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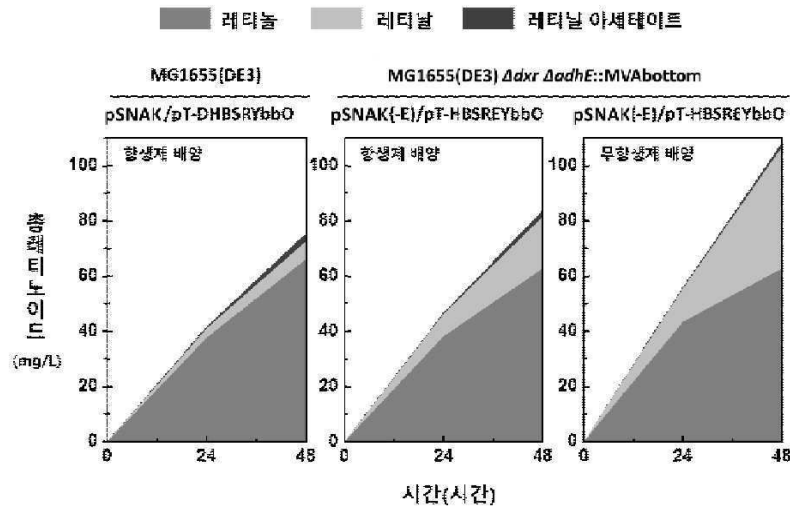
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(54) 발명의 명칭 형질전환 생물체 선별용 마커 조성물, 형질전환 생물체 및 형질전환 방법

(57) 요약

본 발명은 항생제 및 항생제 저항성 유전자 없이 형질 전환 및 목적 산물의 생산이 가능하도록 하는 형질전환 생물체 선별용 마커 조성물, 형질전환 생물체, 형질전환 방법 및 목적 산물의 생산 방법에 관한 것으로, 항생제 및 항생제 저항성 유전자 사용에 기인한 문제를 원천적으로 예방하고, 목적 산물을 높은 수율로 생산할 수 있는 형질전환 생물체 선별용 마커 조성물, 형질전환 생물체, 형질전환 방법 및 목적 산물의 생산 방법에 관한 것이다.

대표도 - 도9



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명세서

청구범위

청구항 1

내재적으로 MVA 경로를 갖지 않으며, 메발론산 이후의 MVA 하부 경로의 효소를 코딩하는 유전자가 염색체에 삽입되고, MEP 경로의 효소를 코딩하는 유전자 중 적어도 하나가 감쇄 또는 결손되고,

MVA 경로의 나머지 전체 효소들을 코딩하는 유전자 또는 상기 감쇄 또는 결손된 유전자와 동일 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환된 생물체.

청구항 2

청구항 1에 있어서, 상기 MVA 하부 경로의 효소를 코딩하는 유전자는 메발로네이트-5-포스페이트 키나아제 (mevalonate-5-phosphate kinase), 메발로네이트-5-디포스페이트 디카르복실라아제 (mevalonate-5-diphosphate decarboxylase) 및 IPP 아이소머라아제 (IPP isomerase)로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 생물체.

청구항 3

청구항 1에 있어서, 상기 MEP 경로의 효소를 코딩하는 유전자는 1-디옥시-D-자이룰로스-5-포스페이트 (DXP) 신타아제, DXP 리덕토이소머라아제, 2-C-메틸-D-에리스리톨-4-포스페이트 (MEP) 시티딜트랜스퍼라아제, 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제, 2-C-메틸-D-에리스리톨-2,4-시클로디포스페이트 (MEcPP) 신타아제, 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제 및 HMBPP 리덕타아제로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 생물체.

청구항 4

청구항 1에 있어서, 상기 감쇄 또는 결손되는 유전자는 DXP 신타아제를 코딩하는 유전자 또는 DXP 리덕토이소머라아제를 코딩하는 유전자인 생물체.

청구항 5

청구항 1에 있어서, 상기 생물체는 항생제 저항성 유전자를 갖지 않는 것인 생물체.

청구항 6

청구항 1에 있어서, 상기 플라스미드는 이소프레노이드, 산탈렌, 비사볼롤 및 레티놀 합성 경로 이루어진 군에서 선택된 경로의 효소를 코딩하는 유전자가 더 도입된 것인 생물체.

청구항 7

내재적으로 MVA 경로를 갖지 않는 형질전환 대상 생물체에 메발론산 이후의 MVA 하부 경로의 효소를 코딩하는 유전자를 염색체에 삽입하는 단계;

상기 생물체에서 MEP 경로의 효소를 코딩하는 유전자 중 적어도 하나를 감쇄 또는 결손시키는 단계; 및

상기 생물체를 MVA 경로의 나머지 효소들을 코딩하는 유전자 또는 상기 감쇄 또는 결손된 유전자와 동일 효소를 코딩하는 유전자가 도입된 재조합 플라스미드로 형질전환시키는 단계;를 포함하고, 항생제 저항성 유전자가 도입된 재조합 플라스미드를 사용하지 않는 생물체의 형질 전환 방법.

청구항 8

청구항 7에 있어서, 상기 MVA 하부 경로의 효소를 코딩하는 유전자는 메발로네이트-5-포스페이트 키나아제 (mevalonate-5-phosphate kinase), 메발로네이트-5-디포스페이트 디카르복실라아제 (mevalonate-5-diphosphate decarboxylase) 및 IPP 아이소머라아제 (IPP isomerase)로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 방법.

청구항 9

청구항 7에 있어서, 상기 MEP 경로의 효소를 코딩하는 유전자는 1-디옥시-D-자이룰로스-5-포스페이트 (DXP) 신타아제, DXP 리덕토이소머라아제, 2-C-메틸-D-에리스리톨-4-포스페이트 (MEP) 시티딜트랜스퍼라아제, 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제, 2-C-메틸-D-에리스리톨-2,4-시클로디포스페이트 (MEcPP) 신타아제, 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제 및 HMBPP 리덕타아제로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 방법.

청구항 10

청구항 7에 있어서, 상기 감쇄 또는 결손되는 유전자는 DXP 신타아제를 코딩하는 유전자 또는 DXP 리덕토이소머라아제를 코딩하는 유전자인 방법.

청구항 11

청구항 7에 있어서, 상기 플라스미드는 이소프레노이드, 산탈렌, 비사볼롤 및 레티놀 합성 경로 이루어진 군에서 선택된 경로의 효소를 코딩하는 유전자를 더 포함하는 생물체의 형질 전환 방법.

청구항 12

청구항 7에 있어서, 적어도 2개의 유전자를 감쇄 또는 결손시키고, 상기 생물체를 이와 동일한 효소를 코딩하는 유전자를 각각 포함하는 적어도 2개의 플라스미드로 형질전환시키는 생물체의 형질 전환 방법.

청구항 13

청구항 1 내지 6 중 어느 한 항의 생물체를 기질이 포함된 배지 상에서 배양하는 단계를 포함하고,

상기 생물체는 목적 산물 생산에 필요한 효소를 코딩하는 유전자로 더 형질전환된 것이고, 상기 기질은 상기 목적 산물 생산에 필요한 기질인, 목적 산물의 생산 방법.

청구항 14

청구항 13에 있어서, 상기 배지는 항생제를 포함하지 않는 것인 목적 산물의 생산 방법.

발명의 설명

기술 분야

[0001] 본 발명은 형질전환 생물체 선별용 마커 조성물, 형질전환 생물체 및 형질전환 방법에 관한 것이다.

배경 기술

[0003] 유용 물질들의 산업적인 생산은 최근에는 대부분 LMO를 이용하고 있다. 이 경우에 대사경로 및 목적산물 생합성 경로의 발현 강화를 위해서 관련 유전자들을 다양한 발현 벡터(플라스미드)들 이용해서 발현을 유도하게 된다. 이 경우에 벡터들은 항생제 저항성 유전자를 선택 마커로 갖고 있으며 배양 과정 동안 숙주 세포 안에서 플라스미드의 안정한 유지를 위해서 배양액에 항생제를 첨가하게 된다. 그러나 배양 공정에서 항생제를 사용하게 되면 고가의 항생제 사용으로 인한 생산비용 상승, 항생제 유출로 인한 환경오염 및 자연계에 항생제 저항성 돌연변이 생물체 발생위험, 최종 산물에 항생제가 잔류하여 추가 분리 정제 공정의 필요, 항생제 저항성 유전자 마커 함유 균주 사용 및 허가 취득의 어려움 등의 문제가 있다.

[0004] 아울러 항생제 사용 배양의 경우에 배양시간이 길어지면 항생제의 분해 및 변형으로 인해서 항생제 저항성 유전자를 선택마커로 갖고 있는 플라스미드의 소실이 발생하고 배양 후반기의 생산성의 급격한 감소를 초래한다. 항생제의 분해 및 변형은 항생제 마커 유전자에서 발현된 효소에 의한 것과 항생제의 불안정성에 의한 자연발생적인 것으로 2차 산물 생산 등의 장시간 발효를 요하는 배양공정에서 심각한 문제를 유발한다.

[0005] 이런 문제를 해결하기 위해서 목적산물 생산에 필요한 외래 유전자들을 숙주 생물체의 염색체에 삽입하는 방법도 있지만, 복수의 유전자 카피를 갖는 플라스미드 도입에 비해 유전자량의 감소에 따른 단백질 발현량 감소, 염색체에의 다수의 유전자 도입 및 발현의 어려움 등의 문제가 있다.

[0006] 상기의 이유들로 항생제 마커프리(antibiotic marker-free) 생물체를 개발하는 것이 최근 생물공정 산업계에서는 이슈가 되고 있다. 그러나 현재까지는 산업계에서 안정적으로 유용하게 사용할 수 있는 항생제 마커프리 시스템은 없거나 극히 제한적이다. 영양 요구성 변이주에서 영양요구성 마커(auxotrophic selection marker)를 항생제 마커 대신에 사용할 수도 있지만 이 경우에는 일반적으로 많이 사용되는 산업용 배지인 복합배지를 사용할 수 없다는 단점이 있다.

[0007] 또 다른 예로 Delphi Genetics사가 개발한 StabyExpress™이 있다(<http://www.genengnews.com/gen-articles/protein-bioproduction-without-antibiotics/3891>). 이것은 세균에 존재하는 antidote/poison 시스템인 ccd operon (ccdA and ccdB)을 이용하는 것이다. 그러나 이것은 일부 세균에서만 작동을 하고, ccdA / ccdB의 발현 비율이 정확하게 안 맞으면 작동을 안 하는 경우가 빈번하다.

선행기술문헌

특허문헌

[0009] (특허문헌 0001) 한국등록특허 제1190255호

발명의 내용

해결하려는 과제

[0010] 본 발명은 항생제 및 항생제 저항성 마커를 대체할 수 있는 형질전환 생물체 선별용 마커 조성물을 제공하는 것을 목적으로 한다.

[0011] 본 발명은 항생제 및 항생제 저항성 마커 사용을 요하지 않는 형질전환 방법 및 형질전환 생물체를 제공하는 것을 목적으로 한다.

과제의 해결 수단

[0013] 1. 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하

나가 도입된 플라스미드를 포함하는 형질전환 생물체 선별용 마커 조성물.

- [0014] 2. 위 1에 있어서, 상기 생물체는 내재적으로 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로를 갖는 것인 형질전환 생물체 선별용 마커 조성물.
- [0015] 3. 위 1에 있어서, 상기 합성 경로는 MEP 경로 또는 MVA 경로인 형질전환 생물체 선별용 마커 조성물.
- [0016] 4. 위 1에 있어서, 상기 합성 경로의 효소를 코딩하는 유전자는 1-디옥시-D-자이룰로스-5-포스페이트(DXP) 신타아제, DXP 리덕토이소머라아제, 2-C-메틸-D-에리스리톨-4-포스페이트(MEP) 시티딜트랜스퍼라아제, 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제, 2-C-메틸-D-에리스리톨-2,4-시클로디포스페이트(MEcPP) 신타아제, 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제, HMBPP 리덕타아제, 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 형질전환 생물체 선별용 마커 조성물.
- [0017] 5. 위 1에 있어서, 상기 조성물은 상기와 동일 효소를 코딩하는 유전자 또는 이의 보완 유전자가 감쇄 또는 결손된 생물체에 형질전환 되는 것인 형질전환 생물체 선별용 마커 조성물.
- [0018] 6. 위 5에 있어서, 상기 유전자는 MEP 경로의 효소를 코딩하는 유전자이고, 상기 보완 유전자는 MVA 경로의 효소 중 적어도 하나를 코딩하는 유전자인 형질전환 생물체 선별용 마커 조성물.
- [0019] 7. 위 6에 있어서, 상기 보완 유전자는 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제를 코딩하는 유전자인 형질전환 생물체 선별용 마커 조성물.
- [0020] 8. 위 5에 있어서, 상기 유전자는 MVA 경로의 효소를 코딩하는 유전자이고, 상기 보완 유전자는 MEP 경로의 효소 중 적어도 하나를 코딩하는 유전자인 형질전환 생물체 선별용 마커 조성물.
- [0021] 9. 위 1에 있어서, 상기 유전자 중 적어도 2개를 포함하고, 이들은 별개의 플라스미드에 각각 도입된 것인 형질전환 생물체 선별용 마커 조성물.
- [0022] 10. 위 1에 있어서, 상기 플라스미드는 이소프레노이드, 산탈렌, 비사볼롤 및 레티놀 합성 경로 이루어진 군에서 선택된 경로의 효소를 코딩하는 유전자가 더 도입된 것인 형질전환 생물체 선별용 마커 조성물.
- [0023] 11. 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나가 감쇄 또는 결손되고, 상기 감쇄 또는 결손된 유전자와 동일 효소를 코딩하는 유전자 또는 그 보완 유전자가 도입된 플라스미드로 형질전환된 생물체.
- [0024] 12. 위 11에 있어서, 상기 생물체는 내재적으로 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로를 갖는 것인 생물체.
- [0025] 13. 위 11에 있어서, 상기 합성 경로는 MEP 경로 또는 MVA 경로인 생물체.
- [0026] 14. 위 11에 있어서, 상기 합성 경로의 효소를 코딩하는 유전자는 1-디옥시-D-자이룰로스-5-포스페이트(DXP) 신타아제, DXP 리덕토이소머라아제, 2-C-메틸-D-에리스리톨-4-포스페이트(MEP) 시티딜트랜스퍼라아제, 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제, 2-C-메틸-D-에리스리톨-2,4-시클로디포스페이트(MEcPP) 신타아제, 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제, HMBPP 리덕타아제, 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 생물체.
- [0027] 15. 위 11에 있어서, 상기 감쇄 또는 결손되는 유전자는 MEP 경로 상의 효소를 코딩하는 유전자인 생물체.
- [0028] 16. 위 15에 있어서, 상기 유전자는 DXP 신타아제 및 DXP 리덕토이소머라아제 중 적어도 하나를 코딩하는 유전자인 생물체.
- [0029] 17. 위 11에 있어서, 상기 감쇄 또는 결손되는 유전자는 MEP 경로 상의 효소를 코딩하는 유전자이고, 상기 보완 유전자는 MVA 경로 상의 효소 중 적어도 하나를 코딩하는 유전자인 생물체.
- [0030] 18. 위 17에 있어서, 상기 보완 유전자는 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-

CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제를 코딩하는 유전자인 생물체.

- [0031] 19. 위 11에 있어서, 상기 감쇄 또는 결손되는 유전자는 MVA 경로 상의 효소를 코딩하는 유전자인 생물체.
- [0032] 20. 위 11에 있어서, 상기 플라스미드는 이소프레노이드, 산탈렌, 비사볼롤 및 레티놀 합성 경로 이루어진 군에서 선택된 경로의 효소를 코딩하는 유전자가 더 도입된 것인 생물체.
- [0033] 21. 형질전환 대상 균주의 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나를 감쇄 또는 결손시키는 단계; 및
- [0034] 상기 균주를 감쇄 또는 결손된 유전자와 동일 효소를 코딩하는 유전자 또는 이의 보완 유전자가 도입된 재조합 플라스미드로 형질전환시키는 단계;를 포함하는 생물체의 형질 전환 방법.
- [0035] 22. 위 21에 있어서, 상기 생물체는 내재적으로 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로를 갖는 것인 생물체의 형질 전환 방법.
- [0036] 23. 위 21에 있어서, 상기 형질 전환은 항생제 없이 수행되는 것인 생물체의 형질 전환 방법.
- [0037] 24. 위 21에 있어서, 상기 합성 경로는 MEP 경로 또는 MVA 경로인 생물체의 형질 전환 방법.
- [0038] 25. 위 21에 있어서, 상기 합성 경로의 효소를 코딩하는 유전자는 1-디옥시-D-자이올로스-5-포스페이트(DXP) 신타아제, DXP 리덕토이소머라아제, 2-C-메틸-D-에리스리톨-4-포스페이트(MEP) 시티딜트랜스퍼라아제, 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제(IspE), 2-C-메틸-D-에리스리톨-2,4-시클로디포스페이트(MecPP) 신타아제, 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제, HMBPP 리덕타아제, 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제 로 이루어진 군에서 선택된 1종 이상의 효소를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0039] 26. 위 21에 있어서, 상기 감쇄 또는 결손되는 유전자는 MEP 경로 상의 효소를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0040] 27. 위 26에 있어서, 상기 유전자는 DXP 신타아제 및 DXP 리덕토이소머라아제 중 적어도 하나를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0041] 28. 위 21에 있어서, 상기 감쇄 또는 결손되는 유전자는 MEP 경로 상의 효소를 코딩하는 유전자이고, 상기 보완 유전자는 MVA 경로 상의 효소 중 적어도 하나를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0042] 29. 위 28에 있어서, 상기 보완 유전자는 아세토아세틸-CoA 신타아제, 3-히드록실-3-메틸글루타리-CoA (HMG-CoA) 신타아제, HMG-CoA 리덕타아제, 메발로네이트 키나아제, 메발로네이트-5-포스페이트 키나아제, 메발로네이트-5-디포스페이트 디카르복실라아제 및 IPP 아이소머라아제를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0043] 30. 위 21에 있어서, 상기 감쇄 또는 결손되는 유전자는 MVA 경로 상의 효소 중 적어도 하나를 코딩하는 유전자인 생물체의 형질 전환 방법.
- [0044] 31. 위 21에 있어서, 상기 플라스미드는 이소프레노이드, 산탈렌, 비사볼롤 및 레티놀 합성 경로 이루어진 군에서 선택된 경로의 효소를 코딩하는 유전자를 더 포함하는 생물체의 형질 전환 방법.
- [0045] 32. 위 21에 있어서, 적어도 2개의 유전자를 감쇄 또는 결손시키고, 상기 균주를 이와 동일한 효소를 코딩하는 유전자를 각각 포함하는 적어도 2개의 플라스미드로 형질전환시키는 생물체의 형질 전환 방법.
- [0046] 33. 위 11 내지 20 중 어느 한 항의 생물체를 기질이 포함된 배지 상에서 배양하는 단계를 포함하는 목적 산물의 생산 방법.
- [0047] 34. 위 33에 있어서, 상기 배지는 항생제를 포함하지 않는 것인 목적 산물의 생산 방법.

발명의 효과

- [0049] 본 발명의 형질전환 생물체 선별용 마커 조성물은 항생제 저항성 유전자를 사용하지 않는다. 이에, 항생제 및 항생제 저항성 유전자의 사용 없이도 형질 전환이 가능하며, 항생제 및 항생제 저항성 유전자 사용에 기인하는

많은 문제들을 원천적으로 방지할 수 있다.

[0050] 본 발명의 형질전환 생물체 선별용 마커 조성물은 생물체의 장시간 배양에도 소실이 적다.

[0051] 본 발명의 생물체는 항생제, 항생제 저항성 유전자 없이 형질전환 및 목적 산물의 생산이 가능하며, 항생제 및 항생제 저항성 유전자 사용에 기인하는 많은 문제들을 원천적으로 방지할 수 있다.

[0052] 본 발명의 형질전환 방법은 항생제, 항생제 저항성 유전자 없이 형질전환이 가능하다.

[0053] 본 발명의 목적 산물의 생산 방법은 높은 수율로 목적 산물을 생산할 수 있다.

도면의 간단한 설명

[0055] 도 1은 IPP와 DMAPP 생합성 경로이다.

도 2는 레티노이드의 HPLC 분석 프로파일(Profile)이다.

도 3은 레티노이드 정량분석을 위한 표준곡선이다.

도 4는 산탈렌의 GC 분석 프로파일이다.

도 5는 비사볼롤의 GC 분석 프로파일이다.

도 6은 비사볼롤 정량분석을 위한 표준곡선

도 7은 발효 부산물 생성 경로이다.

도 8은 MEP 경로 결손주에서 외래 MVA 경로를 보완하여 구축한 항생제 불필요 산탈렌 생산 균주의 항생제 유무에 따른 배양 결과이다.

도 9는 MEP 경로 결손주에서 외래 MVA 경로를 두 플라스미드로 나누어 구축한 항생제가 불필요한 레티노이드 생산 균주의 항생제 유무에 따른 배양 결과이다.

도 10은 항생제 마커가 제거된 레티노이드 생산 균주의 항생제 유무에 따른 배양 결과이다.

도 11은 결손된 MEP 경로 유전자를 선별마커로 이용한 산탈렌 생산 균주의 항생제 유무에 따른 배양 결과이다.

도 12는 결손된 MEP 경로 유전자를 복수 플라스미드에 나누어 구축하여 항생제가 불필요한 이소프레노이드(산탈렌, 비사볼롤) 생산 균주의 배양 결과이다.

도 13은 결손된 MEP 경로 유전자와 함께 외래 MVA 경로가 추가 도입된 항생제 마커가 제거된 레티노이드 생산 균주의 배양 결과이다.

도 14는 결손된 MEP 경로 유전자를 선별마커로 하여 항생제가 불필요한 균주의 형광 단백질 발현 결과이다.

도 15는 pCIN-mvaA-EGFP 재조합 플라스미드 모식도이다.

도 16은 결손된 MVA 경로 유전자를 선별마커로하여 항생제가 불필요한 균주의 형광 단백질 발현 결과이다.

발명을 실시하기 위한 구체적인 내용

[0056] 이하 본 발명을 상세히 설명한다.

[0058] 본 발명은 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나가 도입된 플라스미드를 포함하는 형질전환 생물체 선별용 마커 조성물을 제공한다.

[0059] 이소펜테닐 디포스페이트(IPP) 또는 디메틸알릴 디포스페이트(DMAPP) 합성 경로는 모든 생명체가 필수적으로 갖고 있는 생합성 경로로서, IPP, DMAPP는 세포 내 필수 대사물질로 결핍이 되면 세포는 성장을 할 수 없다. 또한, 이들 물질은 강력한 음의 전하를 갖는 이인산화 물질로 배지 내에 존재해도 세포 내로 유입될 수가 없어서 반드시 세포 내에서 만들어져야 한다.

[0060] 이에, 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도

하나가 도입된 플라스미드를 포함하는 경우에 이를 형질전환 생물체 선별용 마커 조성물로 사용될 수 있다.

