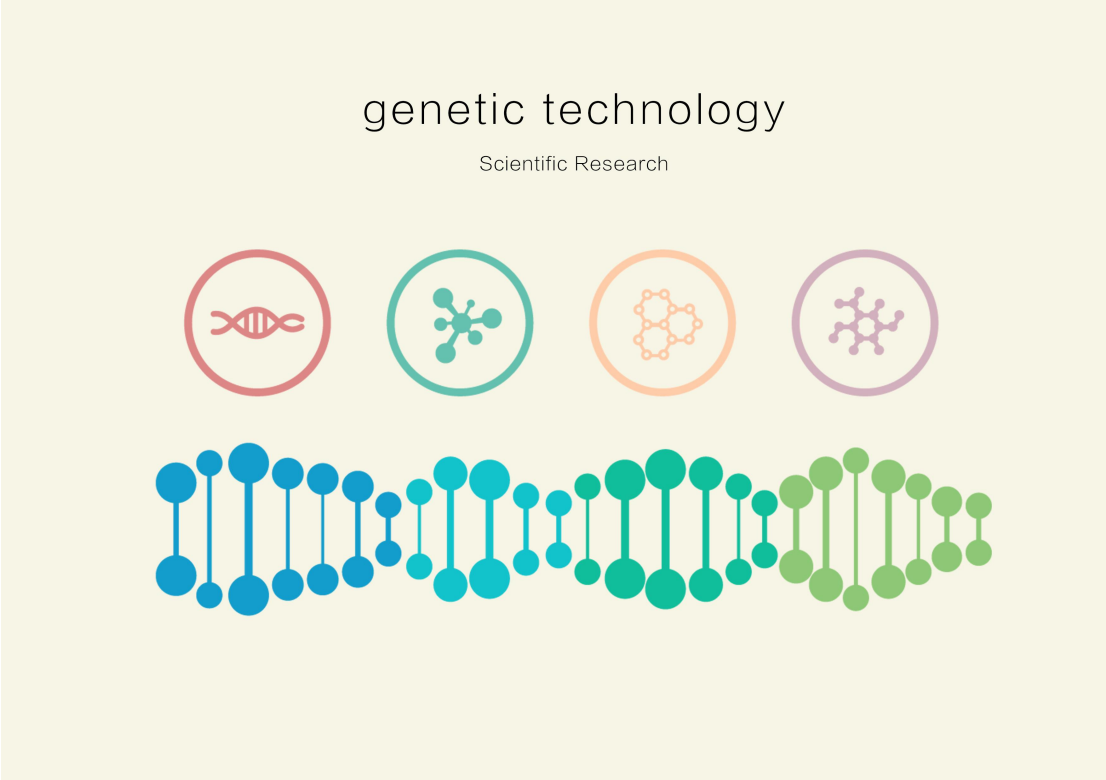
Project Description

Background

Bear bile, one of the most famous animal drugs in Traditional Chinese Medicine (TCM), has been recorded in ancient Chinese medicine book as a significant method to treat hepatic and biliary disorders. UDCA, the effective ingredient of bear bile.Aside from the traditional use of bear bile in Chinese medicine, UDCA(ursodeoxycholic acid), the effective ingredient of bear bile acid, has a much larger pharmaceutical application. As well as the usage of UDCA in dissolving gallstone, its efficacy in primary biliary cirrhosis and primary sclerosing cholangitis (PSC) as an adjunct to medical therapy has been well established. Newer indications include its use in the management of chronic hepatitis, cirrhosis, post liver transplant rejection, graft-versus-host disease and acute viral hepatitis, where it not only relieves symptoms of cholestasis but also arrests ongoing hepatocyte necrosis. However, the increasing demand for bear bile has caused bears to be in an endangered state: bear poaching and illegal animal trade have greatly dwindled the number of the wild Asiatic black bear. Apart from that, bear bile farming industry in Asia extracts bile through “milking” from the bears, which is operated through surgically implanting a permanent catheter in the animal's gallbladder to obtain the drips. It is unquestionable that the bear bile farming process will lead to both physical and psychological damage in bears.

Purpose



To find substitute or alternative for bear bile farming, our team will be working on the biological synthesis of the main effective component of this important medicine, UDCA(Ursodeoxycholic Acid). This biological approach will not only be more efficient but also be cheaper than the original chemical approach, which is widely used in the current UDCA synthesis industry.

Overview of the project

We found that it is possible to convert the main component of poultry bile, CDCA(Chenodeoxycholic Acid), into UDCA, by employing two enzyme-catalyzed the reactions. First, two enzymes was employed to manage the transformation of CDCA to 7oxo-LCA. In the present of 7a-HSD(7alpha-hydroxysteroid dehydrogenase), CDCA is transformed in to 7oxo-LCA by loosing a pair of hydrogen(2H+ and 2e-), the pair of hydrogen is added to NAD+, the cofactor and the acceptor. The NAD+ is transformed into NADH during the reaction. To regenerate the NAD+ and recycle the reaction, to , the LDH(Lactate dehydrogenase) works on pyruvate and take the pair of hydrogen from NADH and transform the pyruvate to lactate and NADH to NAD+.

In the second step, the 7oxo-LCA is transformed to UDCA by 7 β -HSDH(7beta-hydroxysteroid dehydrogenase)and GDH(glutamate dehydrogenase).The 7 β -HSDH works on 7oxo-LCA and take a pair of hydrogen from NADPH(the cofactor for the second step)and add it to 7oxo-LCA and form a beta position 7-hydroxyl group, which is our target product UDCA. The GDH works on glucose and take a pair of hydrogen from it and add it to the NADP+, to form NADPH to manage the regeneration of cofactor NADPH for the second step.

Our goal

Our mission is to expression of the four enzymes 7 α -HSDH (from ecoli DH5a), 7 β -HSDH (from Ruminococcus Torques), GDH (from Bacillus subtilis), and LDH from (Lactobacillus delbruechii subsp. Bulgaricus)and test their activities. By adding the CBD(cellulose binding domain)sequnence to the plasmid we construct, we manage to bind our enzyme on gauze. This specific design excels in 3 specific ways: first, by controlling the presence of the gauze in the solution, we can control the process of the reaction. Second, when the target enzyme is bound to cellulose we manage to purify the protein we express. Third, the enzyme binding gauze is employed to a machine including the reaction efficiency measuring system and the enzyme addition controlling system .