Identification of a New Marine Steroid-degrading Bacterium S19-1 and Isolation of Estradiol-inducible Genes and a Novel Promoter from This Bacterium

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Environmental estrogens in water have been reported to be associated with abnormal sexual development and abnormal feminizing responses in some animals. Estrogen contamination of sea water is an ever growing problem and impacts population dynamics of all kinds of sea animals. Researches about elimination of estrogens from the contaminated environment have become a major issue in environmental research and policy. It has been demonstrated that biological processes play an important role in the removal of these stable compounds. Thus, 12 strains of steroid-degrading bacteria were isolated by our group from the Baltic Sea at Kiel, Germany in 2008.

In the present work, one of these steroid-degrading bacteria, strain S19-1, was identified. Strain S19-1 was characterized to be a Gram-negative bacterium. Phylogenetic analysis based on 16S rRNA sequence showed that strain S19-1 is closely related to the members of the genus *Buttiauxella* of the *Enterobacteriaceae* family. The optimal growth conditions of strain S19-1 require the presence of NaCl (2.1%) and a temperature of 20°C. High performance liquid chromatography (HPLC) results showed that 80% of estradiol could be degraded by S19-1 after 48 h incubation under these conditions. The improved growth of strain S19-1 was obtained by the presence of testosterone, estradiol or cholesterol in the minimal medium. Moreover, S19-1 was identified to be sensitive to antibiotics kanamycin, ampicillin, chloramphenicol, carbenicillin and streptomycin, but resistant against erythromycin. After transformation into strain S19-1, a series of plasmids including pK18 were found to be able to replicate in this bacterium.

Since S19-1 can degrade estradiol, there must be some genes involved in the degradation of estradiol in this bacterium. To analyze the estradiol degradation pathway in this marine isolate, fluorescence microplate assay (FMA) based on substrate-induced gene-expression was used to isolate genes involved in estradiol metabolism, in which chromosomal DNA of strain S19-1 was first digested with restriction enzyme *Sal*I and the resulting fragments were cloned into pKEGFP-2. *E. coli* cells harboring the recombinant plasmids were induced by 4 fmole estradiol and relative fluorescence units (RFU) of bacteria were measured. Compared to negative control pKEGFP-2, 37 plasmids out of meta-genomic library containing 323 plasmids could be induced by estradiol. Relative fluorescence units-increased amount percentage (RFU-IAP) of 6 plasmids among these 37 plasmids was about 6-7%, which is higher than the other 31 inducible plasmids (about 4-5%). So the insert fragments of these 6 plasmids were sequenced and then analyzed by BLAST search. Sequence analysis showed that the inserts of these plasmids comprise transcriptional regulators, substrate transporters, kinases and catabolic enzymes.

Not only catabolic enzymes and relevant regulatory elements, but inducible promoters could also be obtained by fluorescent microplate assay. Unexpectedly, bacteria containing one of those etradiol-inducible plasmids, plasmid p302, became green. This suggested that a strong promoter may exist in this fragment. Since a strong promoter from a new marine bacterium may be very useful in our future work, a series of deletion mutants of p302 were constructed to determine this new promoter. Finally, this promoter was identified to locate adjacent to the EGFP gene in the plasmid p302 and a 108-bp promoter sequence was obtained, the putative -10 region and -35 region of which were predicted.