- [0061] 본 발명에 따른 생물체는 상기 이소펜테닐 디포스페이트(IPP) 또는 디메틸알릴 디포스페이트(DMAPP) 합성 경로를 내재적으로 가지는 것이라면 그 종류는 제한되지 않으며, 동물, 식물, 미생물을 포함하는 것이며, 구체적으로는 미생물일 수 있다.
- [0062] 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로는 구체적으로 도 1에 도시된 MEP 경로 또는 MVA 경로일 수 있다.
- [0063] 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자는 예를 들면 MEP 경로의 1-디옥시-D-자이룰로스-5-포스페이트(DXP) 신타아제(1-deoxy-D-xylulose-5-phosphate synthase), DXP 리덕토이소머라아제(DXP reductoisomerase), 2-C-메틸-D-에리스리톨-4-포스페이트(MEP) 시티딜트랜스퍼라아제(2-C-methyl-D-erythritol-4-phosphate (MEP) cytidyltransferase), 4-디포스포시티딜-2-C-메틸-D-에리스리톨 키나아제(4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, IspE), 2-C-메틸-D-에리스리톨-2,4-시클로 디포스페이트(MECPP) 신타아제(2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase), 4-히드록시-3-메틸-2-부테닐 디포스페이트 (HMBPP) 신타아제(4-hydroxy-3-methyl-2-butenyl diphosphate synthase), HMBPP 리덕타아제(HMBPP reductase), MVA 경로의 아세토아세틸-CoA 신타아제(acetoacetyl-CoA synthase), 3-히드록시-3-메틸글루타리-CoA (HMG-CoA) 신타아제(3-hydroxyl-3-methylglutary-CoA synthase), HMG-CoA 리덕타아제(HMG-CoA reductase), 메발로네이트 키나아제(mevalonate kinase), 메발로네이트-5-포스페이트 키나아제(mevalonate-5-phosphate kinase), 메발로네이트-5-디포스페이트 디카르복실라아제(mevalonate-5-diphosphate decarboxylase) 및 IPP 아이소머라아제(IPP isomerase) 등의 유전자를 들 수 있다.
- [0064] 본 발명의 형질전환 생물체 선별용 마커 조성물은 상기와 동일 효소를 코딩하는 유전자 또는 이의 보완 유전자가 감쇄 또는 결손된 생물체에 형질전환 되는 것일 수 있다.
- [0065] 전술한 바와 같이, 이소펜테닐 디포스페이트 및 디메틸알릴 디포스페이트 합성 경로가 불활성화되면 세포가 생존할 수 없게 되는데, 본 발명의 마커 조성물은 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나가 도입된 플라스미드를 포함하여, 이로 형질전환된 생물체는 결손된 IPP/DMAPP 경로가 다시 활성화되어 생존 가능하므로, 생물체의 생존 여부로 형질 전환 생물체를 확인할 수 있다.
- [0066] 형질전환 대상 생물체는 마커 조성물의 유전자와 동일 효소를 코딩하는 유전자 또는 이의 보완 유전자가 감쇄 또는 결손된 것일 수 있다.
- [0067] 동일 효소를 코딩하는 유전자인 경우 감쇄 또는 결손된 유전자와 동일한 유전자일 수도 있고, 다른 종 유래의 동일 효소를 코딩하는 유전자일 수도 있다.
- [0068] 구체적인 예를 들자면, 형질전환 대상 생물체는 DXP 리덕토이소머라아제를 코딩하는 유전자가 감쇄 또는 결손된 것일 수 있고, 본 발명에 따른 플라스미드는 그와 동일한 유전자 또는 다른 종 유래(유전자는 상이)의 동일한 효소를 코딩하는 유전자가 도입된 것일 수 있다.
- [0069] 보완 유전자는 유전자의 감쇄 또는 결손에 의해 불활성화된 경로 이외의 경로를 활성화시켜 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트를 생산할 수 있도록 하는 유전자를 의미한다. 예를 들면 생물체가 MEP 경로의 유전자가 감쇄 또는 결손된 경우에 마커 조성물의 플라스미드는 MVA 경로 전체의 효소를 코딩하는 유전자가 도입된 것일 수 있고, 생물체가 MVA 경로의 유전자가 감쇄 또는 결손된 경우에 마커 조성물의 플라스미드는 MEP 경로 전체의 효소를 코딩하는 유전자가 도입된 것일 수 있다.
- [0070] 본 발명의 조성물이 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 2개 이상이 도입된 플라스미드를 포함하는 경우에, 하나의 플라스미드에 2개 이상의 유전자가 도입된 것일 수도 있고, 별개의 플라스미드에 각각 유전자가 도입된 것일 수도 있다.
- [0071] 별개의 플라스미드에 각각 유전자가 도입된 경우에는 생물체가 모든 플라스미드로 형질전환되어야 생존할 수 있으므로, 생존하는 생물체를 모든 플라스미드로 형질전환된 생물체로서 선별할 수 있다.
- [0072] 구체적인 예를 들자면, 형질전환 대상 생물체는 DXP 신타아제를 코딩하는 유전자 및 DXP 리덕토이소머라아제를 코딩하는 유전자 중 적어도 하나가 감쇄 또는 결손된 것일 수 있고, 본 발명의 조성물은 그와 동일한 유전자 또는 다른 종 유래(유전자는 상이)의 동일한 효소를 코딩하는 유전자가 각각 도입된 2개의 플라스미드를 포함할

수 있다.

- [0073] 통상 생물체의 형질전환은 항생제가 존재하는 배양액 상에서 수행되어, 항생제 저항성 유전자가 선택 마커로서 사용되나, 그러한 경우에 고가의 항생제 사용에 의한 생산 비용 상승, 항생제 유출에 따른 환경 오염, 항생제 저항성 돌연변이 생물체 발생 위험 등의 문제가 있다.
- [0074] 또한, 항생제 저항성 유전자가 숙주 세포를 완벽하게 보호하는 것은 아니므로, 항생제에 의한 숙주 세포의 손상이 발생할 수 있고, 이를 최소화하는 항생제 사용 최적 농도를 설정하고 이를 유지하는 것은 산업계에서 매우 어려운 일로 인식되며, 이는 숙주 세포의 변경마다 달라질 수 있다.
- [0075] 더욱이, 항생제 사용 배양의 경우에 배양 시간이 길어지면 항생제의 분해 및 변형으로 인해서 항생제 저항성 유전자를 선택마커로 갖고 있는 플라스미드의 소실이 발생하고 배양 후반기의 생산성의 급격한 감소를 초래할 수 있다. 항생제의 분해 및 변형은 항생제 마커 유전자에서 발현된 효소에 의한 것과 항생제의 불안정성에 의한 자연발생적인 것으로 2차 산물 생산 등의 장시간 발효를 요하는 배양공정에서 심각한 문제를 유발한다.
- [0076] 그러나, 본 발명의 상기 형질전환 생물체 선별용 마커 조성물을 사용함으로써 상기 문제점들의 발생을 원천적으로 방지하고, 항생제를 사용하지 않고도 플라스미드를 숙주 생물체 내에서 안전하게 유지하고, 형질전환 생물체를 선별할 수 있다.
- [0077] 또한, 그러한 플라스미드에 목적 산물 생산을 위한 경로의 효소를 코딩하는 유전자를 추가로 도입하여, 목적 산물을 숙주 생물체에서 안정적으로 대량 생산하는 데에 활용할 수도 있다.
- [0079] 또한, 본 발명은 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나가 감쇄 또는 결손되고, 상기 감쇄 또는 결손된 유전자와 동일한 효소를 코딩하는 유전자 또는 그 보완 유전자가 도입된 플라스미드로 형질전환된 생물체를 제공한다.
- [0080] 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로는 MEP 경로 또는 MVA 경로일 수 있고, 이들 효소는 전술한 바와 같다.
- [0081] 본 발명의 생물체에서 감쇄 또는 결손되는 유전자는 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나, 구체적으로 MEP 경로 또는 MVA 경로 상의 효소를 코딩하는 유전자 중 적어도 하나일 수 있다.
- [0082] 본 발명의 생물체는 감쇄 또는 결손된 유전자와 동일한 유전자 또는 다른 종 유래의 동일한 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환된 것일 수 있다.
- [0083] 구체적인 예를 들자면, DXP 신타아제 및 DXP 리덕토이소머라아제를 코딩하는 유전자 중 적어도 하나가 감쇄 또는 결손된 것일 수 있고, 그와 동일한 유전자 또는 다른 종 유래(유전자는 상이)의 동일한 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환된 것일 수 있다.
- [0084] 또한, 본 발명의 생물체는 감쇄 또는 결손된 유전자의 보완 유전자가 도입된 플라스미드로 형질전환된 것일 수 있다.
- [0085] 보완 유전자는 유전자의 감쇄 또는 결손에 의해 불활성화된 경로 이외의 경로를 활성화시켜 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트를 생산할 수 있도록 하는 유전자를 의미한다. 예를 들면 생물체가 MEP 경로의 유전자가 감쇄 또는 결손된 경우에 MVP 경로 전체의 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환된 것일 수 있고, 생물체가 MVA 경로의 유전자가 감쇄 또는 결손된 경우에 MEP 경로 전체의 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환된 것일 수 있다.
- [0086] 본 발명의 생물체는 목적 산물 생산을 위한 목적 산물 합성 경로의 효소를 코딩하는 유전자로 더 형질전환된 것일 수 있다.
- [0087] 그 유전자는 목적하는 산물에 따라 다양하게 선택될 수 있으며, 예를 들면 이소프레노이드 합성 경로, 산탈렌 합성 경로, 레티놀 합성 경로, 비사볼롤 합성 경로 등을 들 수 있으나, 이에 제한되는 것은 아니다. 상기 각 합성 경로는 모두 공지된 경로로서, 공지된 경로의 효소를 코딩하는 유전자로 형질전환될 수 있다.
- [0088] 또한, 본 발명의 생물체는 상기 목적 산물 합성 경로에서 목적 산물의 부산물 생성 경로의 효소를 코딩하는 유전자가 감쇄 또는 결손된 것일 수 있다. 이에 의해 목적 산물 생산 수율이 더욱 향상될 수 있다. 예를 들면 MVA

경로의 출발 물질인 acetyl-CoA를 발효부산물인 acetate, lactate, ethanol로 전환시키는 효소들을 들 수 있고, 구체적으로 아세트알데히드 디하이드로게나아제(adhE), 파루베이트 옥시다아제(PoxB), 락테이트 디하이드로게나아제(ldhA), 아세틸-CoA:아세토아세틸-CoA 신타아제(atoDA) 등을 들 수 있으나, 이에 제한되는 것은 아니다(도 7 참조).

- [0089] 본 발명의 생물체는 항생제 저항성 유전자를 포함하지 않을 수 있다. 항생제 저항성 유전자를 마커로 사용하지 않으므로 이를 포함하는 것이 불필요하다. 물론, 전술한 형질전환 이후에 추후 항생제 저항성 유전자가 도입된 플라스미드로 추가로 형질전환되어 항생제 저항성 유전자를 포함할 수도 있다.
- [0091] 본 발명은 형질전환 대상 생물체의 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나를 감쇄 또는 결손시키는 단계; 및 상기 생물체를 감쇄 또는 결손된 유전자와 동일한 효소를 코딩하는 유전자 또는 그 보완 유전자가 도입된 재조합 플라스미드로 형질전환시키는 단계;를 포함하는 생물체의 형질 전환 방법을 제공한다.
- [0092] 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자는 전술한 범위 내의 유전자일 수 있다.
- [0093] 전술한 바와 같이 통상 생물체의 형질 전환은 항생제를 포함한 배양액 상에서 수행되는 것이지만, 본 발명의 형질 전환 방법은 항생제 저항성 마커를 사용하지 않으므로, 본 발명의 형질 전환은 항생제 없이 수행될 수 있다.
- [0094] 상기 감쇄 또는 결손되는 유전자는 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트 합성 경로의 효소를 코딩하는 유전자 중 적어도 하나일 수 있다. 구체적으로, MEP 경로 또는 MVA 경로 상의 효소를 코딩하는 유전자일 수 있다.
- [0095] 형질전환시에는 감쇄 또는 결손된 유전자와 동일한 유전자 또는 다른 종 유래의 동일한 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환시킬 수 있다.
- [0096] 구체적인 예를 들자면, DXP 신타아제 및 DXP 리덕토이소머라아제 중 적어도 하나를 코딩하는 유전자가 감쇄 또는 결손될 수 있고, 그와 동일한 유전자 또는 다른 종 유래(유전자는 상이)의 동일한 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환될 수 있다.
- [0097] 또한, 감쇄 또는 결손된 유전자의 보완 유전자가 도입된 플라스미드로 형질전환될 수 있다.
- [0098] 보완 유전자는 유전자의 감쇄 또는 결손에 의해 불활성화된 경로 이외의 경로를 활성화시켜 이소펜테닐 디포스페이트 또는 디메틸알릴 디포스페이트를 생산할 수 있도록 하는 유전자를 의미한다. 예를 들면 생물체가 MEP 경로의 유전자가 감쇄 또는 결손된 경우에 MVP 경로 전체의 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환 될 수 있고, 생물체가 MVA 경로의 유전자가 감쇄 또는 결손된 경우에 MEP 경로 전체의 효소를 코딩하는 유전자가 도입된 플라스미드로 형질전환 될 수 있다.
- [0099] 2개 이상의 유전자가 도입되는 경우에 이들은 단일 플라스미드에 도입될 수도 있고, 복수개의 플라스미드에 각각 도입될 수도 있다.
- [0100] 비제한적인 보다 구체적인 결손 및 형질전환 방법의 예시를 들자면, 전술한 바와 같이 IPP, DMAPP가 없으면 생물체가 생존할 수 없으므로, MEP 경로의 dxs 또는 ispC의 결손시에 배지에 2-C-메틸-D-에리스리톨을 첨가하여 ispC의 대사산물인 2-C-메틸-D-에리스리톨 4-포스페이트(MEP)를 생성하여 생물체의 생육을 유지시킨 상태에서 상기 생물체를 이와 동일한 효소를 코딩하는 유전자 또는 그 보완 유전자로 형질전환시킬 수 있다. 또한, 먼저 MVA 하부 경로 유전자를 도입하고, 메바론산이 첨가된 배지에서 MEP 경로 유전자의 감쇄 또는 결손을 수행할 수도 있다.
- [0101] MVA 경로 유전자의 결손시에는 상부 경로, 하부 경로 감쇄 또는 결손에 따라 구체적인 방법이 달라질 수 있다.
- [0102] 상부 경로 결손의 구체적인 예를 들자면 배지에 메바론산을 첨가하여 하부 경로만으로 생육을 유지시킨 상태에서, 상부 경로 유전자를 감쇄 또는 결손시키고 이와 동일한 효소를 코딩하는 유전자 또는 그 보완 유전자로 형질전환시킬 수 있다.
- [0103] 하부 경로 결손의 구체적인 예를 들자면, MEP 경로 유전자 모두를 도입하여 생육을 유지시킨 상태에서, 상부 경로 유전자를 감쇄 또는 결손시키고 이와 동일한 효소를 코딩하는 유전자 또는 그 보완 유전자로 형질전환시킬

수 있다.

- [0105] 본 발명에 따른 플라스미드는 목적 산물 생산을 위한 목적 산물 생산 경로의 효소를 코딩하는 유전자를 더 포함할 수 있다.
- [0106] 그 유전자는 목적하는 산물에 따라 다양하게 선택될 수 있으며, 예를 들면 이소프레노이드 합성 경로, 산탈렌 합성 경로, 레티놀 합성 경로, 비사볼롤 합성 경로 등을 들 수 있으나, 이에 제한되는 것은 아니다. 상기 각 합성 경로는 모두 공지된 경로로서, 공지된 경로의 효소를 코딩하는 유전자가 더 도입될 수 있다.
- [0107] 또한, 본 발명에 따른 생물체는 상기 목적 산물 합성 경로에서 목적 산물의 부산물 생성 경로의 효소를 코딩하는 유전자가 감쇄 또는 결실된 것일 수 있다. 이에 의해 목적 산물 생산 수율이 더욱 향상될 수 있다. 예를 들면 MVA 경로의 출발 물질인 acetyl-CoA를 발효부산물인 acetate, lactate, ethanol로 전환시키는 효소들을 들 수 있고, 구체적으로 아세트알데히드 디하이드로게나아제(adhE), 파루베이트 옥시다아제(PoxB), 락테이트 디하이드로게나아제(ldhA), 아세틸-CoA:아세토아세틸-CoA 신타아제(atoDA) 등을 들 수 있으나, 이에 제한되는 것은 아니다(도 7 참조).
- [0109] 또한, 본 발명은 상기 생물체를 이용한, 또는 상기 형질전환 방법을 포함하는 목적 산물의 생산 방법을 제공한다.
- [0110] 본 발명의 목적 산물의 생산 방법은 상기 생물체를 목적 산물 생산 경로의 효소를 코딩하는 유전자로 형질전환시켜 목적 산물을 생산한다.
- [0111] 목적 산물 생산 경로의 효소를 코딩하는 유전자는 전술한 플라스미드에 도입되어 생물체에 형질전환될 수 있다.
- [0112] 목적 산물은 상기 생물체를 기질을 포함하는 배지 중에서 배양하여 생산할 수 있고, 배양은 항생제를 포함하지 않는 배양 조건에서 수행될 수 있다.
- [0113] 본 발명의 방법은 항생제 및 항생제 저항성 유전자 없이도 형질전환 및 목적 산물의 생산이 가능하여, 항생제 사용에 의한 문제를 원천적으로 방지할 수 있고, 항생제의 분해 및 변형에 의한 플라스미드의 소실 문제가 발생하지 않으므로, 보다 높은 수율로 목적 산물을 생산할 수 있다.
- [0115] 이하, 본 발명을 구체적으로 설명하기 위해 실시예를 들어 상세하게 설명하기로 한다.

[0117] 실시예

[0118] 1. 재료 및 방법

[0119] 1) 실험균주 및 재료

[0120] 실험에 이용된 미생물은 ATCC (American Type Culture Collection), KCTC (Korea Collection for Type Cultures), 및 KCCM (Korea Culture Center of Microorganisms)에서 구입하였으며, 표 1에 정리하였다.

표 1

[0122] 실험균주	설명	정보 또는 참고문헌
DH5a	F ⁻ , λ ⁻ , <i>endA1</i> , <i>glnV44</i> , <i>thi-1</i> , <i>recA1</i> , <i>relA1</i> , <i>gyrA96</i> , <i>deoR</i> , ϕ80, <i>dlacZ</i> Δ15, Δ <i>lacZYA-argF</i> U169, <i>hsdR17</i> (r _K ⁻ m _K ⁺), <i>supE44</i>	NEB C2987
MG1655(DE3)	F ⁻ λ ⁻ <i>ilvG rfb-50 rph-1</i> (DE3)	KCCM 41310
MG1655(DE3) DadhE ::MVA bottom	DadhE (::P _{cTrc} -SN12Didi-ter)	-

MG1655(DE3) DatoDA::MVA bottom	DatoDA (::P _{cTrc} -SN12Didi-ter)	-
MG1655(DE3) DldhA::MVA bottom	DldhA (::P _{cTrc} -SN12Didi-ter)	-
MG1655(DE3) DpoxB::MVA bottom	DpoxB (::P _{cTrc} -SN12Didi-ter)	-
MG1655(DE3) Δ dxr DadhE ::MVA bottom	Δ dxr DadhE (::P _{cTrc} -SN12Didi-ter)	-
MG1655(DE3) Δ dxr/s DadhE ::MVA bottom	Δ dxr, Δ dxs, DadhE (::P _{cTrc} -SN12Didi-ter)	-
<i>E. coli</i> EC1000	RepA ⁺ MC1000, KmR, carrying a single copy of the pWV01 repA gene in the glgB gene; host for pOR128-based plasmids	Leenhouts et al Mol Gen Genet. 1996 Nov 27;253(1-2):217-24
<i>L. lactis</i> subsp. <i>cremoris</i> MG1363	Plasmid-free derivative of <i>L. lactis</i> subsp. <i>Cremoris</i> NCD0712	Plasmid complement of <i>Streptococcus lactis</i> NCD0712 and other lactic streptococci after protoplast-induced curing. J. Bacteriol.154:1-9.
<i>L. lactis</i> subsp. <i>cremoris</i> MG1363 Δ mvaA	Δ mvaA	-

[0123] 유전자 클로닝에는 DH5 α (F-f80d/lacZDM15D(lacZYA-argF)U169 deoR recA1 endA1 hsdR17(r_K⁻, m_K⁺) *phoA supE44* λ⁻ thi⁻¹ *gyrA96 relA1*)를 이용하였고, 목적물질 생산에는 MG1655(DE3)(F⁻ λ⁻ ilvG rfb-50 rph-1(DE3))를 사용하였다. 발현벡터로는 pTrc99A, pSTV28을 사용하였다(표 2). 제한효소 및 기타 효소는 New England Biolabs (미국) 제품을 사용하였다. 또한 PCR을 수행하기 위해서는 *Pfu-X* DNA polymerase는 Solgent (한국), Phusion DNA polymerase는 Thermo Scientific (미국) 제품을 사용하였다. DNA size maker는 Invitrogen (미국) 제품을 사용하였다. IPTG는 Promega(미국), L(+)-arabinose, glucose, lactose는 Sigma (미국), acetone은 Merck (미국), glycerol은 Amresco (미국) 제품을 사용하였다. 그 외 시약들은 Sigma (미국)의 제품을 사용하였다.

[0124] 미생물 배양을 위한 배지의 제조는 각 균주 분양기관의 권장배지조성 및 Difco manual (11th edition, Difco; BD Science, US)에 따랐다. 배지 제조에 사용된 시약은 BD Science (미국) 및 Sigma (미국)로부터 구입하였다. 배양 중의 균체량은 spectrophotometer (Shimadzu UV-1601, Japan)로 600 nm에서 OD (optical density)를 측정 한 결과로 나타내었으며, pH는 pH meter B-212 (HORIBA, Japan)로 측정하였다.

[0125] 유전자 클로닝에는 플라스미드 유지를 위한 항생제로 ampicillin, kanamycin을 사용하였으며, 각각 100 μg/mL, 50 μg/mL 농도로 사용하였다.

[0127] 2) 레티노이드의 추출 및 분석

[0128] 레티노이드는 다음의 방법으로 분석하였다. 배양액 50 ~ 100 μl를 취해서 14,000 rpm에서 40초 동안 원심분리하여 균체를 회수하였다. 회수된 균체에 아세톤 400 μl을 첨가하여 균체를 재현탁시킨 다음 어두운 곳에서 55℃에서 15분 동안 추출하고, 이에 다시 600 μl의 아세톤을 첨가하여 55℃에서 15분 동안 추출하였다. 추출물은 14,000 rpm에서 10분 동안 원심분리 한 후 상층액만을 취하여 HPLC 정량 분석을 실시하였다. 그리고 레티노이드 배양액에 heavy mineral oil 층을 첨가한 경우 heavy mineral oil 층만을 14,000 rpm 에서 10분 동안 원심분리 한 후 레티노이드를 함유하고 있는 heavy mineral oil 층 5 ~ 50 μl를 1 mL 아세톤에 재현탁하여 추출하였으며, 상온에서 15분간 방치하며 5분 간격으로 vortexing하였다. 추출물은 14,000 rpm에서 10분 동안 원심분리 한 후 acetone 층만을 취하여 HPLC 정량 분석을 실시하였다.

[0129] 레티노이드의 분석 시스템은 SHIMADZU LC-20A series with UV/Vis detector (Shimadzu, Kyoto, Japan)을 이용하였으며 분석칼럼은 Symmetry C18 (250x4.6, 5 μm) with Symmetry guard C18 (15x4.6, 5 μm)을 사용하였다. 이동상은 메탄올: 아세토니트릴 혼합용액(95:5, v/v)으로 총 15분간 분석하였다. 유속은 1.5 mL/min으로 하였으며 검출 파장은 각각 레티날은 370 nm, 레티놀과 레티닐 아세테이트는 340 nm에서 측정하였고, 시료 주입량은 20

μ l, 오븐 온도는 40℃로 분석하였다. 레티노이드 표준시료는 에탄올에 녹여 사용하였다. 표준시간의 피크 머무름 시간은 레티놀 약 3.2분, 레티날 약 3.4분, 레티닐 아세테이트는 약 4.0분이며(도 2), 분석결과를 분석된 피크의 면적을 표준시료의 피크 면적으로 계산한 검량선에 대입하고 회석배수를 환산하여 계산하였다(도 3).

