S5-5

Genomic Potential of Marine Microorganisms: Development of Epoxide Hydrolases from Strict Marine Microorganisms

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Ocean covers more than three-quarters of the earth's surface, affecting human life in many ways directly or indirectly. Deep sea comprises as much as 90% of ocean in volumn, supplying reservoirs of unique genetic information and important natural resources for possible development but the research on the deep sea environment has been limited to date because of the adverse environment to the research. However, the recent advances on the state-of-art technology for cruzing the deep sea environment such as AUV, ROV and submarine, and biotechnological technology such as genomics, proteomics and high-throughput robotics facilitated the marine biotechnology.

To develop useful enantioselective enzymes from marine organisms, we are currently collecting samples from a variety of marine environments such as cold seep, hydrothermal vent, deep sea, sediment, tidal flat area and so on. The collected samples were pretreated or enriched with various reagents for the purpose of enhancing the survival of useful target microorganisms and then, the marine bacteria isolated were tested for various enantioselective hydrolyzing activities. Marine samples were also systematically collected to span various physical, chemical and biological environment under agreements with countries involved, and microorganisms were isolated, followed by the analysis of partial sequences of 16s rRNA gene.

To develop an enantioselective epoxide hydrolase (EHase) from marine microorganisms, marine samples collected from a variety of marine environments were analyzed as described above. 181 strains isolated by the capability of living on styrene oxide (SO) were screened for retaining enantioselective EHase activities toward SO. As a result, one strain was selected, and the sequence analysis of 16S rRNA gene showed that the strain felled within the radius of the cluster comprising *Erythrobacter* species (Fig. 1). Nine additional strains belonging to *Erythrobacter* were thereby tested for displaying EHase activities, and most strains showed enantioselective hydrolysis toward SO and glycidyl phenyl ether (GPE). Kinetic resolution of racemic SO using whole cell of *Erythrobacter* sp. JCS358 was performed. Enantiopure

(*S*)-SO could be obtained with an enantiomeric excess (ee) higher than 99 and 10% yield after 16 hr incubation.

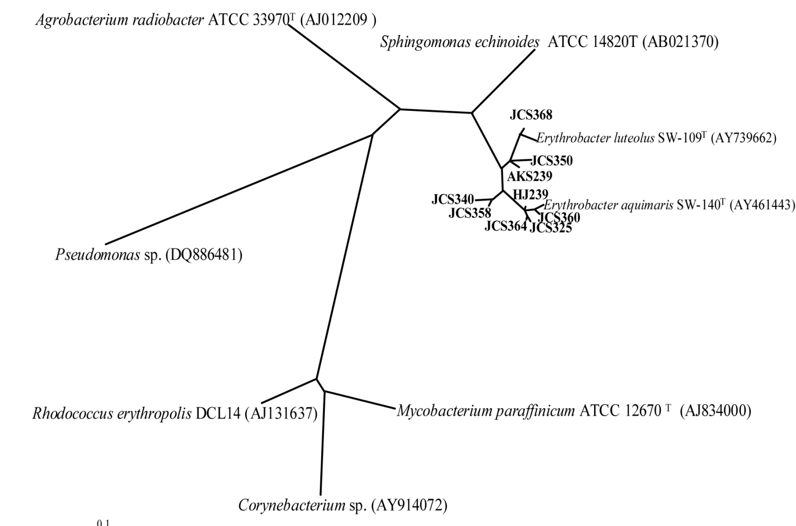

Fig. 1. Phylogenetic analysis of JCS358 and other *Erythrobacter* strains. Phylogenetic analysis of *Erythrobacter* strains with EHase-producing bacteria were constructed by the neighbor-joining method using PHYLIP package (Felsenstein 1993).

