단위동물용 신규 phytase 및 관련 효소의 생산공정 개발

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오태광 2000.2.

연구개요‥미생물이 생산하는 파이타아제를 탐색하여 환경오염을 예방하고 동물의 사료 이용율을 높여서 경제적인 동물사육이 가능한 새로운 형태의 "환경예방용 파이타아제"를 개발함.

개발내용‥국내 토양으로부터 무기인 형태의 파이틱에시드에 대한 분해활성이 강한 신규효소 생산 균주 바실러스 아미로리퀴페시언스 (Bacillus amyloliquefacience DS11)을 탐색 분리를 성공함.

‥신규효소는 기존효소의 단점 요소을 극복한 동물의 소화생리에 적합하여 사용효율이 높을 뿐 아니라, 기존효소가 사료 제조 시 효소의 잔존활성이 미약하여 사용성이 한계성을 가지는 점을 극복한, 뛰어난 유용성을 가진 새로운 개념의 효소 개발함. 특히, 신 효소는 기존효소가 파이틱에시드(Phytic acid)를 분해하여 2–3개의 포스페이트(Phosphate)만 분해하는데 비해서 6개의 포스페이트는 완전히 분해하는 효율성이 100% 되는 효소임.

‥신규효소를 유전공학 기법을 이용하여 신규 유전자를 확보한 후 효소의 대량 과잉생산을 이루어 생산단가를 획기적으로 줄여서 농민의 부담을 줄일 뿐만 아니라 쉽게 효소를 사용할 수 있게 하였음. 국내기술로만 대량생산, 효소의 분리정제, 동물에서 독성 및 안전성을 규명함.

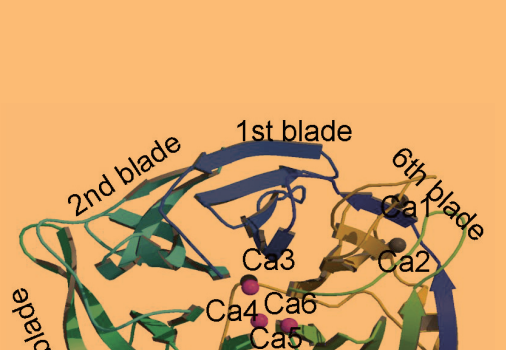
‥동물의 생체특성에 맞는 효소첨가제 개발과 신규 효소의 특성을 규명하기 위하여 효소의3차원 구조를 해석하여 작용기작을 밝히는 모든 분야의 개발과정을 완료함으로써 환경예방효과가 인정되는 환경예방효소제 "트렌스포스" 의 효소제의 개발에 성공함.

활용사례 / 효과

‥네이쳐 스트럭처 바이올로지(*Nature Structutal Biol*)지에 논문이 발표됨. (2000)

‥동물용 의약품으로 등록된 환경예방효소인 파이타아제 : "트렌스포스R" 의 상품화에 성공함.

기술이전‥대성미생물(주)



개발된 효소의 3차 구조

개발된 제품

articles

Crystal structures of a novel, thermostable phytase in partially and fully calcium-loaded states

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Phytases hydrolyze phytic acid to less phosphorylated *myo*-inositol derivatives and inorganic phosphate. A thermostable phytase is of great value in applications for improving phosphate and metal ion availability in animal feed, and thereby reducing phosphate pollution to the environment. Here, we report a new folding architecture of a six-bladed propeller for phosphatase activity revealed by the 2.1 Å crystal structures of a novel, thermostable phytase determined in both the partially and fully Ca^{2+}-loaded states. Binding of two calcium ions to high-affinity calcium binding sites results in a dramatic increase in thermostability (by as much as ~30°C in melting temperature) by joining loop segments remote in the amino acid sequence. Binding of three additional calcium ions to low-affinity calcium binding sites at the top of the molecule turns on the catalytic activity of the enzyme by converting the highly negatively charged cleft into a favorable environment for the binding of phytate.

Phytases (*myo*-inositol-hexakisphosphate-phosphohydrolase, EC 3.1.3.8) are different from other phosphatases in that they prefer phytate (*myo*-inositol-hexakisphosphate) as a substrate. Phytate is responsible for storing more than 80% of the total phosphorus in cereals and legumes[1]. Since monogastric animals such as pigs, poultry and fish are not able to metabolize phytate, the use of phytase in animal feed is highly desirable to reduce phosphorus excretion and to improve the availability of phosphorus and metal ions chelated by phytic acid[2].

Several phytases have been cloned and characterized, including fungal phytases from *Aspergillus ficuum*[3,4], bacterial phytase from *Escherichia coli*[5], and a mammalian phytase (rat hepatic multiple inositol polyphosphate phosphatase)[6]. These phytases

Due to concerns about environmental pollution, 22 countries have adopted the use of phyA as a feed additive[12]. However, phyA has a temperature optimum of 58°C, which is below the optimal temperature range for processing animal feed. Recently, highly thermostable phytases were isolated and cloned from *Bacillus* species by two groups independently[13–15]. The two enzymes are virtually identical, both containing 383 amino acids with 93% sequence identity. They do not align with any other proteins in the sequence data bank, nor do they contain the Arg-His-Gly sequence. Notably, these enzymes are dependent on calcium ions for thermal stability and for catalytic activity[14]. In the presence of 1 mM EDTA, the enzyme from *Bacillus amyloliquefaciens* is severely inhibited, and the enzymatic activity is considerably