[0131] 3) 산탈렌의 추출 및 분석

[0132] 배지에 테케인을 도포하는 2상 배양에서 생산된 산탈렌 분석을 위해서 테케인 층의 gas chromatography (GC)와 gas chromatography-mass spectrometry (GC-MS) 분석을 실시하였다(도 4). GC 분석은 19091N-133 HP-Innowax column(30 m; internal diameter, 0.25 mm; film thickness, 250 nm)이 장착된 GC/FID (Agilent Technologies7890A)를 이용하였다. 컬럼 오븐 온도는 초기 80℃에서 1 min, 그리고 10℃/min의 속도로 250℃까지 올리고 1분 동안 유지하였다. 이동상 기체로 질소를 39 psi의 압력으로 흘려주고, detector temperature는 260℃로 유지하였다. GC-MS 분석은 GCMS-QP2010 Ultra (SHIMADU, Tokyo, Japan)를 사용하였고 이동상 기체로는 helium을 이용하였다.

[0134] 4) 비사볼롤의 추출 및 분석

[0135] 배양 종료 후 테칸층을 원심분리(14,000 rpm, 10 min)를 통해 회수하여 gas chromatography (GC)와 gas chromatography-mass spectrometry (GC-MS)로 분석하였다(도 5). GC 분석은 19091N-133 HP-Innowax column(30 m; internal diameter, 0.25 mm; film thickness, 250 nm)이 장착된 GC/FID (Agilent Technologies 7890A)를 이용하였다. 컬럼 오븐 온도는 초기 50℃에서 20℃/min의 속도로 증가시켰으며, 90℃ 도달 이후 15℃/min의 속도로 증가시켰다. 온도가 150℃에 도달한 후 20℃/min 속도로 190℃까지 온도를 증가시킨 후 10℃/min의 속도로 260℃에 도달하게 한 후 2분 동안 유지하게 하였다. 이동상 기체로 질소를 30 psi의 압력으로 공급하였고, 검출기 온도는 280℃로 유지하였다. GC-MS 분석은 GCMS-QP2010 Ultra (SHIMADU, Japan)를 사용하였고 이동상 기체로는 헬륨을 이용하였다. 측정된 비사볼롤의 피크 면적 값은 미리 작성한 검량선(도 6)에 따라 다음과 같이 계산하였다.

[0137] [수학식 1]

$$\alpha\text{-Bisabolol (mg/L)} = 0.8116 \times \text{GC Peak area} \times \text{회석배수}$$

[0140] 2. MEP 경로 결손 균주 구축 과정

[0141] - MEP 경로 결손 균주 구축 시 MVA 하부경로 유전자를 염색체에 도입하고 구축 작업은 메바론산 첨가 배지에서 진행한 예시

[0142] - MEP 상부경로 유전자 결손

[0143] 1) 대장균 염색체 내 외래 MVA 하부경로 삽입

[0144] 대장균 염색체 내 외래경로 삽입을 위한 방법은 λ -Red recombinase를 이용한 PCR 기반의 상동성 재조합 방법과 P1 형질도입 방법으로 크게 두 가지가 있다. 이번 실험에서는 λ -Red recombinase를 이용한 PCR 기반의 상동성 재조합 방법을 이용하여 외래 MVA 하부경로를 대장균 MG1655(DE3) 균주에 삽입해주었다. MVA 경로에서 mevalonate 이후부터 DMAPP까지의 경로를 하부 경로로 하였다(도 1 참조). 현재 MVA 하부경로는 *Streptococcus pneumoniae*의 *mvaK1*, *mvaK2*, *mvaD*와 *E. coli*의 *idi* 유전자로 이루어져 있으며, 서열번호 1과 서열번호 2의 프라이머를 이용해 상기의 유전자를 증폭해 pTFCC(DPB) 벡터에 클로닝하여 pTFCC(DPB)-SN12Didi를 구축하였다. MVA 하부경로 삽입위치는 *adhE*, *poxB*, *ldhA*, *atoDA* 네 가지 유전자에 각각 삽입하였다. 이는 MVA 경로의 출발물질인 acetyl-CoA를 발효부산물인 acetate, lactate, ethanol로 전환시키는 유전자들로 이를 차단하여 발효생산성 및 탄소원 이용효율을 향상시킬 수 있다(도 7).

[0145] 실험방법은 다음과 같다. 먼저 대장균 염색체 내에 삽입할 경로가 균체 내에서 발현할 수 있도록 promoter, multi-cloning site, terminator, FRT site, 항생제 마커를 포함한 벡터를 구축하였으며, 이는 표 2에 나타내었다.

표 2

[0147]

플라스미드	설명	Genbank 허가번호	제품	서열 번호
pSTV28	p15A origin, <i>lac</i> promoter, <i>lacZ</i> , and <i>cat</i>	M22744	Takara Korea (한국)	71
pTrc99A	pMB1 origin, <i>trc</i> promoter, <i>lacI^d</i> , and <i>bla</i>	U13872.1	Pharmacia (US)	72
pTFKC(DPB)	ColE1 origin, <i>trc</i> promoter without operator region, kanamycin cassette with FRT, and <i>bla</i>	-		73
pTFCC(DPB)	ColE1 origin, <i>trc</i> promoter without operator region, chloramphenicol cassette with FRT, and <i>bla</i>	-		74
pCP20	cI857 λ ts, FLP, <i>cat</i> , and <i>bla</i>	-		75
pKD46	repA101ts, oriR101, <i>exo</i> , <i>bet</i> , <i>gam</i> , tL3, P _{BAD} , <i>araC</i> , and <i>bla</i>	AY048746		76
pT-ispA-STs	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 <i>ispA</i> ; <i>Clausena lansium</i> 유래 santalene synthase 인 STs를 포함하는 Ptrc 발현벡터	-		77
pTAS-NA	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 <i>ispA</i> 및 <i>idi</i> ; <i>Clausena lansium</i> 유래 santalene synthase 인 STs; <i>E. faecalis</i> 유래 <i>mvaE</i> 및 <i>mvaS</i> ; <i>S.pneumonia</i> 유래 <i>mvaK1</i> , <i>mvaK2</i> 및 <i>mvaD</i> 포함하는 Ptrc 발현벡터	-	In this study	78
pSNAK	pSTV28 벡터; lacZ; Km ^r ; <i>E. faecalis</i> 유래 <i>mvaE</i> 및 <i>mvaS</i> ; <i>S.pneumonia</i> 유래 <i>mvaK1</i> , <i>mvaK2</i> 및 <i>mvaD</i> ; <i>E. coli</i> 유래 <i>idi</i> 를 포함하는 Plac 발현 벡터	-		79
pSNAK(-E)	pSTV28 벡터; lacZ; Km ^r ; <i>E. faecalis</i> 유래 <i>mvaS</i> ; <i>S.pneumonia</i> 유래 <i>mvaK1</i> , <i>mvaK2</i> 및 <i>mvaD</i> ; <i>E. coli</i> 유래 <i>idi</i> 를 포함하는 Plac 발현 벡터	-	In this study	80
pSNA(-E)free	pSTV28 벡터; lacZ; <i>E. faecalis</i> 유래 <i>mvaS</i> ; <i>S.pneumonia</i> 유래 <i>mvaK1</i> , <i>mvaK2</i> 및 <i>mvaD</i> ; <i>E. coli</i> 유래 <i>idi</i> 를 포함하는 항생제 마커가 없는 벡터	-	In this study	81
pTEFAmvaE	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. faecalis</i> 유래 <i>mvaE</i> 를 포함하는 Ptrc 발현벡터	-		82
pT-DHBSRYbb0	pTrc99A 벡터; lacIq; Amp ^r ; <i>P.agglomerans</i> 유래 <i>crtE</i> , <i>crtB</i> 및 <i>crtI</i> ; <i>P.ananatis</i> 유래의 <i>crtY</i> ; 코돈 최적화 된 <i>uncultured marine bacterium</i> 66A03 유래 SR; <i>E.coli</i> 의 <i>dxs</i> 및 <i>Ybb0</i>	-		83
pT-HBSRYbb0	pTrc99A 벡터; lacIq; Amp ^r ; <i>P.agglomerans</i> 유래 <i>crtE</i> , <i>crtB</i> 및 <i>crtI</i> ; <i>P.ananatis</i> 유래의 <i>crtY</i> ; 코돈 최적화 된 <i>uncultured marine bacterium</i> 66A03 유래 SR; <i>E.coli</i> 의 <i>Ybb0</i>	-	In this study	84

pT-HBSREYbbO	pTrc99A 벡터; lacIq; Amp ^r ; <i>P.agglomerans</i> 유래 crtE, crtB 및 crtI; <i>P.ananatis</i> 유래의 crtY; 코돈 최적화 된 <i>uncultured marine bacterium</i> 66A03 유래 SR; <i>E.coli</i> 의 YbbO; <i>E. faecalis</i> 유래 mvaE	-	In this study	85
pT-HBSREYbbOfree	pTrc99A 벡터; lacIq; <i>P.agglomerans</i> 유래 crtE, crtB 및 crtI; <i>P.ananatis</i> 유래의 crtY; 코돈최적화 된 <i>uncultured marine bacterium</i> 66A03 유래 SR; <i>E.coli</i> 의 YbbO; <i>E. faecalis</i> 유래 mvaE를 포함하는 항생제 마커가 없는 벡터	-	In this study	86
pT-dxr	pTrc99A 벡터; lacIq; Amp ^r ; <i>E.coli</i> 유래의 dxr를 포함하는 Ptrc 발현벡터	-		87
pTAS-dxr	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 ispA 및 dxr; <i>Clausena lansium</i> 유래 santalene synthase 인 STS 포함하는 Ptrc 발현 벡터	-	In this study	88
pT-dxr/s	pTrc99A 벡터; lacIq; Amp ^r ; <i>E.coli</i> 유래의 dxr 및 dxs를 포함하는 Ptrc 발현벡터	-		89
pT-ispA-MrBBS	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 ispA; 코돈최적화 된 <i>Matricaria recutita</i> 유래의 α-bisabolol synthase 인 MrBBS를 포함하는 Ptrc 발현벡터	-	In this study	90
pTAS-dxs	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 ispA 및 dxs; <i>Clausena lansium</i> 유래 santalene synthase 인 STS 포함하는 Ptrc 발현 벡터	-	In this study	91
pTAB-idi-dxr	pTrc99A 벡터; lacIq; Amp ^r ; <i>E. coli</i> 유래 FPP Synthase인 ispA 및 dxr; 코돈최적화 된 <i>Matricaria recutita</i> 유래의 α-bisabolol synthase 인 MrBBS를 포함하는 Ptrc 발현벡터	-	In this study	92
pSNAK(-E)-dxs	pSTV28 벡터; lacZ; Km ^r ; <i>E. faecalis</i> 유래 mvaS; <i>S.pneumonia</i> 유래 mvaK1, mvaK2 및 mvaD; <i>E. coli</i> 유래 idi 및 dxs를 포함하는 Plac 발현 벡터	-	In this study	93
pSNA(-E)-dxsfree	pSTV28 벡터; lacZ; <i>E. faecalis</i> 유래 mvaS; <i>S.pneumonia</i> 유래 mvaK1, mvaK2 및 mvaD; <i>E. coli</i> 유래 idi 및 dxs를 포함하는 항생제 마커가 없는 벡터	-	In this study	94
pT-HBSREYbbOdxr free	pTrc99A 벡터; lacIq; <i>P.agglomerans</i> 유래 crtE, crtB 및 crtI; <i>P.ananatis</i> 유래의 crtY; 코돈최적화 된 <i>uncultured marine bacterium</i> 66A03 유래 SR; <i>E.coli</i> 의 YbbO 및 dxr; <i>E. faecalis</i> 유래 mvaE를 포함하는 항생제 마커가 없는 벡터	-	In this study	95
pEGFP	pUC origin, lac promoter; EGFP, and Amp ^R	-	Clontech (US)	96
pEGFP-dxr	pUC origin, lac promoter; EGFP, <i>E.coli</i> 의 dxr, and Amp ^R	-	In this study	97
pORI19	Em ^r Ori ⁺ RepA ⁻ lacZ' derivative of pORI28	A System To Generate Chromosomal Mutations in <i>Lactococcus lactis</i> Which Allows Fast Analysis of Targeted Genes	-	98

pORI19-mvaA	pORI19-mvaA	-	In this study	99
pCI372	E. coli/L. lactis shuttle vector, CamR	Identification of the minimal replicon of <i>lactococcus lactis</i> subsp. <i>lactis</i> UC317 plasmid pCI305	-	100
pCIN	pCI372-FP promoter, MCS, rrnp terminator	-	In this study	101
pCIN-mvaA	pCIN-mvaA	-	In this study	102
pCIN-mvaA-EGFP	pCIN-mvaA, EGFP	-	In this study	103

[0148] 구축된 벡터는 모두 *trc* promoter를 사용하거나, *trc* promoter를 항상 발현할 수 있도록 변형하여 사용하였다. 따라서 구축된 pBFKC의 경우 *trc* promoter를 가지며, pTFKC(DPB)와 pTFCC(DPB)의 경우 항상 발현할 수 있도록 변형된 *trc* promoter를 가진다. 구축된 벡터의 multi-cloning site를 이용하여 원하는 경로를 삽입하였으며, 각각의 구축된 plasmid를 주형으로 대장균 삽입할 부분과 50 bp의 상동성을 가지는 프라이머를 이용하여 PCR을 수행하였다. 각 프라이머 정보는 표 4에 서열번호 3부터 서열번호 10으로 나타내었다. 얻어진 PCR 산물은 정제하여 pKD46을 포함한 대장균 MG1655(DE3) 수용성 세포(competent cell)에 1mm 간격의 큐벳을 통해 1.8 kV로 electro-transformation하고, 곧바로 SOC medium (2% Bacto Tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) 1mL을 첨가하여, 37℃에서 1시간 동안 진탕 배양 한 후, 항생제를 첨가한 평판배지에 도말하여 37℃에서 배양 후 단일 콜로니를 얻었다. 여기서 얻은 단일 콜로니들을 소량의 증류수에 현탁하여 10분간 가열하고, 이를 원심 분리한 후 상층액을 주형으로 하여 서열번호 11부터 서열번호 18까지의 각 프라이머로 PCR을 수행하여 경로삽입 여부를 확인하였다. 항생제 마커 제거를 위해서는 외래경로 삽입이 확인된 균주에 pCP20 플라스미드를 형질전환하여 30℃에서 배양 후 단일 콜로니를 얻었으며, 여기서 얻은 단일 콜로니들을 주형으로 하여 상기 확인용 프라이머를 통해 PCR을 수행하고 항생제 마커가 제거된 것을 확인하였다. 확인된 콜로니는 43℃에 배양하여 온도민감성 플라스미드인 pCP20 플라스미드를 제거하여 완성된 외래경로 삽입균주를 얻을 수 있었다.

[0150] 2) MG1655(DE3) Δ*adhE*::MVAbottom 균주에서 *dxr*, *dxs* 유전자 결실

[0151] 유전자 결실 또한 Datsenko와 Wanner의 방법을 이용하여 대장균 염색체 내 *dxr* 유전자를 결손하였다.

[0152] *dxr* 유전자를 상동성 재조합에 의하여 선별 마커 유전자인 카나마이신 유전자로 대체하기 위하여, pTFKC(DPB) 플라스미드의 카나마이신 유전자에 결합하면서 *dxr* 유전자의 상류 말단과 하류 말단의 염기 서열을 갖는 PCR 프라이머를 제조하였다. 정방향 프라이머는 *dxr* 유전자의 상류 말단의 50bp 염기서열 다음에, 카나마이신 유전자 결합 염기 서열 15bp로 구성되어 있으며, 역방향 프라이머는 *dxr* 유전자의 하류 말단의 50bp 염기서열 다음에, 카나마이신 유전자 결합 염기 서열 20bp로 구성되어 있다. 사용한 프라이머는 하기 표 7에 표시하였다. 서열번호 19과 서열번호 20의 올리고뉴클레오티드를 프라이머로 하고, pTFKC(DPB)을 주형으로 PCR 반응을 수행하였다.

[0153] 상기의 정제된 PCR 반응산물은 pKD46 (Datsenko KA and Wanner BL, 2000 Proc Natl Acad Sci U.S.A., 97(12):6640-6645)을 포함한 MG1655(DE3) Δ*adhE*::MVAbottom 수용성 세포 (competent cell)로 형질전환하였다. 3.3mM mevalonate와 카나마이신을 포함한 평판 배지에서 콜로니를 얻고, 이 콜로니는 PCR을 통하여 카나마이신 유전자가 상동성 재조합에 의해서 *dxr* 유전자를 대체했는지를 확인할 수 있다. 또한 mevalonate가 없는 평판 배지에 도말하여 콜로니가 자라지 못하는 것으로 *dxr* 유전자의 결손에 따른 MEP 경로가 차단된 것을 확인할 수 있다. 콜로니 PCR에 사용된 프라이머는 대장균 MG1655(DE3) 염색체 상에서 *dxr* 유전자 바로 인접한 영역에 결합하도록 제조하였다. 사용한 확인용 프라이머는 서열번호 21, 22, 23의 올리고뉴클레오티드의 프라이머이다.

[0154] 염색체 상의 항생제 마커 제거를 위해서는 외래경로 삽입이 확인된 균주에 pCP20 플라스미드를 형질전환하여 30℃에서 배양 후 단일 콜로니를 얻었으며, 여기서 얻은 단일 콜로니들을 주형으로 하여 확인용 프라이머를 통해 PCR을 수행하고 항생제 마커가 제거된 것을 확인하였다. 확인된 콜로니는 43℃에 배양하여 온도민감성 플라스미드인 pCP20 플라스미드를 제거하여 완성된 MG1655(DE3) Δ*dxr* Δ*adhE*::MVAbottom 균주를 얻었다.

[0155] 상기 방법과 동일한 방법으로 MG1655(DE3) Δ*dxr* Δ*adhE*::MVAbottom 균주에서 *dxs* 결손을 진행하여

MG1655(DE3) $\Delta dxr/s \Delta adhE::MVA_{bottom}$ 균주를 얻을 수 있다. 상동성 재조합을 위해 서열번호 24과 서열번호 25의 프라이머를 이용하여, pTFKC(DPB)을 주형으로 PCR 반응을 수행하였다. 이후 확인용 프라이머는 서열번호 26과 서열번호 27, 그리고 서열번호 28의 올리고뉴클레오타이드를 이용하였다.

[0156]

[0157] 3. 플라스미드 구축 및 배양방법(MEP 경로 결손주)

[0158] - 염색체 내 외래 MVA 하부경로 삽입하고 MEP 상부경로 유전자를 결손시킨 균주 이용

[0159] - 상기 MEP 경로 결손주에 사용되는 플라스미드의 선별마커는 결손된 MEP 경로를 보완할 수 있는 활성화된 MVA 경로 또는 염색체의 결손된 MEP 경로 유전자가 될 수 있다. 상기 결손주에 도입할 수 있는 플라스미드의 수는 단일 또는 복수가 될 수 있으며, 단일 플라스미드 방식에서는 도입하는 단일 플라스미드에 의해 숙주의 MEP 경로 결손이 보완될 수 있어야 하고, 복수 플라스미드 방식에서는 도입하는 복수의 플라스미드가 모두 존재할 때에만 숙주의 MEP 경로 결손이 보완될 수 있도록 하는 것이다

[0161] 1) 외래 MVA 경로 기반의 이소프레노이드 생합성 플라스미드

[0162] A. 단일 플라스미드 방식 (산탈렌 생산 예시)

[0163] - 숙주의 MEP 경로 결손을 보완하는 활성화된 MVA 경로가 단일 플라스미드에 모두 존재하는 방식으로 이 플라스미드에는 MVA 경로와 목적산물의 생합성 경로가 함께 존재하도록 구축한다.

[0164] i. 플라스미드 구축

[0165] 상기 MEP 경로 결손주에서 외래 MVA 경로를 항생제 마커를 대체하는 선별마커로 사용하기 위하여, 산탈렌을 생산하는 플라스미드에 MVA 경로를 도입하여 재조합 플라스미드를 구축한다. 상기 결손주는 재조합 플라스미드가 도입될 때 생육이 가능하고 산탈렌이 생산된다.

[0166] 산탈렌 생산 플라스미드인 pT-ispA-STs는 pTrc99A 벡터에 대장균(*Escherichia coli*)의 FPP synthase인 *ispA* 유전자와 *Clausena lansium*의 santalene synthase 유전자인 *STs*를 도입한 것이다. 상기 플라스미드에서 *STs* 유전자 뒤에 존재하는 제한효소부위 BglII와 SbfI을 이용해서 pSNAK 플라스미드에 있는 외래 MVA 경로 오페론을 클로닝하였다. pSNAK 플라스미드는 스트렙토코커스 뉴모니아(*Streptococcus pneumoniae*)의 *mvaK1*, *mvaD* 그리고 *mvaK2*와 엔테로코커스 패칼리스(*Enterococcus faecalis*)의 *mvaE*와 *mvaS*, 그리고 대장균(*Escherichia coli*)의 *idi* 유전자를 도입한 것이다. MVA 오페론은 양말단의 제한효소 BamHI과 SbfI으로 절단하여 pT-ispA-STs 벡터에 도입하였다. 제한효소 BamHI과 BglII는 동일한 외가닥 말단(cohesive end)을 지닌다. 최종적으로 산탈렌 생산 오페론과 MVA 경로 오페론이 함께 도입된 pTAS-NA를 구축하였다.

[0167] 상기 플라스미드의 클로닝 과정에서 형질전환체의 선별은 대장균 DH5 α 또는 대장균 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 를 이용할 수 있다. 대장균 DH5 α 를 이용하는 경우에는 상기 구축하는 플라스미드에 존재하는 항생제 마커를 이용해서 앰피실린 플레이트에서 선별할 수 있다. 대장균 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 를 이용하는 경우에는 숙주 대장균의 MEP 경로가 불활성화 되어서 상기에 구축하는 MVA 경로가 활성화된 플라스미드가 도입되면 생육이 가능하기 때문에 항생제 없이도 형질전환체의 선별이 가능하다.

[0169] ii. 플라스미드 도입

[0170] 상기 결손주는 재조합 플라스미드가 도입될 때 생육이 가능하고 산탈렌이 생산된다. 상기 구축된 플라스미드 pTAS-NA를 MEP 경로가 결손된 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주에 도입하여 mevalonate가 없는 플레이트에서 형질전환체를 얻음으로써, 플라스미드의 외래 MVA경로를 이용하는 재조합 균주를 선별할 수 있다. 이후 무항생제의 배지에서 배양하여 산탈렌의 생산성을 확인함으로써, MVA 경로 기반의 선별마커를 지니는 플라스미드의 유지 및 활성 능력을 확인 할 수 있다.

[0171] 상기 항생제 프리 산탈렌 생산 관련 플라스미드를 갖는 재조합 균주를 무항생제 조건에서 종균배양하고, 생산배지인 2% (v/v) 글리세롤 및 0.2mM IPTG를 함유한 2YT배지(리터 당 16g 트립톤, 10g 이스트 추출물, 및 5g NaCl) 4mL와 데칸 1mL의 혼합 배지 중에서 배양한다. 배양은 길이 15 cm, 지름 25 mm의 홈이 있는 시험관에 상기 혼합 배지를 넣고 각 균주를 접종한 후, 진탕 배양기(shaking incubator)에 약 250 rpm으로 교반하면서 30

℃에서 배양한다.

iii. 배양 결과

야생형 대장균 MG155(DE3) 균주를 대조군으로 하여 새롭게 구축한 재조합 균주인 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 에서의 산탈렌 생산량을 비교하였다. 배양 결과는 도 8과 표 3에 나타내었다.

