heavy metals, and a highly acidic ph.

Collected DNA was fractionated and transformed into E. coli bacteria, which expressed the DNA fragments. Colonies were grown on a lipolytic substrate that leaves a visible change in appearance upon enzymatic hydrolysis. From this a unique, previously unrecognized lipase sequence was isolated and named EstATII.
Extracted from an extreme environment, the enzyme is potentially valuable in that it has sufficient activity at a temperature of 45-75°C and retains activity at is the enzyme thermo-stable and halotolerant, it also shows high activity in alkaline conditions, despite being recovered from acidic waters. And when exposed to a battery of metal ions it showed resistance to every candidate, making it the first esterase to show resistance to high concentrations of multiple, toxic heavy metals.

temperatures as high as 80°C. It’s also “salt-tolerant”, retaining excellent activity at 4.5M NaCl.

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