To clone and characterize EHases from *Erythrobacter*, *E. litoralis* HTCC2594 was selected since the whole genome sequencing was finished recently, facilitating the rapid cloning and characterization. Three genes encoding putative EHases were identified by analyzing open reading frames (ORFs) of *E. litoralis* HTCC2594. Despite low similarities to reported EHases, the phylogenetic analysis of the three genes showed that *eeh1* was similar to microsomal EHase while *eeh2* and *eeh3* could be grouped with soluble EHases (Fig. 2). The three EHase genes were cloned, and the recombinant proteins (rEEH1, rEEH2 and rEEH3) were purified. The functionality of purified proteins was proved by hydrolytic activities toward styrene oxide. EEH1 preferentially hydrolyzed (*R*)-styrene oxide whereas EEH3 preferred to hydrolyze (*S*)-styrene oxide, representing enatioselective hydrolysis of styrene oxide. On the other hand, EEH2 could hydrolyze (*R*)- and (*S*)-styrene oxide at an equal rate. The optimal pH and temperature for the EHases occurred largely at neutal pHs and 40-55°C. This is the first representation that a strict marine microorganism possessed three EHases with different enantioselectivity toward styrene oxide.

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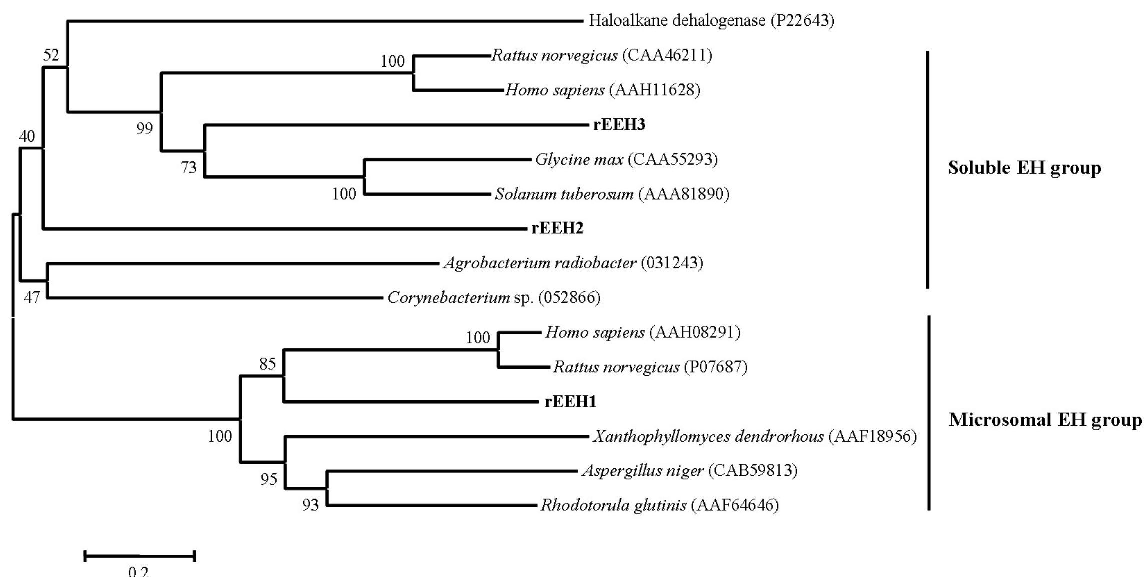

Fig. 2. Phylogenetic analysis of EHase. Sequence alignment on the desired amino acid sequence was performed using the Clustal W software package. *Rhodotorula glutinis* (AAF64646), *Rattus norvegicus* (P07687), *Homo sapiens* (AAH08291), *Xanthophyllomyces dendrorhous* (AAF18956), *Aspergillus niger* (CAB59813), *Homo sapiens* (AAH11628), *Rattus norvegicus* (CAA46211), *Solanum tuberosum* (AAA81890), *Glycine max* (CAA55293), *Agrobactrium radiobacter* sEH (O31243), *Corynebacterium* sp. sEH (O52866), and Haloalkane dehalogenase (P22643)