상세히 설명하자면, MG1655(DE3)와 MG1655(DE3) $\Delta adhE::MVA_{bottom}$ 균주에서는 항생제를 첨가하여 배양하였으며, 항생제 프리 시스템을 적용할 수 있는 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주는 항생제 첨가 유무에 변화를 주어 배양을 진행하였다. 48시간의 배양 후 데칸층을 회수하여 GC를 통하여 산탈렌 생산량을 분석하였다. 그 결과, 79.8mg/L의 산탈렌을 생산한 MG1655(DE3)균주에 비하여 신규 재조합 균주인 MG1655(DE3) $\Delta adhE::MVA_{bottom}$ 균주에서 5배 이상 높은 산탈렌 생산성(420.2mg/L)을 나타내었다. 또한, 무항생제 배양을 한 경우는 항생제를 첨가하였을 때 보다 더 높은 산탈렌 생산성(646.7mg/L)을 보였다. 이러한 산탈렌 생산량의 증가가 염색체에 추가로 도입된 MVA 하부경로에서 기인하였을 가능성을 확인하기 위하여 MG1655(DE3) $\Delta adhE::MVA_{bottom}$ 균주에서 같은 실험을 진행하였지만, 이 경우 오히려 산탈렌을 생산하지 못하는 양상을 나타내었다.

표 3

균주 (48h)	MG1655(DE3)	MG1655(DE3) $\Delta adhE::MVA_{bottom}$	MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$	
	pTAS-NA	pTAS-NA	pTAS-NA	
항생제 첨가	+	+	+	-
균체 성장량 (OD _{600nm})	10.9 ± 0.5	9.1 ± 1.0	10.7 ± 0.3	12.7 ± 0.3
산탈렌 (mg/L)	79.8 ± 0.9	2.9 ± 1.3	420.2 ± 69.3	646.7 ± 6.6

B. 복수 플라스미드 방식 (레티노이드 생산 예시)

- 숙주의 MEP 경로 결손을 보완하는 MVA 경로가 복수의 플라스미드들에 분산되어 존재하는 방식으로 이들 복수의 플라스미드가 모두 존재하는 경우에만 MVA 경로가 활성화 될 수 있도록 하는 것이다. 즉 이들 플라스미드들에는 MVA 경로와 목적산물의 생합성 경로가 각각의 플라스미드의 크기와 구성 유전자들의 필요한 발현량을 고려해서 효율적으로 분산 배치되도록 구축한다.

i. 플라스미드 구축

MVA 하부경로가 염색체에 도입된 결손주에서 사용할 수 있는 외래 MVA경로 기반의 플라스미드 선별마커는 MVA 상부경로 유전자인 *mvaE* 유전자와 *mvaS* 유전자가 있다. *mvaE* 유전자는 *atoB* 유전자와 *mvaA* 유전자가 융합된 유전자로 필요 시 상기 두 유전자로 나누어 선별마커로 사용할 수 있다. 이번 실험에서는 외래 MVA 경로를 발현하는 pSNAK 플라스미드와 레티노이드를 생산하는 pT-DHBSRYbb0를 이용하여 두 플라스미드를 무항생제 조건에서 유지하는 플라스미드 시스템을 구축한다. 선별마커인 *mvaE* 유전자와 *mvaS* 유전자를 두 플라스미드에 분산 배치하여 두 플라스미드가 함께 도입되어야 숙주의 MEP 경로 결손이 보완되도록 한다. MVA 경로 선별마커 유전자 하나를 비교적 낮은 복제개수(copy number)와 lac 프로모터를 지니는 pSNAK 플라스미드에서 높은 복제개수와 trc 프로모터를 이용하는 pT-DHBSRYbb0 플라스미드로 옮겨야 하므로, 더 높은 발현량을 요구하는 *mvaE* 유전자를 옮겼다. 즉 *mvaS* 유전자가 *mvaE*를 제외한 MVA 경로를 발현하는 플라스미드의 선별마커로 사용되고, *mvaE* 유전자가 레티노이드 생산 플라스미드의 선별마커로 작용한다.

pSNAK 플라스미드의 제한효소부위 HpaI을 이용하여 *mvaE* 유전자를 절단 후 self-ligation으로 *mvaE* 유전자가 제거된 pSNAK(-E) 플라스미드를 구축했다. pSNAK(-E) 플라스미드에서 5' 말단에 인산기를 지니는 서열번호 29와 서열번호 30의 프라이머를 이용한 PCR을 통해 Kanamycin 항생제 마커 유전자를 제거한 후 self-ligation으로 pSNA(-E)free를 구축했다.

[0184] pT-DHBSRYbb0는 pTrc99A 벡터에 판토에아 아글루메란스(*Pantoea agglomerans*) 유래의 *crtE*, *crtB* 및 *crtI*, 판토에아 아나나티스(*Pantoea ananatis*) 유래의 *crtY*, 대장균의 *dxs* 및 *Ybb0*, 그리고 코돈최적화 된 *uncultured marine bacterium* 66A03 유래의 *SR* 유전자가 도입된 플라스미드이다. 상기 플라스미드에서 5' 말단에 인산기를 지니는 서열번호 31과 32의 프라이머를 이용한 PCR을 통해 *dxs* 유전자를 제거하여 pT-HBSRYbb0를 구축했다.

[0185] pTEFAMvaE 플라스미드는 pTrc99A 벡터에 엔테로코커스 패칼리스(*Enterococcus faecalis*)의 *mvaE* 유전자를 도입하여 제작된 플라스미드로 이를 주형으로 하여, 서열번호 33과 34의 프라이머를 이용한 PCR을 통해 *trc* 프로모터와 *mvaE* 유전자를 함께 증폭한다. 이를 제한효소 NotI과 SalI으로 절단하여 pT-HBSRYbb0 벡터의 동일 제한효소 부위로 삽입해서, *idi* 유전자(*ipiHP1*)와 *crtY* 유전자 사이에 위치시킨 플라스미드 pT-HBSREYbb0를 제작했다. pT-HBSREYbb0에서 서열번호 35와 36, 그리고 서열번호 37과 38의 프라이머를 이용한 두 번의 PCR을 통해 암피실린 항생제 마커를 제거하면서 HindIII 제한효소 사이트를 도입한 pT-HBSREYbb0free를 구축했다.

[0187] ii. 플라스미드 도입 및 배양 결과

[0188] ① 항생제 마커를 제거하지 않은 두개의 신규 플라스미드 pSNAK(-E)와 pT-HBSREYbb0를 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주에 함께 도입하여, 무항생제 배지에서 플라스미드를 유지할 수 있는 신규 재조합 균주를 구축하였다. 기존의 pSNAK와 pT-DHBSRYbb0 플라스미드를 지니는 MG1655(DE3) 균주를 대조군으로 하여 신규 재조합 균주를 항생제 첨가 유무에 따른 레티놀 생산성을 확인하였다.

[0189] 배양 결과는 표 4와 도 9에 나타내었다. 상세히 설명하면, 기존의 MG1655(DE3) 균주보다 신규 재조합 균주인 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 에서 레티노이드의 생산성이 증가하였음을 알 수 있다. 신규 균주의 항생제 유무에 따른 배양 결과를 비교해보면, 48시간의 배양시간 동안 무항생제 조건에서 균체 성장량이 항생제 조건에 비해서 약간 낮았지만 레티노이드의 생산성은 더 높았다. 이것으로 항생제의 선택압력 없이 플라스미드가 소실되지 않고 잘 유지 및 작동을 하고 있다는 것을 알 수 있으며, 대사흐름도 균체 성장에서 레티노이드 생산 쪽으로 더 많이 유도되고 있음을 알 수 있다. 또한 항생제 유무에 따른 레티노이드의 생산량 차이는 플라스미드에서 발현되는 항생제 저항성 유전자가 항생제의 독성을 완전히 보완하지 못한다는 것을 알 수 있다. 이번 배양에서 사용한 항생제는 암피실린과 카나마이신으로 암피실린은 세포벽의 합성을 저해하는 작용을 하고, 카나마이신은 리보솜의 30s 소단위체를 저해하여 전반적인 단백질 생산을 감소시키는 역할을 한다. 따라서 무항생제 조건의 배양에서는 상기 세포에 악영향을 미치는 인자의 부재로 단백질 합성이 보다 잘 되어 생산량이 증가 하였다고 판단된다.

표 4

균주 (48h)	MG1655(DE3)	MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$	
	pSNAK / pT-DHBSRYbb0	pSNAK(-E) / pT-HBSREYbb0	
항생제 첨가	+	+	-
균체 성장량 (OD _{600nm})	18.3 ± 0.5	11.4 ± 0.1	11.5 ± 0.2
총 레티노이드 (mg/L)	75.4 ± 0.1	83.7 ± 2	108 ± 1

[0192] ② 또한 항생제 저항성 유전자들이 제거된 플라스미드들인 pSNA(-E)free와 pT-HBSREYbb0free를 함께 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주에 형질 전환하여 무항생제 조건에서 배양을 진행하였다. 배양은 상기 레티노이드 배양조건으로 진행하였으며, 대장균의 테스트 튜브 배양 최대 시간인 72시간까지 진행하였다.

[0193] 배양 결과는 표 5와 도 10에 나타내었다. 상세히 설명하면, 항생제 마커를 완전히 제거한 플라스미드가 도입된 재조합 균주에서의 레티노이드 생산속도가 가장 빠르고, 48시간에 최대 생산성을 보였으며 72시간에는 탄소원 고갈의 영향으로 약간 감소하는 것을 볼 수 있다. 따라서 플라스미드에서 항생제 마커를 제거함으로써 숙주세포의 대사적인 부담이 감소하는 것으로 추정된다.

표 5

균주 (72h)	MG1655(DE3)	MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$	
	pSANK / pT-DHBSRYbbO	pSNAK(-E) / pT-HBSREYbbO	pSNA(-E)free / pT-HBSREYbbOfree
항생제 첨가	+	-	-
균체 성장량 (OD _{600nm})	22.1 $\pm$ 0.03	12.9 $\pm$ 0.08	13.0 $\pm$ 0.4
총 레티노이드 (mg/L)	173.3 $\pm$ 6	218.1 $\pm$ 1	204.2 $\pm$ 3

2) 염색체의 결손된 MEP 경로 유전자를 선별 마커로 한 MEP 경로 기반의 이소프레노이드 생합성 플라스미드

A. 단일 플라스미드 방식 (산탈렌 생산 예시)

- 염색체의 결손된 MEP 경로 유전자를 선별 마커로 갖는 단일 플라스미드를 구축하고 숙주에 도입하는 방식으로, 이 경우에 숙주의 MEP 경로 결손은 도입된 플라스미드에 의해 보완되게 된다.

i. 플라스미드 구축

산탈렌 생산 플라스미드인 pT-ispA-STs의 산탈렌 생합성 오페론 뒤에 상기 결손주에서 결손된 MEP 경로 유전자인 *dxr*를 도입하여 pTAS-dxr를 구축하였다. 상세히 설명하자면, pTrc99A 플라스미드에 대장균의 *dxr* 유전자가 도입되어 있는 pT-dxr 플라스미드를 주형으로 하여 서열번호 39과 서열번호 40의 프라이머를 이용한 PCR을 통해 *dxr* 유전자의 양말단에 제한효소부위 BglII과 XhoI를 도입하면서 증폭하였다. 이를 pT-ispA-STs벡터의 STS 유전자 뒤에 존재하는 동일 제한효소부위 BglII와 SalI을 이용한 클로닝을 통해 결손된 *dxr* 유전자를 지니는 산탈렌 생산 플라스미드 pTAS-dxr를 구축하였다. 제한효소 SalI과 XhoI은 동일한 외가닥 말단(cohesive end)을 지닌다.

상기 플라스미드의 클로닝 과정에서 형질전환체의 선별은 대장균 DH5 α 또는 대장균 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 를 이용할 수 있다. 대장균 DH5 α 를 이용하는 경우에는 상기 구축하는 플라스미드에 존재하는 항생제 마커를 이용해서 앰피실린 플레이트에서 선별할 수 있다. 대장균 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 를 이용하는 경우에는 숙주 대장균의 MEP 경로가 불활성화 되어서 상기에 구축하는 *dxr* 유전자가 도입된 플라스미드가 도입되면 생육이 가능하기 때문에 항생제 없이도 형질전환체의 선별이 가능하다.

ii. 플라스미드 도입

상기 구축된 플라스미드 pTAS-dxr을 MEP 경로가 차단되어 있는 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주에 도입하여 Mevalonate가 없는 플레이트에서 균주를 얻음으로써, 플라스미드의 *dxr* 유전자를 이용하여 내재 MEP 경로를 이용하는 균주를 선별할 수 있다. 이후 무항생제의 배지에서 배양을 진행하여 산탈렌을 생산성을 확인함으로써, 결손된 MEP 경로 유전자를 이용한 플라스미드의 유지 및 활성을 확인 할 수 있다.

상기 항생제 불필요 산탈렌 생산 플라스미드를 갖는 재조합 균주를 무항생제 조건에서 종균배양하고, 생산배지인 2% (v/v) 글리세롤 및 0.2mM IPTG를 함유한 2YT 배지(리터 당 16g 트립톤, 10g 이스트 추출물, 및 5g NaCl) 4mL와 데칸 1mL의 혼합 배지 중에서 배양한다. 배양은 길이 15 cm, 지름 25 mm의 홈이 있는 시험관에 상기 혼합 배지를 넣고 각 균주를 접종한 후, 진탕 배양기 (shaking incubator)에 약 250 rpm으로 교반하면서 30℃에서 배양한다.

iii. 배양 결과

야생형 대장균 MG155(DE3) 균주를 대조군으로 하여 신규 구축 재조합 균주인 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 에서의 산탈렌 생산량을 비교하였다. 배양 결과는 도 11과 표 6에 나타내었다. 상세히 설명하자면, MG1655(DE3)와 MG1655(DE3) $\Delta adhE::MVA_{bottom}$ 균주에서는 항생제를 첨가하여 배양하였으며, 항생제 프리 시스템을 적용할 수 있는 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주는 항생제 첨가 유무에 변화를 주어 배양을 진행하였다. 48시간의 배양 후 데칸층을 회수하여 GC를 통하여 산탈렌 생산량을 분석하였다. 그 결과 MG1655(DE3)의 경우 0.6mg/L의 산탈렌을 생산한 반면, 신규구축한 MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$ 균주는

4.5mg/L의 산탈렌을 생산하였다. MG1655(DE3) $\Delta adhE::MVAbottom$ 균주에서의 산탈렌 생산량 또한 3.4mg/L로 야생형 균주에 비하여 많은 양의 산탈렌을 생산하였다. 증가한 산탈렌의 양이 MVA 하부경로의 추가도입에서 기인하였을 가능성도 있지만, 메발로네이트를 추가 도입해주지 않았기 때문에 MVA 하부경로를 통한 IPP와 DMAPP의 증가로 보기 힘들다. *adhE*가 결손되면서 Acetyl-CoA가 부산물인 에탄올을 생산하지 않으면서 축적되게 되고, 이로 인해 Glucose의 해당과정의 흐름이 변하여 MEP경로의 전구물질인 G3P와 Pyruvate의 양이 증가하였을 수 있다. 따라서 산탈렌의 생산량이 증가하였을 가능성이 있다.

표 6

[0211]

균주 (48h)	MG1655(DE3)	MG1655(DE3) $\Delta adhE::MVAbottom$	MG1655(DE3) Δdxr $\Delta adhE::MVAbottom$	
	pTAS-dxr	pTAS-dxr	pTAS-dxr	
항생제 첨가	+	+	+	-
균체 성장량 (OD _{600nm})	4.9 ± 0.1	5.5 ± 0.2	5.0 ± 0.2	4.8 ± 0.2
산탈렌 (mg/L)	0.6 ± 0.0	3.4 ± 0.1	4.5 ± 0.2	3.9 ± 0.1

[0212]

B. 복수 플라스미드 방식 (산탈렌 + 비사볼롤 생산 예시)

[0213]

- 염색체의 결손된 MEP 경로 유전자들을 선별 마커들로 갖는 복수의 플라스미드를 구축하고 숙주에 도입하는 방식으로, 이 경우에 숙주의 MEP 경로 결손은 복수의 플라스미드가 모두 존재하는 경우에만 보완되도록 결손된 MEP 경로 유전자들이 각각의 플라스미드에 고르게 분산되도록 한다. 특히 숙주로 사용하는 결손주에서 염색체의 MEP 경로 유전자들은 도입하는 플라스미드의 숫자 이상으로 결손이 되어야 한다. 즉, 2개의 플라스미드를 사용하는 경우에는 최소한 2개 이상의 염색체의 MEP 경로 유전자들이 결손되어야 하고 이들 유전자들은 선별마커로 각각의 플라스미드에 분산배치 되게 된다.

[0215]

i. 플라스미드 구축

[0216]

산탈렌 생산 플라스미드인 pT-ispA-STS의 산탈렌 생합성 오페론 뒤에 *dxs* 유전자를 도입하여 pTAS-dxs를 구축하였다. 상세히 설명하자면, pTrc99A 벡터에 대장균의 *dxs* 유전자와 *dxr* 유전자가 도입된 pT-dxs/r 플라스미드를 주형으로 하여 서열번호 41과 서열번호 42의 프라이머를 이용한 PCR을 통해 *dxs* 유전자의 양말단에 제한효소부위 BglII와 XhoI을 도입하면서 증폭하였다. 상기 PCR 산물과 pT-ispA-STS벡터의 STS 유전자 뒤에 존재하는 동일 제한효소부위 BglII와 SalI을 이용한 클로닝을 통해 *dxs* 유전자를 지니는 산탈렌 생산 플라스미드 pTAS-dxs를 구축하였다. 제한효소 SalI과 XhoI은 동일한 외가닥 말단(cohesive end)을 지닌다.

[0217]

마찬가지로 pTrc99A 벡터에 대장균(*Escherichia coli*)의 FPP Synthase인 *ispA* 유전자와 *Matricaria recutita*의 α -bisabolol synthase 유전자인 *MrBBS*로 구성된 비사볼롤 생산 플라스미드인 pT-ispA-MrBBS의 비사볼롤 생합성 오페론 뒤에 *dxr* 유전자와 *idi* 유전자(b2889)를 도입하여 pTAB-idi-dxr를 구축하였다. *idi* 유전자는 IPP 이소머라제로 상기 구축한 플라스미드에 의하여 MEP경로가 강화되었을때, IPP와 DMAPP의 균형을 맞추어 주어 이소프레노이드의 생산성을 향상시킬 수 있다. 구축과정을 상세히 설명하자면 pT-ispA-MrBBS벡터의 *MrBBS* 유전자 뒤에 존재하는 제한효소부위 BglII와 SalI을 이용하여, 서열번호 43과 서열번호 44의 프라이머를 이용한 PCR을 통해 제한효소부위 BamHI과 SalI을 도입하면서 증폭한 *idi* 유전자와 서열번호 45과 서열번호 46의 프라이머를 이용한 PCR을 통해 제한효소부위 SalI과 HindIII를 도입하면서 증폭한 *dxr* 유전자를 비사볼롤 생산 플라스미드에 도입하여 pTAB-idi-dxr를 구축하였다.

[0218]

상기 플라스미드의 클로닝 과정에서 형질전환체의 선별은 대장균 MG1655(DE3) $\Delta dxr/s \Delta adhE::MVAbottom$ 를 이용할 수 있다. 대장균 MG1655(DE3) $\Delta dxr/s \Delta adhE::MVAbottom$ 를 이용하는 경우에는 숙주 대장균의 MEP 경로가 불활성화 되어서 상기에 구축하는 *dxr* 유전자와 *dxs* 유전자가 각각 도입된 두 플라스미드가 함께 도입될 때 생육이 가능하기 때문에 항생제 없이도 형질전환체의 선별이 가능하다.

[0220] ii. 플라스미드 도입

[0221] 상기 구축한 *dxs* 유전자를 선별마커로 지니는 플라스미드 pTAS-*dxs*와 *dxr* 유전자를 선별마커로 지니는 플라스미드 pTAB-*idi-dxr*를 *dxr*와 *dxs* 유전자가 모두 결손되어 있는 MG1655(DE3) $\Delta dxr/s$ $\Delta adhE::MVAbottom$ 균주에 함께 형질 전환하여 재조합 균주를 구축한다. 상기 재조합 균주를 무항생제 배지환경에서 배양하여 산탈렌과 비사볼롤의 생산을 확인함으로써 결손된 MEP 유전자들 각각이 복수의 플라스미드들의 선별 마커로 작동함을 확인 할 수 있다.

[0222] 상기 복수 플라스미드를 갖는 재조합 균주를 무항생제 조건에서 중균배양하고, 생산배지인 2% (v/v) 글리세롤 및 0.2mM IPTG를 함유한 2YT 배지(리터 당 16g 트립톤, 10g 이스트 추출물, 및 5g NaCl) 4mL와 데칸 1mL의 혼합 배지 중에서 배양한다. 배양은 길이 15 cm, 지름 25 mm의 홈이 있는 시험관에 상기 혼합 배지를 넣고 각 균주를 접종한 후, 진탕 배양기 (shaking incubator)에 약 250 rpm으로 교반하면서 30°C에서 배양한다.

[0224] iii. 배양 결과

[0225] 상기 구축한 재조합 균주를 항생제 첨가 유무에 따른 배지를 이용하여 배양을 진행하였다. 58시간의 배양 후 데칸층을 회수하여 GC를 통하여 산탈렌과 비사볼롤 생산량을 분석하였다. 배양 결과는 도 12와 표 7에 나타내었다. 상세히 설명하자면 항생제를 첨가하지 않은 배지에서도 산탈렌과 비사볼롤이 각 7.7mg/L, 40.7mg/L로 생산되었다. 이를 통해 산탈렌 생합성 유전자와 비사볼롤 생합성 유전자를 각각 지니고 있는 두 플라스미드가 모두 잘 유지되고 유전자를 발현하고 있다는 것을 알 수 있다.

표 7

균주 (58h)	MG1655(DE3) $\Delta dxs/r$ $\Delta adhE::MVAbottom$	
	pTAS- <i>dxs</i> / pTAB- <i>idi-dxr</i>	
항생제 첨가	+	-
균체 성장량 (OD _{600nm})	10.1 ± 0.8	10.7 ± 0.7
산탈렌 (mg/L)	14.3 ± 1.6	7.7 ± 2.5
비사볼롤 (mg/L)	49.7 ± 5.0	40.7 ± 7.1

[0228] C. 외래 MVA 경로 추가 방식 (레티노이드 생산 예시)

[0229] - 이소프레노이드 생산에 외래 MVA 경로를 도입하는 것이 유리하다는 선행결과를 통해서 외래 MVA 경로를 플라스미드에 도입하면서도 MEP 경로 결손 유전자를 선별마커로 사용하는 방법을 제공한다. 이 경우에 숙주 균주는 MEP 경로와 MVA 경로가 모두 활성화 되기 때문에 높은 이소프레노이드 생산성을 기대할 수 있다. 다만 복수의 플라스미드를 사용하는 경우에 추가로 도입되는 외래 MVA 경로가 어느 한쪽에 집중되지 않고 사용하는 플라스미드들에 고르게 분산되도록 해서 모든 플라스미드들이 존재할 때에만 숙주의 결손된 MEP 경로가 보완이 되도록 한다.

[0231] i. 플라스미드 구축

[0232] 상기 구축한 MVA 경로 유전자를 두 플라스미드에 나누어 발현하는 무항생제 레티노이드 생산 균주를 이용하여 MEP 경로의 결손 유전자를 선별마커로 사용하면서 외래 MVA경로도 함께 이용하는 재조합 플라스미드를 구축한다. 플라스미드의 사이즈가 큰 상기 *mvaE* 유전자가 도입된 레티노이드 생산 플라스미드 pT-HBSREYbb0free에는 비교적 사이즈가 작은 *dxr* 유전자를 도입하고, 사이즈에 여유가 있는 *mvaE* 유전자를 제외한 MVA 경로를 발현하는 플라스미드 pSNAK(-E)에는 *dxs* 유전자를 도입한다.

[0233] pT-*dxs/r* 플라스미드를 주형으로 하여 서열번호 47과 서열번호 48의 프라이머를 이용한 PCR을 통해 MEP 상부경로 유전자인 *dxs*를 양 말단에 BglIII와 XhoI을 도입하면서 증폭한다. pSNAK(-E)를 주형으로 하여 서열번호 49와 서열번호 50의 프라이머를 이용한 PCR로 양 말단에 BglIII와 XhoI 제한효소서열을 도입하면서 벡터를 증폭한다. 두 PCR 산물을 제한효소 BglIII와 XhoI으로 절단하여 벡터의 *idi* 유전자(b2889)와 *mvaS* 유전자 사이에 *dxs* 유전자를 삽입하여 pSNAK(-E)-*dxs* 구축한다. 이후 항생제 제거 프라이머를 이용한 PCR을 통해 카나마이신 항생제 저

항성 유전자를 제거하여 pSNA(-E)-dxs_{free}를 구축한다.

[0234] pT-dxr 플라스미드를 주형으로 하여 서열번호 51과 서열번호 52의 프라이머를 이용한 PCR을 통해 MEP 상부경로 유전자인 *dxr*의 양 말단에 제한효소 *NheI*과 *ScaI*을 도입하면서 증폭하고, pT-HBSREYbbO_{free} 플라스미드를 서열번호 36과 서열번호 53, 그리고 서열번호 37과 서열번호 54의 프라이머를 이용한 두 번의 PCR을 통해 제한효소 *XhoI* 부위를 기점으로 각 7.1kb, 5.6kb의 두 조각으로 증폭한다. 두 조각은 *xhoI*으로 다시 연결하고, *dxr* 유전자는 제한효소 *ScaI*과 *NheI*으로 절단하여 레티노이드 오페론의 말단에 삽입하여 pT-HBSREYbbOdxr_{free}를 구축한다.

[0236] ii. 플라스미드 도입

[0237] MEP 경로의 *dxr* 유전자와 MVA 경로의 *mvaE* 유전자를 지니는 pT-HBSREYbbOdxr_{free} 플라스미드와 MEP 경로의 *dxs* 유전자와 *mvaE* 유전자를 제외한 모든 MVA 경로 유전자를 지니는 pSNA(-E)-dxs_{free}를 대장균 MG1655(DE3) $\Delta dxr/s \Delta adhE::MVA_{bottom}$ 에 함께 도입하여 MVA경로와 MEP경로를 동시에 이용하는 무항생제 레티놀 생산 재조합 균주의 레티놀 생산성을 확인한다.

[0238]

[0239] iii. 배양 결과

[0240] MEP 경로 결손주에서 외래 MVA 경로만 이용하는 무항생제 레티놀 생산 재조합 균주와 함께 상기 구축된 MVA 경로와 MEP 경로를 동시에 이용하는 무항생제 레티놀 생산 재조합 균주를 무항생제 조건에서 배양하여 레티놀 생산성을 비교하였다. 배양 결과는 표 8과 도 13에 나타내었다. 상세히 설명하면, 외래 MVA 경로만으로 MEP 경로 결손을 보완하였던 레티노이드 생산 균주와 비교하여, 상기 구축한 MVA 경로와 MEP 경로를 동시에 이용하는 무항생제 레티놀 생산 재조합 균주가 항생제 저항성 유전자를 제거 하였을 때, 보다 높은 레티놀 생산성을 나타내었다. 이를 통해 외래 MVA 경로를 추가적으로 보완하면서, 결손된 MEP 경로 유전자를 선별마커로 이용할 수 있다는 것을 확인하였다.

표 8

[0242]

균주 (72h)	MG1655(DE3) $\Delta dxr \Delta adhE::MVA_{bottom}$	MG1655(DE3) $\Delta dxs/r \Delta adhE::MVA_{bottom}$	
	pSNA(-E) _{free} / pT-HBSREYbbO _{free}	pSNAdxs(-A) / pT-HBSRAYbbOdxr	pSNAdxs(-A) _{free} / pT-HBSRAYbbOdxr _{free}
항생제 첨가	-	-	-
균체 성장량 (OD _{600nm})	12.7 ± 0.24	12.6 ± 0.28	12.1 ± 0.12
총 레티노이드 (mg/L)	153.2 ± 2	124.0 ± 3	207.2 ± 1

[0243] 3) 염색체의 결손된 MEP 경로 유전자를 선별 마커로 한 단백질 발현용 플라스미드

[0244] - GFP단백질을 이용하여 단백질 발현의 안정성 확인

[0245] - 단일 단백질 발현에만 국한 되는 것이 아니라 다양한 대사산물 생합성 경로 발현에도 이용할 수 있다.

[0246] i. 플라스미드 구축

[0247] 녹색 형광을 발현하는 벡터 pEGFP의 EGFP 유전자 뒤에 결손주의 결손된 MEP 경로 유전자인 *dxr*을 도입하여 pEGFP-dxr을 구축하였다. 상세히 설명하자면, 상기 벡터의 EGFP 유전자 뒤의 제한효소부위 *StuI*과 *SpeI* 사이트를 이용하여, 서열번호 51과 서열번호 52의 프라이머를 이용한 PCR을 통해 증폭한 *dxr* 유전자를 도입해 주었다. 증폭된 PCR 산물은 양 말단에 제한효소부위 *ScaI*과 *NheI*이 도입되었다. 제한효소 *StuI*과 제한효소 *ScaI*은 평활 말단(blunt end)를 지니며, 제한효소 *SpeI*과 *NheI*은 동일한 외가닥 말단(cohesive end)를 지닌다. 최종적으로 *dxr* 유전자를 지니며 녹색형광을 발현하는 플라스미드인 pEGFP-dxr을 구축한다.

[0249] ii. 플라스미드 도입

[0250] 상기 구축된 pEGFP-dxr 플라스미드를 MG1655(DE3) $\Delta dxr \Delta adhE::MVA$ bottom 균주에 형질전환한다. 이를 항생제를 포함하지 않은 LB 평판배지에 도말하여 형광을 관찰한다.

[0251] 상기 구축된 재조합 균주의 목표 단백질의 발현을 확실히 하기 위하여, 유도물질인 IPTG를 1mM 넣어주어 배양을 진행한다. 상세히 설명하자면 균주를 무항생제 조건에서 종균배양하고, 생산배지인 LB 배지 5ml에 OD_{600nm} 0.1이 되도록 접종하여 배양한다. 배양 후 OD_{600nm} 0.6이 되었을 시점에 1mM IPTG를 첨가해준다. 배양은 길이 15 cm, 지름 25 mm의 홈이 있는 시험관에 상기 혼합 배지를 넣고 각 균주를 접종한 후, 진탕 배양기 (shaking incubator)에 약 250 rpm으로 교반하면서 30℃에서 배양한다. 이 배양액을 채취하여 무항생제 배지 조건에서 플라스미드에 함유된 유전자의 발현의 안정성을 SDS-PAGE와 형광측정을 통하여 확인한다.

[0253] iii. 배양 결과

[0254] SDS-PAGE와 형광발현 결과는 도 14에 나타내었다. 상세히 설명하자면, 형광현미경을 통하여 녹색 형광을 관찰한 결과 항생제를 선별마커로 지니는 대조군보다 결손된 MEP경로 유전자를 플라스미드의 선별마커로 사용한 경우, 보다 선명한 형광을 나타내는 것을 확인하였다. 또한 SDS-PAGE를 통한 단백질 발현량을 비교해 보아도 대조군에 비하여 새롭게 구축한 균주의 무항생제 배양에서 강한 단백질 발현양을 나타내었다. IPTG와 같은 유도제를 처리하지 않아도, 균주의 결손된 MEP경로 유전자의 선택압력에 의해서 플라스미드를 충분히 유지하고 유전자를 발현하고 있는 것을 확인하였다.

[0256] 4. MVA 경로 변이 균주 구축 방법

[0257] 1) 유산균 HMG-CoA reductase를 코딩하는 유전자(mvaA)의 불활성화

[0258] 유산균의 내재하는 MVA 경로에서 율속제한효소(rate-limiting enzyme)인 Hydroxymethylglutaryl-CoA reductase (mvaA) 유전자를 Homologous recombination system을 이용하여 불활성화 하였다. *Lactococcus lactis* MG1363의 mvaA 유전자 변이를 위해 우선 MG1363에서 mvaA 유전자 앞 1Kb 부분을 서열번호 55과 서열번호 56의 프라이머를 이용하여 증합효소연쇄반응(PCR)을 통해 증폭하고 mvaA 유전자 뒤 1Kb 부분을 서열번호 57과 서열번호 58의 프라이머를 이용하여 PCR을 통해 증폭한 후 두 PCR 산물을 서열번호 59과 서열번호 60의 프라이머를 이용하여 Splicing by overhang extension(SOE) PCR을 통해 증폭하였다. 또한 pORI19 플라스미드를 서열번호 61과 서열번호 62의 프라이머를 이용하여 PCR을 통해 증폭한 후 두 PCR 산물을 Ligation한 후 EC1000 수용성 세포 (competent cell) 100ul에 2mm 간격의 큐벳을 통해 25 μ F, 200 Ω , 2,500V 조건에서 electroporation을 수행하였다. Electroporation한 후에 LB 배지 1ml을 첨가하고 37℃에서 30분간 배양 후 300ug/ml 에리트로마이신 항생제가 첨가된 LB 고체 평판배지에 100 μ l 도말하고 37℃에서 12시간 배양하여 pORI19-mvaA 플라스미드를 얻었다 (표 2).

[0259] 유산균의 수용성 세포(competent cell)를 제작하기 위해 플라스미드 pVE6007를 함유한 *L. lactis* MG1363(pVE6007) 균주를 0.5% (v/v) glucose와 5ug/ml 클로람페니콜 항생제가 첨가된 5ml의 M17배지(1리터당 5.0g Pancreatic Digest of Casein, 5.0g Soy peptone, 5.0g Beef Extract, 2.5g Yeast Extract, 0.5g Ascorbic Acid, 0.25g Magnesium Sulfate, 10.0g Disodium- β -glycerophosphate)에 접종하고, 온도 30℃에서 16-24시간 종균배양한다. 0.5% (v/v) glucose, 0.5M sucrose, 1.5% (w/v) glycine, 5ug/ml 클로람페니콜이 첨가된 9ml의 M17 배지에 종균배양액 1ml을 접종 후 30℃에서 16-24시간 배양한다. 상기배양액 5ml을 동일한 새 배지 35ml에 접종하고 다시 30℃에서 OD_{600nm}, 0.25까지 배양한다. 수용성 세포를 만들기 위해 상기 배양액을 얼음에서 5분간 냉각한 후에 4℃, 5,000rpm에서 15분간 원심분리해서 상층액을 제거하고, 배양액과 동일한 양의 40ml 세척완충액(0.5M sucrose, 10% glycerol)으로 2회 세척한다. 세척한 균체를 0.4ml의 세척완충액에 현탁해서 수용성 세포를 얻었다.

[0260] 상기에서 얻은 수용성 세포 100ul에 pORI19-mvaA 플라스미드 3-5 μ g을 2mm간격의 큐벳을 통해 25 μ F, 200 Ω , 2,500V 조건에서 electroporation을 수행한다. Electroporation한 후에 5 μ g/ml 클로람페니콜이 첨가된 GM17배지 1ml을 첨가하고 30℃에서 2시간 배양 후 5 μ g/ml 클로람페니콜과 5 μ g/ml 에리트로마이신이 첨가된 GM17 고체

평판배지에 100 μ l 도말하여 MG1363(pORI19-mvaA, pVE6007)균주를 얻었다.

[0261] pVE6007 플라스미드 제거를 위해 상기에서 얻은 균주를 5 μ g/ml 클로람페니콜과 5 μ g/ml 에리트로마이신이 첨가된 5ml의 GM17 배지에 접종하여 30℃에서 16-24시간 배양 후 배양액 1ml을 cell down하여 GM17 배지 1ml로 세척 2번 진행한 후 5 μ g/ml 에리트로마이신이 첨가된 10ml의 GM17 배지에 0.1% (v/v)으로 접종하여 30℃에서 16-24시간 배양한다. 동일한 배지에 0.1% (v/v)으로 접종하여 37℃에서 12시간 간격으로 계대배양을 3회 실시한 후 5 μ g/ml 에리트로마이신이 첨가된 GM17 고체 평판배지에 10⁵⁻⁷ 희석해 도말하여 30℃에서 16-24시간 배양한다.

[0262] Single cross over(SCO)가 일어난 균주를 PCR을 통해서 선별하였다. 200개의 콜로니를 서열번호 63와 서열번호 64의 프라이머와 함께 upstream 확인을 위한 서열번호 65 프라이머와 downstream 확인을 위한 서열번호 66 프라이머를 추가로 각각 넣어주어 총 3개의 프라이머를 이용하여 PCR을 진행하였다. Upstream에서 SCO가 일어난 경우 서열번호 63과 서열번호 65의 프라이머에 의해 확인이 가능하며 downstream에서 SCO가 일어난 경우 서열번호 64와 서열번호 66의 프라이머에 의해 확인이 가능하다. 그리고 SCO가 일어나지 않은 wild type의 경우 서열번호 63와 서열번호 64의 프라이머에 의해 확인이 가능하다. SCO가 일어난 균주를 5 μ g/ml 에리트로마이신이 첨가된 5ml의 GM17 배지에 접종하여 30℃에서 16-24시간 배양 후 배양액 1ml을 cell down하여 GM17 배지 1ml로 세척 2번 진행한 후 3.3mM mevalonate가 첨가된 10ml의 GM17 배지에 0.1% (v/v)으로 접종하여 30℃에서 16-24시간 배양한다. 유전자 *mvaA*가 변이된 변이주의 생육을 위해서는 배지에 mevalonate를 첨가한다. 동일한 배지에 0.1% (v/v)으로 접종하여 30℃에서 12시간 간격으로 계대배양을 3회 이상 실시한 후 3.3mM mevalonate가 첨가된 GM17 고체 평판배지에 10⁵⁻⁷ 희석해 도말하여 30℃에서 16-24시간 배양한다.

[0263] Double cross over(DCO)가 일어난 균주를 PCR을 통해서 선별하였다. 400개의 콜로니를 서열번호 63와 서열번호 64의 프라이머를 이용하여 PCR을 진행하여 *Lactococcus lactis* MG1363 Δ *mvaA* 균주를 구축하였다.

[0264]

[0265] 5. 플라스미드 구축 및 배양방법 (MVA 경로 변이주)

[0266] - 숙주의 염색체 내 MVA 상부경로 유전자를 변이시킨 균주 이용

[0267] 1) 대장균-유산균 재조합 서틀 벡터 구축

[0268] 기존 대장균-유산균 서틀벡터 pCI372를 제한효소 AgeI과 NheI으로 절단하고, promoter, multi-cloning site, terminator를 도입한 pCIN벡터를 구축하였다(표 2).

[0270] 2) 염색체의 변이된 MVA 경로 유전자를 선별 마커로 한 단백질 발현용 플라스미드

[0271] i. 플라스미드 구축

[0272] 선별마커로 이용하기 위한 *mvaA* 유전자를 MG1363 균주로부터 서열번호 67와 서열번호 68의 프라이머를 이용하여 증합효소연쇄반응(PCR)을 통해 증폭하고 제한효소 BamHI과 XbaI를 절단한 후에 pCIN벡터의 동일한 제한효소부위에 도입해서 pCIN-mvaA벡터를 구축하였다. 녹색 형광 단백질 발현확인을 위한 EGFP 유전자를 pEGFP벡터로부터 서열번호 69와 서열번호 70의 프라이머를 이용하여 증합효소연쇄반응(PCR)을 통해 증폭하고 제한효소 SalI과 SphI으로 절단한 후에 pCIN-mvaA벡터의 동일한 제한효소부위에 도입해서 pCIN-mvaA-EGFP를 구축하였다(도 10). 상기 구축한 벡터들은 표 2에 나타내었다.

[0274] ii. 플라스미드 도입

[0275] 상기 pCIN-mvaA-EGFP 재조합 플라스미드를 *L. lactis* MG1363 Δ *mvaA* 균주에 형질전환하여 *mvaA*를 선별마커로 갖는 유산균 형질전환체를 제작한다.

[0276] pCIN-mvaA-EGFP 재조합 서틀벡터를 *L. lactis* MG1363 Δ *mvaA* 균주에 형질전환하기 위하여 수용성 세포(competent cell)를 제작한다. MG1363 Δ *mvaA* 균주를 0.5% (v/v) glucose와 3.3mM mevalonate가 첨가된 5ml의 M17 배지에 접종하고, 온도 30℃에서 16-24시간 종균배양한다. 0.5% (v/v) glucose, 0.5M sucrose, 1.5% (w/v) glycine, 3.3mM mevalonate가 첨가된 9ml M17 배지에 종균배양액 1ml을 접종 후 30℃에서 16-24시간 배양한다. 상기 배양액 5ml을 동일한 새 배지 35ml에 접종하고 다시 30℃에서 OD₆₀₀, 0.25까지 배양한다. 수용성 세포를 만

들기 위해 상기 배양액을 얼음에서 5분간 냉각한 후에 온도 4℃, 5000rpm에서 15분간 원심분리해서 상층액을 제거하고, 배양액과 동일한 양의 40ml의 세척완충액 (0.5M sucrose, 10% glycerol)으로 2회 세척한다. 세척한 균체를 0.4ml의 세척완충액에 현탁해서 수용성 세포를 얻는다.

[0277] 상기 얻어진 수용성 세포 100ul에 pCIN-mvaA-EGFP 플라스미드를 5ul 넣고 섞은 뒤 혼합액을 electroporation cuvette (2mm 간격)에 넣고, 25 μ F, 200 Ω , 2,500V 조건에서 electroporation을 수행한다. Electroporation 한 후에 0.5% (v/v) glucose가 첨가된 M17 배지를 1ml 첨가하고 30℃에서 1시간 배양한다. Electroporation으로 pCIN-mvaA-EGFP 플라스미드를 도입한 세포 시료를 0.5% (v/v) glucose를 첨가한 M17 고체 평판배지에 10^1 - 10^3 희석하여 도말하여 MG1363 Δ mvaA(pCIN-mvaA-EGFP) 형질전환체를 얻는다.

[0279] iii. GFP 단백질을 이용하여 단백질 발현의 안정성 확인

[0280] 상기 구축된 MG1363 Δ mvaA(pCIN-mvaA-EGFP) 균주를 항생제를 포함하지 않은 0.5% (v/v) glucose를 첨가한 M17 고체 평판배지에 도말하여 형광을 관찰한다.

[0281] 상기 구축된 재조합 균주의 목표 단백질의 발현을 확실히 하기 위하여 배양을 진행한다. 상세히 설명하자면 균주를 무항생제 조건에서 종균배양하고, 생산배지인 0.5% (v/v) glucose가 첨가된 M17 배지 5ml에 OD_{600nm} 0.1이 되도록 접종하여 30℃에서 배양한다. 이 배양액을 채취하여 무항생제 배지 조건에서 플라스미드에 함유된 유전자의 발현의 안정성을 형광측정을 통하여 확인한다.

표 9

[0283]

서열번호	프라이머 이름	서열번호	프라이머 이름
1	SN12Didi-F	36	RET-R
2	SN12Didi-R	37	RET-F
3	IS poxB-Ptrc-F	38	dAmp(RET)-R
4	KO poxB -R	39	dxr_1-F
5	IS ldhA-Ptrc-F	40	dxr_1-R
6	KO ldhA-R	41	dxs_1-F
7	IS adhE-Ptrc-F	42	dxs_1-R
8	KO adhE-R	43	idi-F
9	IS atoDA-Ptrc-F	44	idi-R
10	KO atoDA-R	45	dxr_3-F
11	KOpoxBCF-F	46	dxr_3-R
12	KOpoxBCF-R	47	dxs_2-F
13	KO1dhACF-F	48	dxs_2-R
14	KO1dhACF-R	49	dmvaE-F
15	KOadhECF-F	50	dmvaE-R
16	KOadhECF-R	51	dxr_2-F
17	KOatoDACF-F	52	dxr_2-R
18	KOatoDACF-R	53	RET_2-F
19	KO dxr-F	54	RET_2-R
20	KO dxr-R	55	mvaA u/s-Fwd
21	KOdxrCF-F	56	mvaA u/s-Rev
22	KOdxrCF-R	57	mvaA d/s-Fwd
23	dxrCF-R	58	mvaA d/s-Rev
24	KO dxs-F	59	mvaA ex-Fwd
25	KO dxs-R	60	mvaA ex-Rev
26	KOdxsCF-F	61	pORI19-Rev
27	KOdxsCF-R	62	pORI19-Fwd
28	KmCF-F	63	DCO-Fwd
29	dCmp(NA)-F	64	DCO-Rev
30	dCmp(NA)-R	65	SCO u/s-Rev
31	ddxs(RET)-F	66	SCO u/s-Fwd
32	ddxs(RET)-R	67	mvaA-Fwd

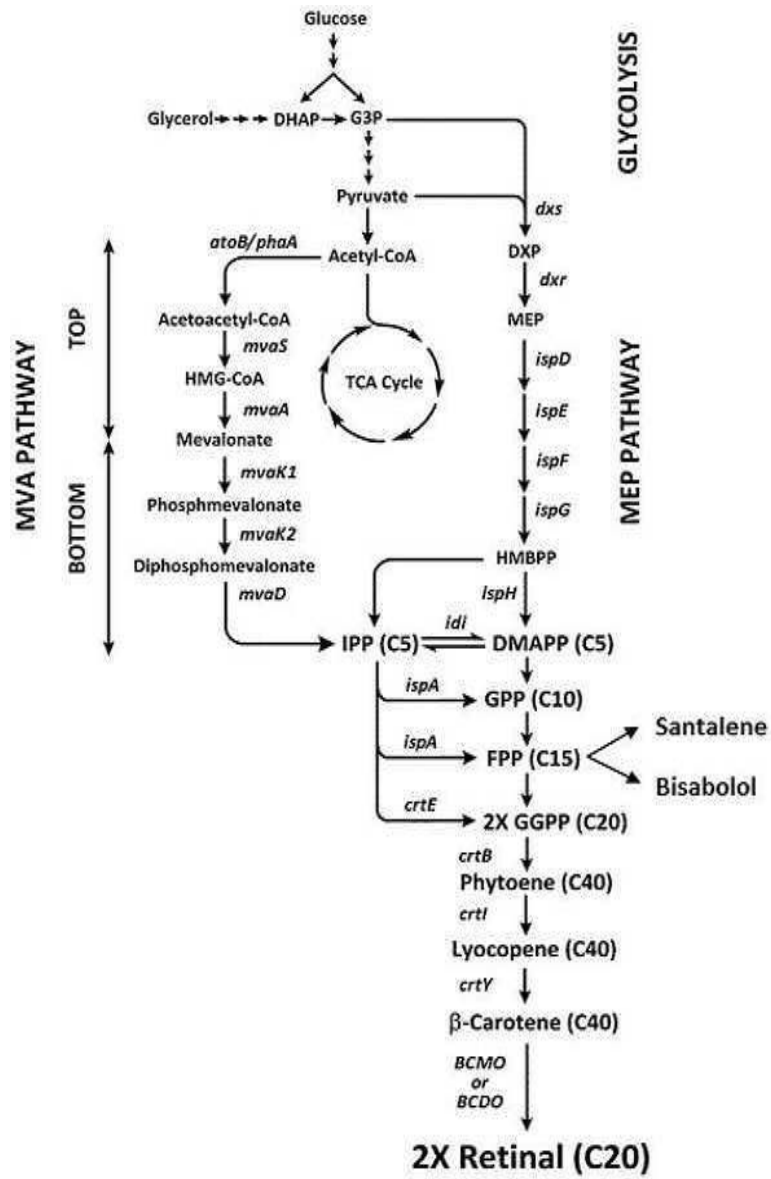
33	P1mvaE-F	68	mvaA-Rev
34	P1mvaE-R	69	EGFP-Fwd
35	dAmp(RET)-F	70	EGFP-Rev

표 10

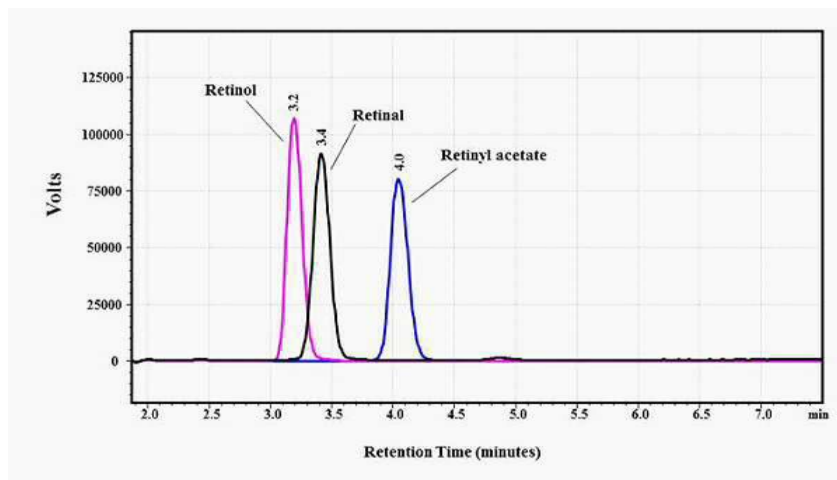
유전 자명	효소	유래	Genbank 허가번호	염기 서열	아미 노산 서열
				서열 번호	서열 번호
mvaE	아세틸-CoA 아세틸트랜스퍼라제 /하이드록시메틸글루타릴 (HMG)-CoA 리덕타제	엔테로코커스 패칼리스(<i>Enterococcus faecalis</i>)	AF290092	104	124
mvaS	HMG-CoA 신타제	엔테로코커스 패칼리스(<i>Enterococcus faecalis</i>)	AF290092	105	125
mvaK1	메발로네이트 키나제	스트렙토코커스 뉴모니아(<i>Streptococcus pneumoniae</i>)	AF290099	106	126
mvaK2	포스포메발로네이트 키나제	스트렙토코커스 뉴모니아 (<i>Streptococcus pneumoniae</i>)	AF290099	107	127
mvaD	메발로네이트 디포스페이트 데카 르복실라제	스트렙토코커스 뉴모니아 (<i>Streptococcus pneumoniae</i>)	AF290099	108	128
Idi	IPP 이소머라제	대장균(<i>Escherichia coli</i>)	U00096	109	129
ipiHpl	IPP 이소포머라제	헤마토코커스 플루비알리스 (<i>Haematococcuspluvialis</i>)	AF082325	110	130
crtE	제라닐제라닐 피로포스페이트 (GGPP) 신타제	판토에아 아글루메란스 (<i>pantoea agglomerans</i>)	M87280	111	131
crtB	피토엔 신타제	판토에아 아글루메란스 (<i>pantoea agglomerans</i>)	M87280	112	132
crtI	피토엔 데히드로게나 제	판토에아 아글루메란스 (<i>pantoea agglomerans</i>)	M87280	113	133
crtY	라이코펜-β-시클라제	판토에아 아나나티스 (<i>pantoea ananatis</i>)	D90087	114	134
SR	β-카로틴모노옥시게나제	배양되지 않은 해양 박테리 아 (unculturedmarine bacterium) 66A03	b1h의 대장균 코 돈최적화 서열	115	135
YbbO	산화환원효소	야생형 대장균(<i>Escherichia coli</i> MG1655; taxid 511145)	1786701	116	136
dxs	1-데옥시자일롤로즈-5-포스페이 트 (DXP) 신타제	대장균(<i>Escherichia coli</i>)	AF035440.1	117	137
dxr	1-데옥시-D-자일롤로즈-50포스페 이트 리덕토이소머라제(1-deoxy-D-xylo ulose 5-phosphate reductoisomerase)	대장균(<i>Escherichia coli</i>)	AB013300.1	118	138
ispA	파네실 파이로포스페이트 신타제	대장균(<i>Escherichia coli</i>)	-	119	139
STS	산탈렌 신타제	<i>Clausena lansium</i>	-	120	140
MrBBS	α-비사볼롤 신타제	<i>Matricaria recutita</i>	KM259907.1의 대 장균 코돈최적화 서열	121	141
EGFP	enhanced green fluorescent protein	Synthetic construct	KX130867.1	122	142
mvaA	Hydroxymethylglutaryl-CoA reductase	<i>Lactococcus lactis</i> subsp. <i>cremoris</i> MG1363	(WP_011834877.1)	123	143

도면

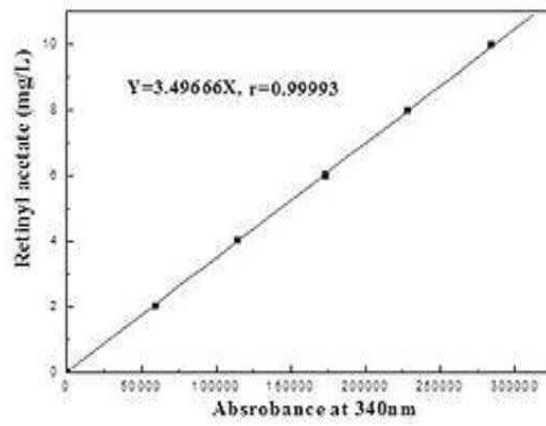
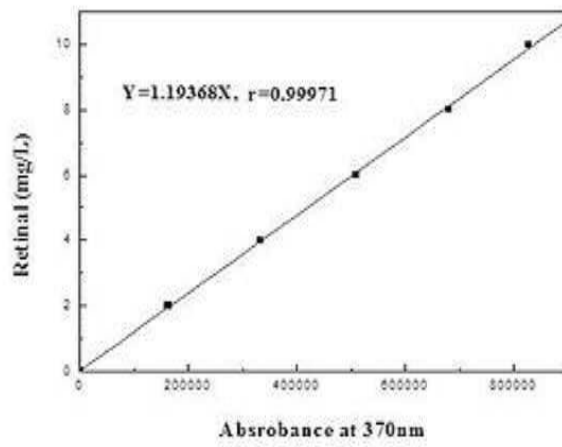
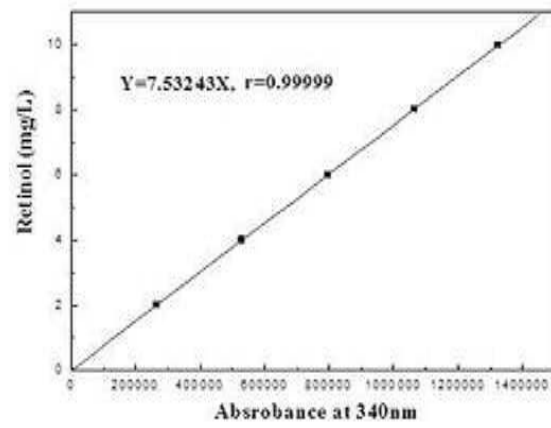
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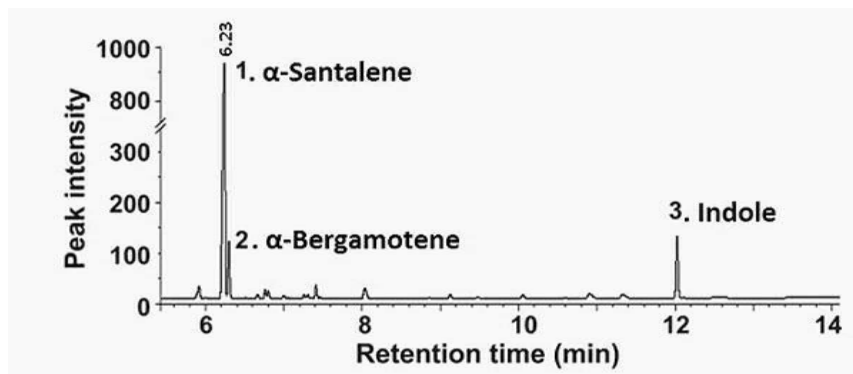
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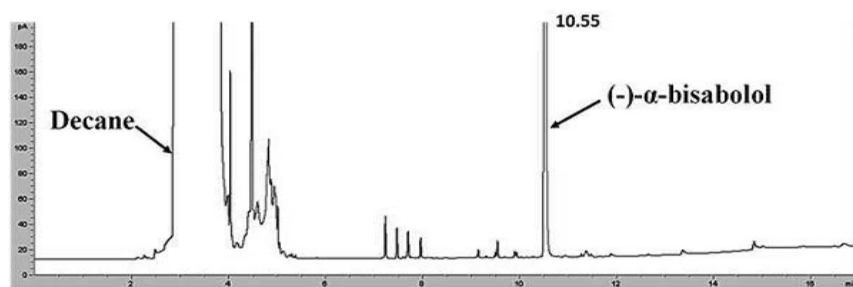
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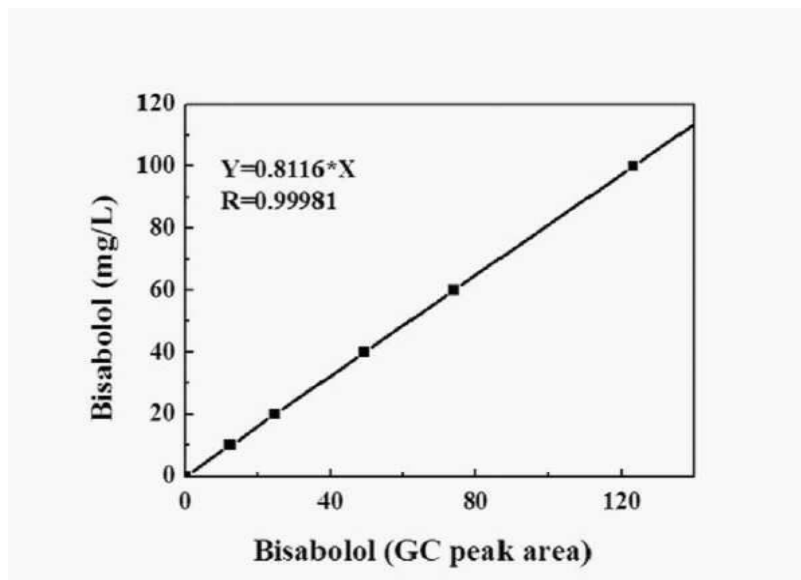
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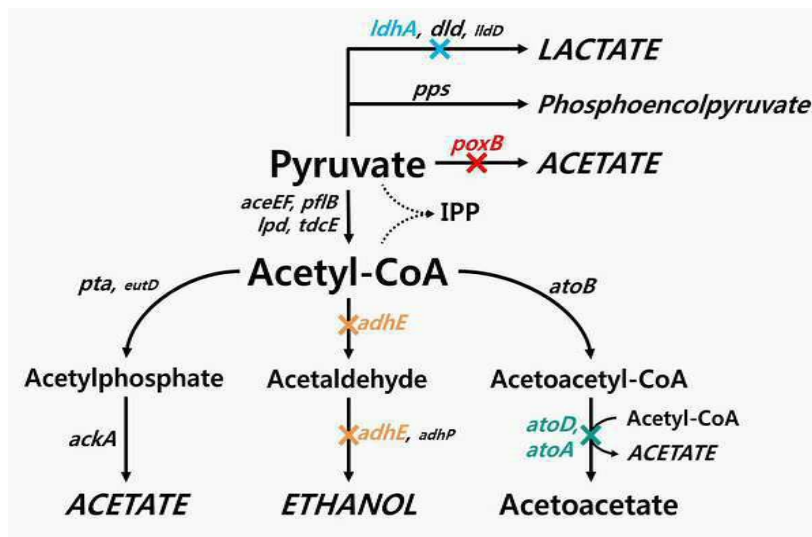
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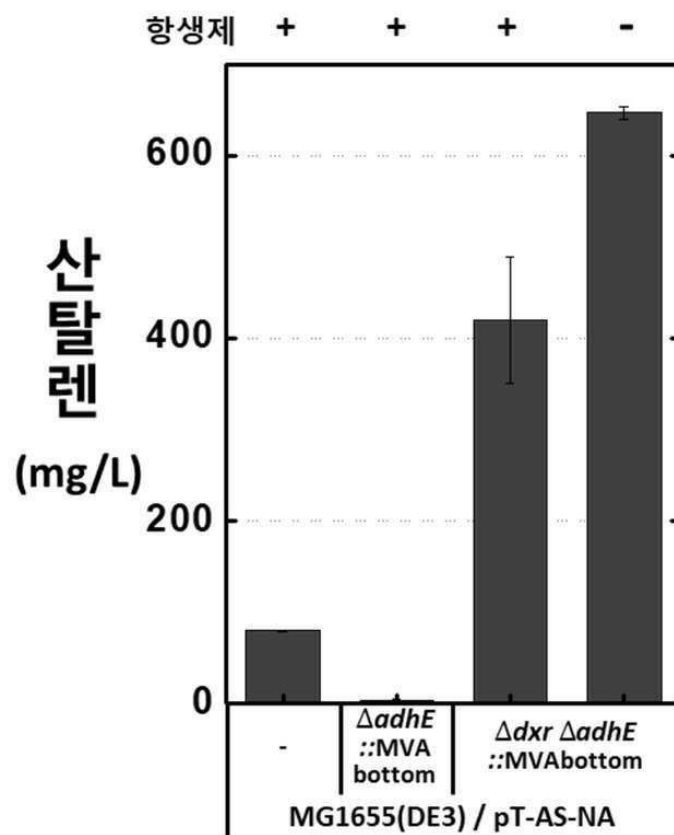
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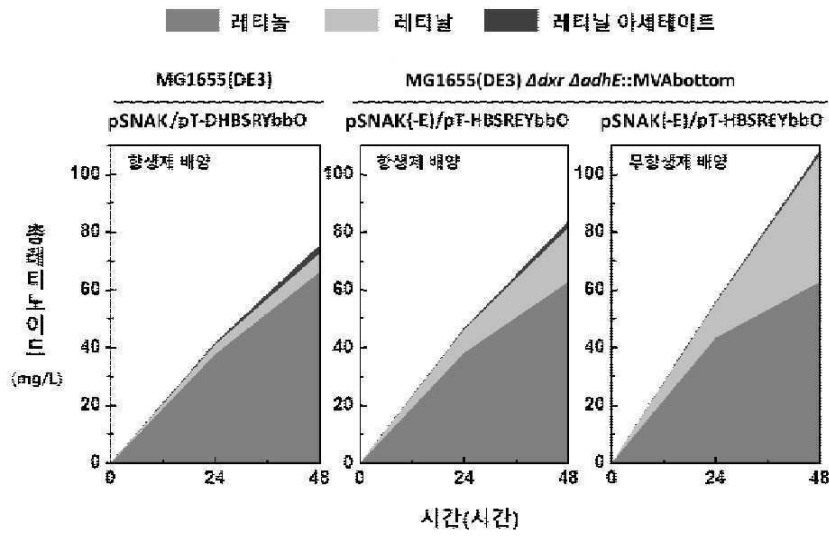
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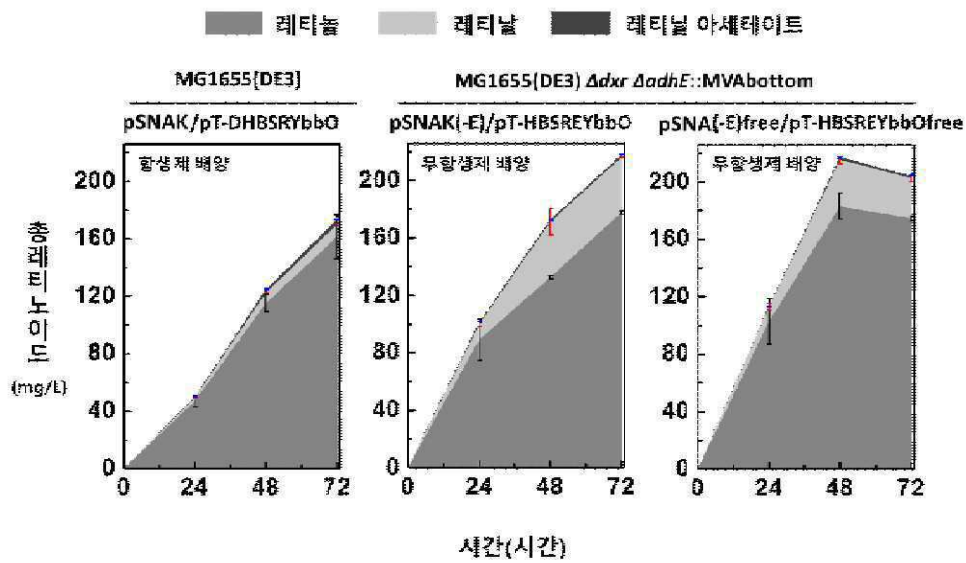
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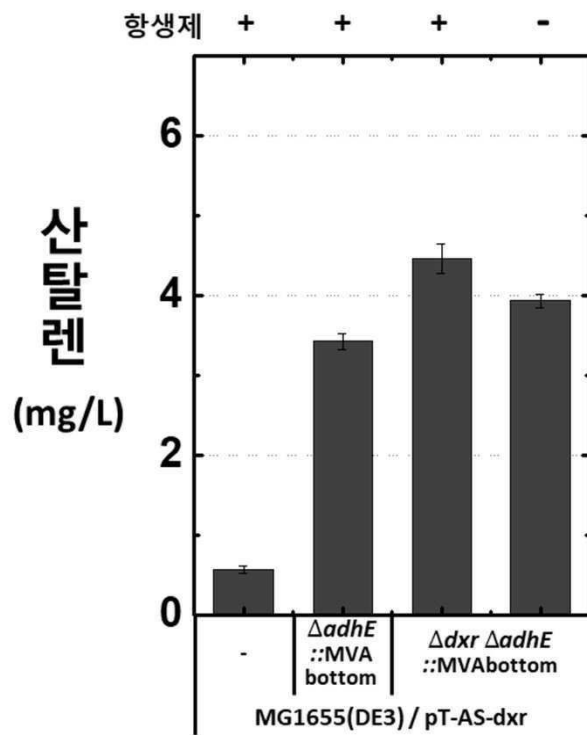
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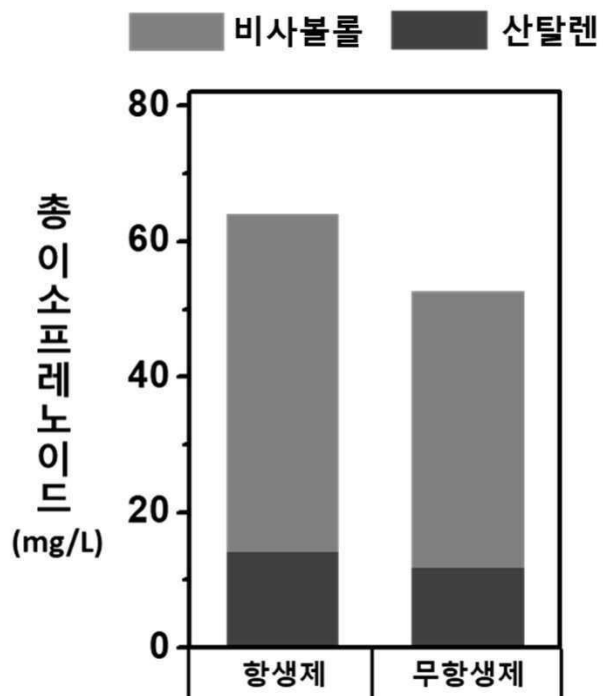
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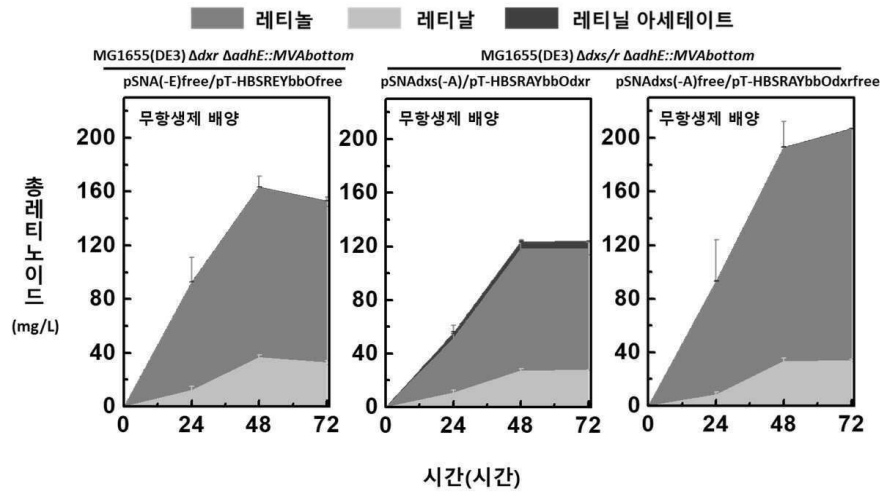
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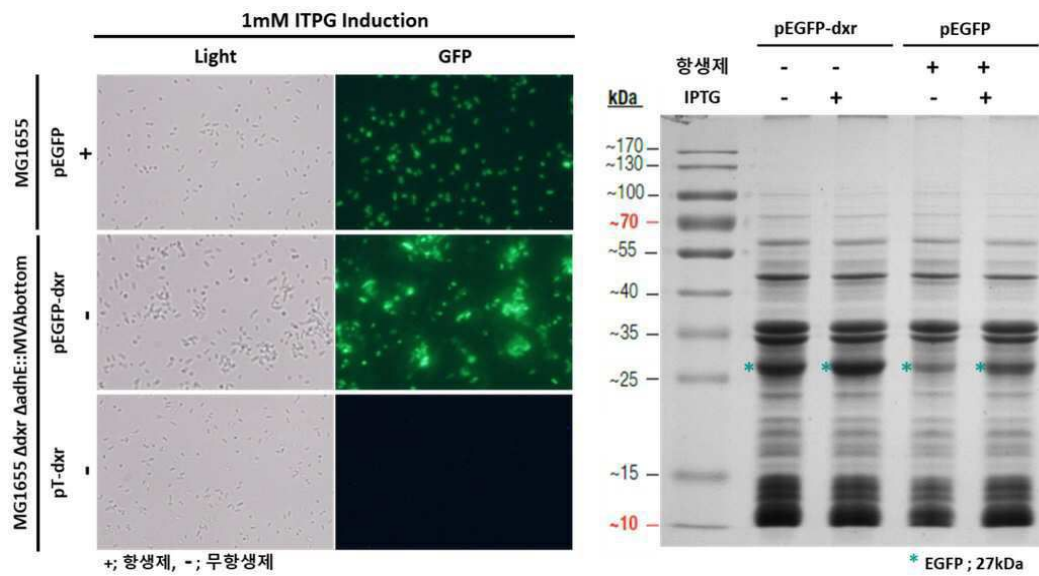
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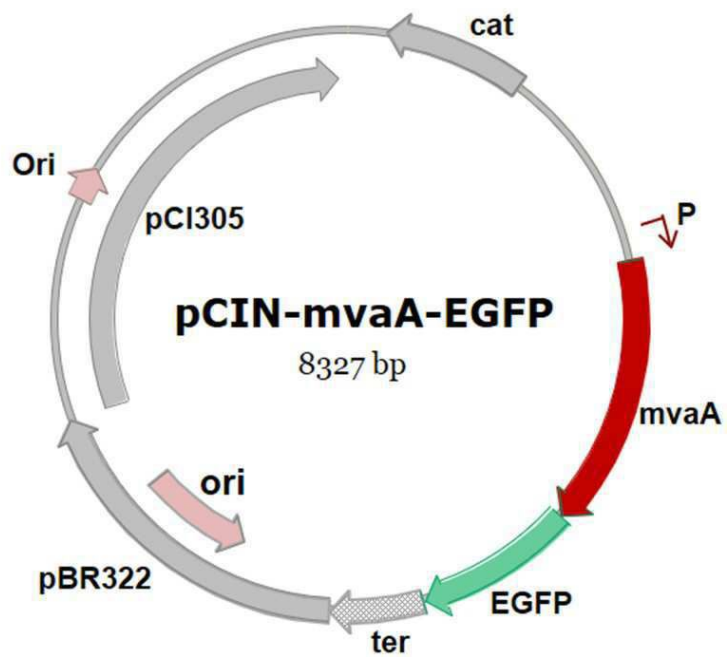
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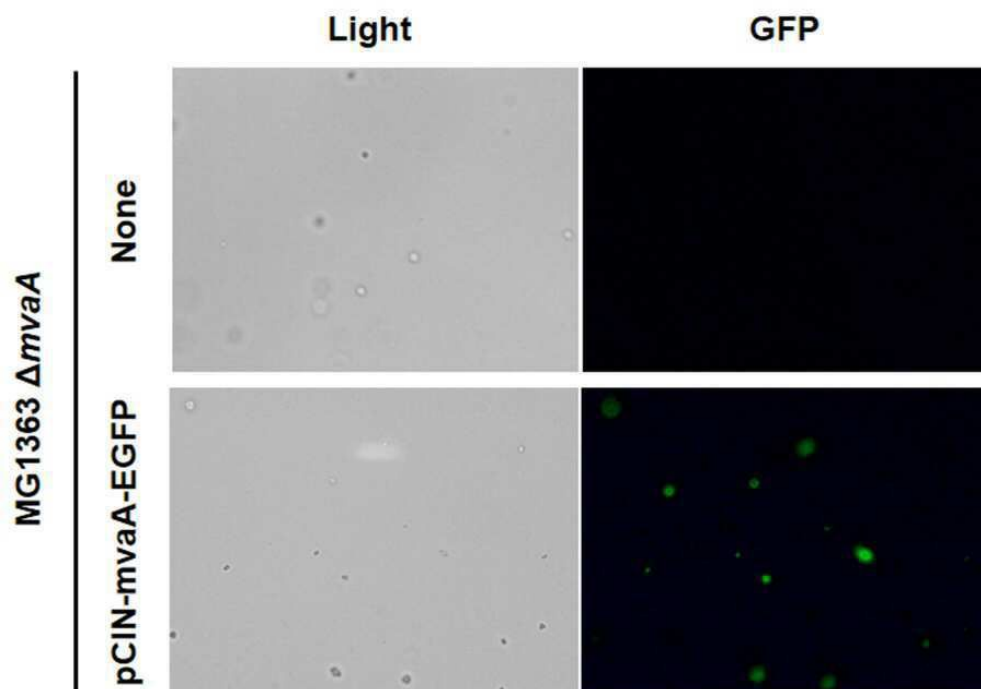
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<211> 810

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<400> 116

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<211> 1197

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<213> Artificial Sequence	

<220><223> Matricaria recutita MrBBS

<400> 121

atgtcaacc tgtcagtc cagccgtcc ttctcatcgt cgccgtgtc ctcagtgaac	60
aaaaatagca cgaacaaca cgttacgcgc aatagtgtga tctttcatga ttccatttgg	120
ggcgaccagt tcttgaata caaagaaaaa ttcaacgttg caaccgaaaa acaactgatt	180
gaagaactga aagaagaagt ccgcaatgaa ctgatgatcc gtgcgtgcaa cgaagccagt	240
cgctatatta aactgatcca gctgattgat gtggttgaac gtctgggcct ggcctaccac	300
tttgaaaaag aaatcgaaga atccctgcaa catatttatg ttacctacgg tcacaaatgg	360
acgaactaca acaacatcga aagcctgtct ctgtggtttc gcctgctgcg tcagaacggt	420
tttaatgtca gctctgatat cttcgaaaac catattgacg aaaaaggcaa tttccaagaa	480
agtcctgtga acgatccgca aggcatgctg gcaactgtatg aagcggccta catgcgcgtg	540
gaaggcgaaa ttatcctgga caaagctctg gaatttacca aactgcacct gggtattatc	600
agcaacgatc cgtcttgtga cagttccctg cgtacggaaa ttaaacaggc gctgaaacag	660
ccgctgcgtc gccgtctgcc gcgtctggaa gcagttcgtt atattgctat ctaccagcaa	720
aaagcgagtc attccgaagt cctgctgaaa ctggccaaac tggatttcaa tgtgctgcag	780
gaaatgcaca aagacgaact gtcacaaatt tgtaaatggt ggaaagacct ggacatccgt	840
aacaaactgc cgtatgttcg cgatcgtctg attgaaggct atttttggat tctgggtatt	900
tacttcgaac cgcagcattc gcgcacccgt atgtttctga tgaaaacgtg catgtggctg	960
atcgtcctgg atgacacett tgataattat ggacagtcacg aagaactgga aattttcacc	1020
caggcgggtg aacgctggag catcacgtgt ctggatgaac tgccggaata catgaaactg	1080
atctaccatg aacagttccg tgtgcaccaa gaaatggaag aatctctgga aaaagaaggt	1140
aaagcatacc agatccatta catcaaagaa atggctaaag aaggcaccg ctctctgctg	1200
ctggaagcga aatggctgaa agaaggttat atgccgacgc tggatgaata cctgtcaaac	1260
tcgtgggtta cctgcggcta tgcactgatg acggctcgtc catacgttgc ccgtgatgac	1320
ggatattgtca ccgaagatgc gtttaaatgg gtggccacgc acccgccgat cgttaaagca	1380
gcttgtaaaa ttctgcgtct gatggatgac atcgcgaccc ataaagaaga acaggaacgc	1440
ggccacatcg cctcatcgat tgaatgctat cgtaaagaaa ccggtgcac tgaagaagaa	1500
gcgtgtatgg attttctgaa acaggtcgaa gacggctgga aagtatcaa tcaagaatca	1560
ctgatgccga ccgatgtgcc gttcccgtg ctgatcccg cgattaacct ggcgccggtt	1620
tcggacacgc tgtataaaga taacgacggt tacaatcatg ctgataaaga agtgattggt	1680

tacattaaaa gtctgtttgt ccacccgatg attgtgtga 1719

<210> 122

<211> 717

<212> DNA

<213> Artificial Sequence

<220><223> EGFP

<400> 122

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ggcgacgtaa acggccacaa gttcagcgtg tccggcgagg gcgagggcga tgccacctac 120

ggcaagctga ccctgaagtt catctgcacc accggcaagc tgcccgtgcc ctggcccacc 180

ctcgtgacca ccctgacctc cggcgtgcag tcttcagcc gctaccccga ccacatgaag 240

cagcagcact tcttcaagtc cgccatgccc gaaggctacg tccaggagcg caccatcttc 300

ttcaaggacg acggcaacta caagaccgcg gccgaggtga agttcgaggg cgacaccctg 360

gtgaaccgca tcgagctgaa gggcatcgac ttcaaggagg acggcaacat cctggggcac 420

aagctggagt acaactacaa cagccacaac gtctatatca tggccgacaa gcagaagaac 480

ggcatcaagg tgaacttcaa gatccgccac aacatcgagg acggcagcgt gcagctcgcc 540

gaccactacc agcagaacac ccccatcggc gacggccccc tgctgctgcc cgacaaccac 600

tacctgagca cccagtccgc cctgagcaaa gaccccaacg agaagcgcgga tcacatggtc 660

ctgctggagt tcgtgaccgc cgccggggtc actctcggca tggacgagct gtacaag 717

<210> 123

<211> 1233

<212> DNA

<213> Artificial Sequence

<220><223> Lactococcus lactis subsp. cremoris MG1363

Hydroxymethylglutaryl-CoA reductase

<400> 123

atgagaaaaa aattttatca aatgtcgcca caagagcgat tgaattcggt aaacttatcc 60

gaaaagagtc aagaaatttt aagtgagatg gcctagata caactatttt ggacaatctc 120

atagaaaatc aaatttctga atttgaattg ccaatgggaa ttgcccacaa ttttgttatt 180

aatggacaaa gttttctaata accaatgggt acagaggaac cgtcagtaat tgccgcagca 240

agtaatggag caaaaatagc tggaaatttt gtagctgaaa ttaaagaacg ttaaatgcgt 300

ggTcaaatg tatTTtatga tgTtaaaaat tcagataaga ttgcaaatga aatacttgaa 360
aagcaagaaa aaatctttga acaagccgaa ctttcttacc cttcaatcgt taaacgaggt 420
ggcggtttga gagaggtttc tagtcggatt tttcttagtc aaaagttttt atctgttgat 480
gtcaaggttg atgttaaaga cgccatgggt gcaaatatta ttaattcaat cttagaggga 540
attgctgaac tctttcggag gtggtttcct gatgaaaaaa ttctgttttag tatTTtatca 600
aattatgcta ctgaatcact agtgaaagtg acctgtgaaa ttccggttga acgcctttca 660
aaaaaagcag atggatatga aattggtcaa aaaattatgg ctgcttccca atattcaaaa 720

attgatcctt atctgtgctag caccataac aaagggatta tgaacggtat caatgcggtc 780
atTTtagcta caggaaatga tactcgggcc atctcagctg ctattcatgc ttatgcggca 840
aaagatggag cctaccaagg gttagccaac tgggaacttc aagagaaaat gttagtggc 900
gaactcgaat ttcttttacc agtggaact gtcggagggg gagtcaaggt ttTccaaaa 960
gcacaagcgg ccatggagat tctgggtatc agtgatgcta aggagctcgc aaaagtatt 1020
gctgcagtgg gactagctca aaacttagcc gctttacgtg ccctagtTtc agaaggaatt 1080
caacagggac acatgagtct acaagctcgt tctttggctt tgagtgtagg agcacaagcg 1140

gatgaaatcg cgatattaag tcaacaattg cgcaaagaaa aagtatgaa tcaagaagta 1200
gccccaaatt tactaaaaaa tttgagaaaa taa 1233

<210> 124
<211> 803
<212> PRT
<213> Enterococcus faecalis mvaE
<400> 124

Leu Lys Thr Val Val Ile Ile Asp Ala Leu Arg Thr Pro Ile Gly Lys
1 5 10 15
Tyr Lys Gly Ser Leu Ser Gln Val Ser Ala Val Asp Leu Gly Thr His
20 25 30
Val Thr Thr Gln Leu Leu Lys Arg His Ser Thr Ile Ser Glu Glu Ile
35 40 45
Asp Gln Val Ile Phe Gly Asn Val Leu Gln Ala Gly Asn Gly Gln Asn
50 55 60
Pro Ala Arg Gln Ile Ala Ile Asn Ser Gly Leu Ser His Glu Ile Pro
65 70 75 80
Ala Met Thr Val Asn Glu Val Cys Gly Ser Gly Met Lys Ala Val Ile

85	90	95	
Leu Ala Lys Gln Leu Ile Gln Leu Gly Glu Ala Glu Val Leu Ile Ala			
100	105	110	
Gly Gly Ile Glu Asn Met Ser Gln Ala Pro Lys Leu Gln Arg Phe Asn			
115	120	125	
Tyr Glu Thr Glu Ser Tyr Asp Ala Pro Phe Ser Ser Met Met Tyr Asp			
130	135	140	
Gly Leu Thr Asp Ala Phe Ser Gly Gln Ala Met Gly Leu Thr Ala Glu			
145	150	155	160
Asn Val Ala Glu Lys Tyr His Val Thr Arg Glu Glu Gln Asp Gln Phe			
165	170	175	
Ser Val His Ser Gln Leu Lys Ala Ala Gln Ala Gln Ala Glu Gly Ile			
180	185	190	
Phe Ala Asp Glu Ile Ala Pro Leu Glu Val Ser Gly Thr Leu Val Glu			
195	200	205	
Lys Asp Glu Gly Ile Arg Pro Asn Ser Ser Val Glu Lys Leu Gly Thr			
210	215	220	
Leu Lys Thr Val Phe Lys Glu Asp Gly Thr Val Thr Ala Gly Asn Ala			
225	230	235	240
Ser Thr Ile Asn Asp Gly Ala Ser Ala Leu Ile Ile Ala Ser Gln Glu			
245	250	255	
Tyr Ala Glu Ala His Gly Leu Pro Tyr Leu Ala Ile Ile Arg Asp Ser			
260	265	270	
Val Glu Val Gly Ile Asp Pro Ala Tyr Met Gly Ile Ser Pro Ile Lys			
275	280	285	
Ala Ile Gln Lys Leu Leu Ala Arg Asn Gln Leu Thr Thr Glu Glu Ile			
290	295	300	
Asp Leu Tyr Glu Ile Asn Glu Ala Phe Ala Ala Thr Ser Ile Val Val			
305	310	315	320
Gln Arg Glu Leu Ala Leu Pro Glu Glu Lys Val Asn Ile Tyr Gly Gly			
325	330	335	

Gly Ile Ser Leu Gly His Ala Ile Gly Ala Thr Gly Ala Arg Leu Leu
340 345 350

Thr Ser Leu Ser Tyr Gln Leu Asn Gln Lys Glu Lys Lys Tyr Gly Val
355 360 365

Ala Ser Leu Cys Ile Gly Gly Gly Leu Gly Leu Ala Met Leu Leu Glu
370 375 380

Arg Pro Gln Gln Lys Lys Asn Ser Arg Phe Tyr Gln Met Ser Pro Glu
385 390 395 400

Glu Arg Leu Ala Ser Leu Leu Asn Glu Gly Gln Ile Ser Ala Asp Thr
405 410 415

Lys Lys Glu Phe Glu Asn Thr Ala Leu Ser Ser Gln Ile Ala Asn His
420 425 430

Met Ile Glu Asn Gln Ile Ser Glu Thr Glu Val Pro Met Gly Val Gly
435 440 445

Leu His Leu Thr Val Asp Glu Thr Asp Tyr Leu Val Pro Met Ala Thr
450 455 460

Glu Glu Pro Ser Val Ile Ala Ala Leu Ser Asn Gly Ala Lys Ile Ala
465 470 475 480

Gln Gly Phe Lys Thr Val Asn Gln Gln Arg Leu Met Arg Gly Gln Ile
485 490 495

Val Phe Tyr Asp Val Ala Asp Ala Glu Ser Leu Ile Asp Glu Leu Gln
500 505 510

Val Arg Glu Thr Glu Ile Phe Gln Gln Ala Glu Leu Ser Tyr Pro Ser
515 520 525

Ile Val Lys Arg Gly Gly Gly Leu Arg Asp Leu Gln Tyr Arg Ala Phe
530 535 540

Asp Glu Ser Phe Val Ser Val Asp Phe Leu Val Asp Val Lys Asp Ala
545 550 555 560

Met Gly Ala Asn Ile Val Asn Ala Met Leu Glu Gly Val Ala Glu Leu
565 570 575

Phe Arg Glu Trp Phe Ala Glu Gln Lys Ile Leu Phe Ser Ile Leu Ser
580 585 590

Asn Tyr Ala Thr Glu Ser Val Val Thr Met Lys Thr Ala Ile Pro Val
595 600 605

Ser Arg Leu Ser Lys Gly Ser Asn Gly Arg Glu Ile Ala Glu Lys Ile

610 615 620

Val Leu Ala Ser Arg Tyr Ala Ser Leu Asp Pro Tyr Arg Ala Val Thr

625 630 635 640

His Asn Lys Gly Ile Met Asn Gly Ile Glu Ala Val Val Leu Ala Thr

645 650 655

Gly Asn Asp Thr Arg Ala Val Ser Ala Ser Cys His Ala Phe Ala Val

660 665 670

Lys Glu Gly Arg Tyr Gln Gly Leu Thr Ser Trp Thr Leu Asp Gly Glu

675 680 685

Gln Leu Ile Gly Glu Ile Ser Val Pro Leu Ala Leu Ala Thr Val Gly

690 695 700

Gly Ala Thr Lys Val Leu Pro Lys Ser Gln Ala Ala Ala Asp Leu Leu

705 710 715 720

Ala Val Thr Asp Ala Lys Glu Leu Ser Arg Val Val Ala Ala Val Gly

725 730 735

Leu Ala Gln Asn Leu Ala Ala Leu Arg Ala Leu Val Ser Glu Gly Ile

740 745 750

Gln Lys Gly His Met Ala Leu Gln Ala Arg Ser Leu Ala Met Thr Val

755 760 765

Gly Ala Thr Gly Lys Glu Val Glu Ala Val Ala Gln Gln Leu Lys Arg

770 775 780

Gln Lys Thr Met Asn Gln Asp Arg Ala Leu Ala Ile Leu Asn Asp Leu

785 790 795 800

Arg Lys Gln

<210> 125

<211> 383

<212> PRT

<213> Enterococcus faecalis mvaS

<400> 125

Met Thr Ile Gly Ile Asp Lys Ile Ser Phe Phe Val Pro Pro Tyr Tyr
1 5 10 15

Ile Asp Met Thr Ala Leu Ala Glu Ala Arg Asn Val Asp Pro Gly Lys
20 25 30

Phe His Ile Gly Ile Gly Gln Asp Gln Met Ala Val Asn Pro Ile Ser
35 40 45

Gln Asp Ile Val Thr Phe Ala Ala Asn Ala Ala Glu Ala Ile Leu Thr
50 55 60

Lys Glu Asp Lys Glu Ala Ile Asp Met Val Ile Val Gly Thr Glu Ser
65 70 75 80

Ser Ile Asp Glu Ser Lys Ala Ala Ala Val Val Leu His Arg Leu Met
85 90 95

Gly Ile Gln Pro Phe Ala Arg Ser Phe Glu Ile Lys Glu Ala Cys Tyr
100 105 110

Gly Ala Thr Ala Gly Leu Gln Leu Ala Lys Asn His Val Ala Leu His
115 120 125

Pro Asp Lys Lys Val Leu Val Val Ala Ala Asp Ile Ala Lys Tyr Gly
130 135 140

Leu Asn Ser Gly Gly Glu Pro Thr Gln Gly Ala Gly Ala Val Ala Met
145 150 155 160

Leu Val Ala Ser Glu Pro Arg Ile Leu Ala Leu Lys Glu Asp Asn Val
165 170 175

Met Leu Thr Gln Asp Ile Tyr Asp Phe Trp Arg Pro Thr Gly His Pro
180 185 190

Tyr Pro Met Val Asp Gly Pro Leu Ser Asn Glu Thr Tyr Ile Gln Ser
195 200 205

Phe Ala Gln Val Trp Asp Glu His Lys Lys Arg Thr Gly Leu Asp Phe
210 215 220

Ala Asp Tyr Asp Ala Leu Ala Phe His Ile Pro Tyr Thr Lys Met Gly
225 230 235 240

Lys Lys Ala Leu Leu Ala Lys Ile Ser Asp Gln Thr Glu Ala Glu Gln
 245 250 255
 Glu Arg Ile Leu Ala Arg Tyr Glu Glu Ser Ile Ile Tyr Ser Arg Arg
 260 265 270
 Val Gly Asn Leu Tyr Thr Gly Ser Leu Tyr Leu Gly Leu Ile Ser Leu
 275 280 285
 Leu Glu Asn Ala Thr Thr Leu Thr Ala Gly Asn Gln Ile Gly Leu Phe
 290 295 300

 Ser Tyr Gly Ser Gly Ala Val Ala Glu Phe Phe Thr Gly Glu Leu Val
 305 310 315 320
 Ala Gly Tyr Gln Asn His Leu Gln Lys Glu Thr His Leu Ala Leu Leu
 325 330 335
 Asp Asn Arg Thr Glu Leu Ser Ile Ala Glu Tyr Glu Ala Met Phe Ala
 340 345 350
 Glu Thr Leu Asp Thr Asp Ile Asp Gln Thr Leu Glu Asp Glu Leu Lys
 355 360 365
 Tyr Ser Ile Ser Ala Ile Asn Asn Thr Val Arg Ser Tyr Arg Asn

 370 375 380
 <210> 126
 <211> 292
 <212> PRT
 <213> Streptococcus pneumoniae mvaK1
 <400> 126
 Met Thr Lys Lys Val Gly Val Gly Gln Ala His Ser Lys Ile Ile Leu
 1 5 10 15
 Ile Gly Glu His Ala Val Val Tyr Gly Tyr Pro Ala Ile Ser Leu Pro
 20 25 30
 Leu Leu Glu Val Glu Val Thr Cys Lys Val Val Pro Ala Glu Ser Pro
 35 40 45

 Trp Arg Leu Tyr Glu Glu Asp Thr Leu Ser Met Ala Val Tyr Ala Ser
 50 55 60
 Leu Glu Tyr Leu Asn Ile Thr Glu Ala Cys Ile Arg Cys Glu Ile Asp

65						70						75						80
Ser	Ala	Ile	Pro	Glu	Lys	Arg	Gly	Met	Gly	Ser	Ser	Ala	Ala	Ile	Ser			
					85						90						95	
Ile	Ala	Ala	Ile	Arg	Ala	Val	Phe	Asp	Tyr	Tyr	Gln	Ala	Asp	Leu	Pro			
					100						105						110	
His	Asp	Val	Leu	Glu	Ile	Leu	Val	Asn	Arg	Ala	Glu	Met	Ile	Ala	His			
					115						120						125	
Met	Asn	Pro	Ser	Gly	Leu	Asp	Ala	Lys	Thr	Cys	Leu	Ser	Asp	Gln	Pro			
					130						135						140	
Ile	Arg	Phe	Ile	Lys	Asn	Val	Gly	Phe	Thr	Glu	Leu	Glu	Met	Asp	Leu			
145						150						155						160
Ser	Ala	Tyr	Leu	Val	Ile	Ala	Asp	Thr	Gly	Val	Tyr	Gly	His	Thr	Arg			
					165						170						175	
Glu	Ala	Ile	Gln	Val	Val	Gln	Asn	Lys	Gly	Lys	Asp	Ala	Leu	Pro	Phe			
					180						185						190	
Leu	His	Ala	Leu	Gly	Glu	Leu	Thr	Gln	Gln	Ala	Glu	Val	Ala	Ile	Ser			
					195						200						205	
Gln	Lys	Asp	Ala	Glu	Gly	Leu	Gly	Gln	Ile	Leu	Ser	Gln	Ala	His	Leu			
					210						215						220	
His	Leu	Lys	Glu	Ile	Gly	Val	Ser	Ser	Pro	Glu	Ala	Asp	Phe	Leu	Val			
225						230						235						240
Glu	Thr	Thr	Leu	Ser	His	Gly	Ala	Leu	Gly	Ala	Lys	Met	Ser	Gly	Gly			
					245						250						255	
Gly	Leu	Gly	Gly	Cys	Ile	Ile	Ala	Leu	Val	Thr	Asn	Leu	Thr	His	Ala			
					260						265						270	
Gln	Glu	Leu	Ala	Glu	Arg	Leu	Glu	Glu	Lys	Gly	Ala	Val	Gln	Thr	Trp			
					275						280						285	
Ile	Glu	Ser	Leu															
					290													
<210>	127																	
<211>	336																	
<212>	PRT																	

<213> Streptococcus pneumoniae mvaK2

<400> 127

Met Ile Ala Val Lys Thr Cys Gly Lys Leu Tyr Trp Ala Gly Glu Tyr

1 5 10 15

Ala Ile Leu Glu Pro Gly Gln Leu Ala Leu Ile Lys Asp Ile Pro Ile

20 25 30

Tyr Met Arg Ala Glu Ile Ala Phe Ser Asp Ser Tyr Arg Ile Tyr Ser

35 40 45

Asp Met Phe Asp Phe Ala Val Asp Leu Arg Pro Asn Pro Asp Tyr Ser

50 55 60

Leu Ile Gln Glu Thr Ile Ala Leu Met Gly Asp Phe Leu Ala Val Arg

65 70 75 80

Gly Gln Asn Leu Arg Pro Phe Ser Leu Lys Ile Cys Gly Lys Met Glu

85 90 95

Arg Glu Gly Lys Lys Phe Gly Leu Gly Ser Ser Gly Ser Val Val Val

100 105 110

Leu Val Val Lys Ala Leu Leu Ala Leu Tyr Asn Leu Ser Val Asp Gln

115 120 125

Asn Leu Leu Phe Lys Leu Thr Ser Ala Val Leu Leu Lys Arg Gly Asp

130 135 140

Asn Gly Ser Met Gly Asp Leu Ala Cys Ile Val Ala Glu Asp Leu Val

145 150 155 160

Leu Tyr Gln Ser Phe Asp Arg Gln Lys Ala Ala Ala Trp Leu Glu Glu

165 170 175

Glu Asn Leu Ala Thr Val Leu Glu Arg Asp Trp Gly Phe Phe Ile Ser

180 185 190

Gln Val Lys Pro Thr Leu Glu Cys Asp Phe Leu Val Gly Trp Thr Lys

195 200 205

Glu Val Ala Val Ser Ser His Met Val Gln Gln Ile Lys Gln Asn Ile

210 215 220

Asn Gln Asn Phe Leu Ser Ser Ser Lys Glu Thr Val Val Ser Leu Val

225 230 235 240

Glu Ala Leu Glu Gln Gly Lys Ala Glu Lys Val Ile Glu Gln Val Glu

245 250 255

Val Ala Ser Lys Leu Leu Glu Gly Leu Ser Thr Asp Ile Tyr Thr Pro

260 265 270

Leu Leu Arg Gln Leu Lys Glu Ala Ser Gln Asp Leu Gln Ala Val Ala

275 280 285

Lys Ser Ser Gly Ala Gly Gly Gly Asp Cys Gly Ile Ala Leu Ser Phe

290 295 300

Asp Ala Gln Ser Ser Arg Asn Thr Leu Lys Asn Arg Trp Ala Asp Leu

305 310 315 320

Gly Ile Glu Leu Leu Tyr Gln Glu Arg Ile Gly His Asp Asp Lys Ser

325 330 335

<210> 128

<211> 317

<212> PRT

<213> Streptococcus pneumoniae mvaD

<400> 128

Met Asp Arg Glu Pro Val Thr Val Arg Ser Tyr Ala Asn Ile Ala Ile

1 5 10 15

Ile Lys Tyr Trp Gly Lys Lys Lys Glu Lys Glu Met Val Pro Ala Thr

20 25 30

Ser Ser Ile Ser Leu Thr Leu Glu Asn Met Tyr Thr Glu Thr Thr Leu

35 40 45

Ser Pro Leu Pro Ala Asn Val Thr Ala Asp Glu Phe Tyr Ile Asn Gly

50 55 60

Gln Leu Gln Asn Glu Val Glu His Ala Lys Met Ser Lys Ile Ile Asp

65 70 75 80

Arg Tyr Arg Pro Ala Gly Glu Gly Phe Val Arg Ile Asp Thr Gln Asn

85 90 95

Asn Met Pro Thr Ala Ala Gly Leu Ser Ser Ser Ser Ser Gly Leu Ser

100 105 110

Ala Leu Val Lys Ala Cys Asn Ala Tyr Phe Lys Leu Gly Leu Asp Arg
115 120 125

Ser Gln Leu Ala Gln Glu Ala Lys Phe Ala Ser Gly Ser Ser Ser Arg
130 135 140

Ser Phe Tyr Gly Pro Leu Gly Ala Trp Asp Lys Asp Ser Gly Glu Ile
145 150 155 160

Tyr Pro Val Glu Thr Asp Leu Lys Leu Ala Met Ile Met Leu Val Leu
165 170 175

Glu Asp Lys Lys Lys Pro Ile Ser Ser Arg Asp Gly Met Lys Leu Cys
180 185 190

Val Glu Thr Ser Thr Thr Phe Asp Asp Trp Val Arg Gln Ser Glu Lys
195 200 205

Asp Tyr Gln Asp Met Leu Ile Tyr Leu Lys Glu Asn Asp Phe Ala Lys
210 215 220

Ile Gly Glu Leu Thr Glu Lys Asn Ala Leu Ala Met His Ala Thr Thr
225 230 235 240

Lys Thr Ala Ser Pro Ala Phe Ser Tyr Leu Thr Asp Ala Ser Tyr Glu
245 250 255

Ala Met Ala Phe Val Arg Gln Leu Arg Glu Lys Gly Glu Ala Cys Tyr
260 265 270

Phe Thr Met Asp Ala Gly Pro Asn Val Lys Val Phe Cys Gln Glu Lys
275 280 285

Asp Leu Glu His Leu Ser Glu Ile Phe Gly Gln Arg Tyr Arg Leu Ile
290 295 300

Val Ser Lys Thr Lys Asp Leu Ser Gln Asp Asp Cys Cys
305 310 315

<210> 129
<211> 182
<212> PRT

<213> Escherichia coli idi
<400> 129

Met Gln Thr Glu His Val Ile Leu Leu Asn Ala Gln Gly Val Pro Thr

1 5 10 15
 Gly Thr Leu Glu Lys Tyr Ala Ala His Thr Ala Asp Thr Arg Leu His
 20 25 30
 Leu Ala Phe Ser Ser Trp Leu Phe Asn Ala Lys Gly Gln Leu Leu Val
 35 40 45
 Thr Arg Arg Ala Leu Ser Lys Lys Ala Trp Pro Gly Val Trp Thr Asn
 50 55 60

Ser Val Cys Gly His Pro Gln Leu Gly Glu Ser Asn Glu Asp Ala Val
 65 70 75 80
 Ile Arg Arg Cys Arg Tyr Glu Leu Gly Val Glu Ile Thr Pro Pro Glu
 85 90 95
 Ser Ile Tyr Pro Asp Phe Arg Tyr Arg Ala Thr Asp Pro Ser Gly Ile
 100 105 110
 Val Glu Asn Glu Val Cys Pro Val Phe Ala Ala Arg Thr Thr Ser Ala
 115 120 125
 Leu Gln Ile Asn Asp Asp Glu Val Met Asp Tyr Gln Trp Cys Asp Leu

130 135 140
 Ala Asp Val Leu His Gly Ile Asp Ala Thr Pro Trp Ala Phe Ser Pro
 145 150 155 160
 Trp Met Val Met Gln Ala Thr Asn Arg Glu Ala Arg Lys Arg Leu Ser
 165 170 175
 Ala Phe Thr Gln Leu Lys

180

<210> 130

<211> 305

<212> PRT

<213> Haematococcus pluvialis ipiHp1

<400> 130

Met Leu Arg Ser Leu Leu Arg Gly Leu Thr His Ile Pro Arg Val Asn

1 5 10 15
 Ser Ala Gln Gln Pro Ser Cys Ala His Ala Arg Leu Gln Phe Lys Leu
 20 25 30

Arg Ser Met Gln Met Thr Leu Met Gln Pro Ser Ile Ser Ala Asn Leu
 35 40 45
 Ser Arg Ala Glu Asp Arg Thr Asp His Met Arg Gly Ala Ser Thr Trp
 50 55 60
 Ala Gly Gly Gln Ser Gln Asp Glu Leu Met Leu Lys Asp Glu Cys Ile
 65 70 75 80

 Leu Val Asp Val Glu Asp Asn Ile Thr Gly His Ala Ser Lys Leu Glu
 85 90 95
 Cys His Lys Phe Leu Pro His Gln Pro Ala Gly Leu Leu His Arg Ala
 100 105 110
 Phe Ser Val Phe Leu Phe Asp Asp Gln Gly Arg Leu Leu Leu Gln Gln
 115 120 125
 Arg Ala Arg Ser Lys Ile Thr Phe Pro Ser Val Trp Thr Asn Thr Cys
 130 135 140
 Cys Ser His Pro Leu His Gly Gln Thr Pro Asp Glu Val Asp Gln Leu

 145 150 155 160
 Ser Gln Val Ala Asp Gly Thr Val Pro Gly Ala Lys Ala Ala Ala Ile
 165 170 175
 Arg Lys Leu Glu His Glu Leu Gly Ile Pro Ala His Gln Leu Pro Ala
 180 185 190
 Ser Ala Phe Arg Phe Leu Thr Arg Leu His Tyr Cys Ala Ala Asp Val
 195 200 205
 Gln Pro Ala Ala Thr Gln Ser Ala Leu Trp Gly Glu His Glu Met Asp
 210 215 220

 Tyr Ile Leu Phe Ile Arg Ala Asn Val Thr Leu Ala Pro Asn Pro Asp
 225 230 235 240
 Glu Val Asp Glu Val Arg Tyr Val Thr Gln Glu Glu Leu Arg Gln Met
 245 250 255
 Met Gln Pro Asp Asn Gly Leu Gln Trp Ser Pro Trp Phe Arg Ile Ile
 260 265 270
 Ala Ala Arg Phe Leu Glu Arg Trp Trp Ala Asp Leu Asp Ala Ala Leu
 275 280 285

Asn Thr Asp Lys His Glu Asp Trp Gly Thr Val His His Ile Asn Glu

290 295 300

Ala

305

<210> 131

<211> 309

<212> PRT

<213> pantoea agglomerans crtB

<400> 131

Met Ser Gln Pro Pro Leu Leu Asp His Ala Thr Gln Thr Met Ala Asn

1 5 10 15

Gly Ser Lys Ser Phe Ala Thr Ala Ala Lys Leu Phe Asp Pro Ala Thr

20 25 30

Arg Arg Ser Val Leu Met Leu Tyr Thr Trp Cys Arg His Cys Asp Asp

35 40 45

Val Ile Asp Asp Gln Thr His Gly Phe Ala Ser Glu Ala Ala Ala Glu

50 55 60

Glu Glu Ala Thr Gln Arg Leu Ala Arg Leu Arg Thr Leu Thr Leu Ala

65 70 75 80

Ala Phe Glu Gly Ala Glu Met Gln Asp Pro Ala Phe Ala Ala Phe Gln

85 90 95

Glu Val Ala Leu Thr His Gly Ile Thr Pro Arg Met Ala Leu Asp His

100 105 110

Leu Asp Gly Phe Ala Met Asp Val Ala Gln Thr Arg Tyr Val Thr Phe

115 120 125

Glu Asp Thr Leu Arg Tyr Cys Tyr His Val Ala Gly Val Val Gly Leu

130 135 140

Met Met Ala Arg Val Met Gly Val Arg Asp Glu Arg Val Leu Asp Arg

145 150 155 160

Ala Cys Asp Leu Gly Leu Ala Phe Gln Leu Thr Asn Ile Ala Arg Asp

165 170 175

Ile Ile Asp Asp Ala Ala Ile Asp Arg Cys Tyr Leu Pro Ala Glu Trp

180 185 190
 Leu Gln Asp Ala Gly Leu Thr Pro Glu Asn Tyr Ala Ala Arg Glu Asn
 195 200 205
 Arg Ala Ala Leu Ala Arg Val Ala Glu Arg Leu Ile Asp Ala Ala Glu
 210 215 220
 Pro Tyr Tyr Ile Ser Ser Gln Ala Gly Leu His Asp Leu Pro Pro Arg
 225 230 235 240
 Cys Ala Trp Ala Ile Ala Thr Ala Arg Ser Val Tyr Arg Glu Ile Gly
 245 250 255
 Ile Lys Val Lys Ala Ala Gly Gly Ser Ala Trp Asp Arg Arg Gln His
 260 265 270
 Thr Ser Lys Gly Glu Lys Ile Ala Met Leu Met Ala Ala Pro Gly Gln
 275 280 285
 Val Ile Arg Ala Lys Thr Thr Arg Val Thr Pro Arg Pro Ala Gly Leu
 290 295 300
 Trp Gln Arg Pro Val
 305
 <210> 132
 <211> 492
 <212> PRT
 <213> pantoea agglomerans crtI
 <400> 132
 Met Lys Lys Thr Val Val Ile Gly Ala Gly Phe Gly Gly Leu Ala Leu
 1 5 10 15
 Ala Ile Arg Leu Gln Ala Ala Gly Ile Pro Thr Val Leu Leu Glu Gln
 20 25 30
 Arg Asp Lys Pro Gly Gly Arg Ala Tyr Val Trp His Asp Gln Gly Phe
 35 40 45
 Thr Phe Asp Ala Gly Pro Thr Val Ile Thr Asp Pro Thr Ala Leu Glu
 50 55 60
 Ala Leu Phe Thr Leu Ala Gly Arg Arg Met Glu Asp Tyr Val Arg Leu
 65 70 75 80

Leu Pro Val Lys Pro Phe Tyr Arg Leu Cys Trp Glu Ser Gly Lys Thr

85 90 95

Leu Asp Tyr Ala Asn Asp Ser Ala Glu Leu Glu Ala Gln Ile Thr Gln

100 105 110

Phe Asn Pro Arg Asp Val Glu Gly Tyr Arg Arg Phe Leu Ala Tyr Ser

115 120 125

Gln Ala Val Phe Gln Glu Gly Tyr Leu Arg Leu Gly Ser Val Pro Phe

130 135 140

Leu Ser Phe Arg Asp Met Leu Arg Ala Gly Pro Gln Leu Leu Lys Leu

145 150 155 160

Gln Ala Trp Gln Ser Val Tyr Gln Ser Val Ser Arg Phe Ile Glu Asp

165 170 175

Glu His Leu Arg Gln Ala Phe Ser Phe His Ser Leu Leu Val Gly Gly

180 185 190

Asn Pro Phe Thr Thr Ser Ser Ile Tyr Thr Leu Ile His Ala Leu Glu

195 200 205

Arg Glu Trp Gly Val Trp Phe Pro Glu Gly Gly Thr Gly Ala Leu Val

210 215 220

Asn Gly Met Val Lys Leu Phe Thr Asp Leu Gly Gly Glu Ile Glu Leu

225 230 235 240

Asn Ala Arg Val Glu Glu Leu Val Val Ala Asp Asn Arg Val Ser Gln

245 250 255

Val Arg Leu Ala Asp Gly Arg Ile Phe Asp Thr Asp Ala Val Ala Ser

260 265 270

Asn Ala Asp Val Val Asn Thr Tyr Lys Lys Leu Leu Gly His His Pro

275 280 285

Val Gly Gln Lys Arg Ala Ala Ala Leu Glu Arg Lys Ser Met Ser Asn

290 295 300

Ser Leu Phe Val Leu Tyr Phe Gly Leu Asn Gln Pro His Ser Gln Leu

305 310 315 320

Ala His His Thr Ile Cys Phe Gly Pro Arg Tyr Arg Glu Leu Ile Asp

325 330 335
 Glu Ile Phe Thr Gly Ser Ala Leu Ala Asp Asp Phe Ser Leu Tyr Leu
 340 345 350
 His Ser Pro Cys Val Thr Asp Pro Ser Leu Ala Pro Pro Gly Cys Ala
 355 360 365
 Ser Phe Tyr Val Leu Ala Pro Val Pro His Leu Gly Asn Ala Pro Leu

370 375 380
 Asp Trp Ala Gln Glu Gly Pro Lys Leu Arg Asp Arg Ile Phe Asp Tyr
 385 390 395 400
 Leu Glu Glu Arg Tyr Met Pro Gly Leu Arg Ser Gln Leu Val Thr Gln
 405 410 415
 Arg Ile Phe Thr Pro Ala Asp Phe His Asp Thr Leu Asp Ala His Leu
 420 425 430
 Gly Ser Ala Phe Ser Ile Glu Pro Leu Leu Thr Gln Ser Ala Trp Phe
 435 440 445

Arg Pro His Asn Arg Asp Ser Asp Ile Ala Asn Leu Tyr Leu Val Gly
 450 455 460
 Ala Gly Thr His Pro Gly Ala Gly Ile Pro Gly Val Val Ala Ser Ala
 465 470 475 480
 Lys Ala Thr Ala Ser Leu Met Ile Glu Asp Leu Gln
 485 490

<210> 133
 <211> 382
 <212> PRT
 <213> pantoea ananatis crtY
 <400> 133

Met Gln Pro His Tyr Asp Leu Ile Leu Val Gly Ala Gly Leu Ala Asn
 1 5 10 15
 Gly Leu Ile Ala Leu Arg Leu Gln Gln Gln Pro Asp Met Arg Ile
 20 25 30
 Leu Leu Ile Asp Ala Ala Pro Gln Ala Gly Gly Asn His Thr Trp Ser
 35 40 45

Phe His His Asp Asp Leu Thr Glu Ser Gln His Arg Trp Ile Ala Pro
 50 55 60
 Leu Val Val His His Trp Pro Asp Tyr Gln Val Arg Phe Pro Thr Arg
 65 70 75 80
 Arg Arg Lys Leu Asn Ser Gly Tyr Phe Cys Ile Thr Ser Gln Arg Phe
 85 90 95
 Ala Glu Val Leu Gln Arg Gln Phe Gly Pro His Leu Trp Met Asp Thr
 100 105 110
 Ala Val Ala Glu Val Asn Ala Glu Ser Val Arg Leu Lys Lys Gly Gln
 115 120 125
 Val Ile Gly Ala Arg Ala Val Ile Asp Gly Arg Gly Tyr Ala Ala Asn
 130 135 140
 Ser Ala Leu Ser Val Gly Phe Gln Ala Phe Ile Gly Gln Glu Trp Arg
 145 150 155 160
 Leu Ser His Pro His Gly Leu Ser Ser Pro Ile Ile Met Asp Ala Thr
 165 170 175
 Val Asp Gln Gln Asn Gly Tyr Arg Phe Val Tyr Ser Leu Pro Leu Ser
 180 185 190
 Pro Thr Arg Leu Leu Ile Glu Asp Thr His Tyr Ile Asp Asn Ala Thr
 195 200 205
 Leu Asp Pro Glu Cys Ala Arg Gln Asn Ile Cys Asp Tyr Ala Ala Gln
 210 215 220
 Gln Gly Trp Gln Leu Gln Thr Leu Leu Arg Glu Glu Gln Gly Ala Leu
 225 230 235 240
 Pro Ile Thr Leu Ser Gly Asn Ala Asp Ala Phe Trp Gln Gln Arg Pro
 245 250 255
 Leu Ala Cys Ser Gly Leu Arg Ala Gly Leu Phe His Pro Thr Thr Gly
 260 265 270
 Tyr Ser Leu Pro Leu Ala Val Ala Val Ala Asp Arg Leu Ser Ala Leu
 275 280 285
 Asp Val Phe Thr Ser Ala Ser Ile His His Ala Ile Thr His Phe Ala

290 295 300
 Arg Glu Arg Trp Gln Gln Gln Gly Phe Phe Arg Met Leu Asn Arg Met
 305 310 315 320
 Leu Phe Leu Ala Gly Pro Ala Asp Ser Arg Trp Arg Val Met Gln Arg
 325 330 335
 Phe Tyr Gly Leu Pro Glu Asp Leu Ile Ala Arg Phe Tyr Ala Gly Lys
 340 345 350
 Leu Thr Leu Thr Asp Arg Leu Arg Ile Leu Ser Gly Lys Pro Pro Val
 355 360 365
 Pro Val Leu Ala Ala Leu Gln Ala Ile Met Thr Thr His Arg

 370 375 380
 <210> 134
 <211> 275
 <212> PRT
 <213> uncultured marine bacterium 66A03 SR
 <400> 134
 Met Gly Leu Met Leu Ile Asp Trp Cys Ala Leu Ala Leu Val Val Phe
 1 5 10 15
 Ile Gly Leu Pro His Gly Ala Leu Asp Ala Ala Ile Ser Phe Ser Met
 20 25 30
 Ile Ser Ser Ala Lys Arg Ile Ala Arg Leu Ala Gly Ile Leu Leu Ile
 35 40 45

 Tyr Leu Leu Leu Ala Thr Ala Phe Phe Leu Ile Trp Tyr Gln Leu Pro
 50 55 60
 Ala Phe Ser Leu Leu Ile Phe Leu Leu Ile Ser Ile Ile His Phe Gly
 65 70 75 80
 Met Ala Asp Phe Asn Ala Ser Pro Ser Lys Leu Lys Trp Pro His Ile
 85 90 95
 Ile Ala His Gly Gly Val Val Thr Val Trp Leu Pro Leu Ile Gln Lys
 100 105 110
 Asn Glu Val Thr Lys Leu Phe Ser Ile Leu Thr Asn Gly Pro Thr Pro

115 120 125
 Ile Leu Trp Asp Ile Leu Leu Ile Phe Phe Leu Cys Trp Ser Ile Gly
 130 135 140
 Val Cys Leu His Thr Tyr Glu Thr Leu Arg Ser Lys His Tyr Asn Ile
 145 150 155 160
 Ala Phe Glu Leu Ile Gly Leu Ile Phe Leu Ala Trp Tyr Ala Pro Pro
 165 170 175
 Leu Val Thr Phe Ala Thr Tyr Phe Cys Phe Ile His Ser Arg Arg His
 180 185 190

 Phe Ser Phe Val Trp Lys Gln Leu Gln His Met Ser Ser Lys Lys Met
 195 200 205
 Met Ile Gly Ser Ala Ile Ile Leu Ser Cys Thr Ser Trp Leu Ile Gly
 210 215 220
 Gly Gly Ile Tyr Phe Phe Leu Asn Ser Lys Met Ile Ala Ser Glu Ala
 225 230 235 240
 Ala Leu Gln Thr Val Phe Ile Gly Leu Ala Ala Leu Thr Val Pro His
 245 250 255
 Met Ile Leu Ile Asp Phe Ile Phe Arg Pro His Ser Ser Arg Ile Lys

 260 265 270
 Ile Lys Asn
 275
 <210> 135
 <211> 269
 <212> PRT
 <213> Escherichia coli MG1655 Ybb0
 <400> 135
 Met Thr His Lys Ala Thr Glu Ile Leu Thr Gly Lys Val Met Gln Lys
 1 5 10 15
 Ser Val Leu Ile Thr Gly Cys Ser Ser Gly Ile Gly Leu Glu Ser Ala
 20 25 30
 Leu Glu Leu Lys Arg Gln Gly Phe His Val Leu Ala Gly Cys Arg Lys
 35 40 45

Pro Asp Asp Val Glu Arg Met Asn Ser Met Gly Phe Thr Gly Val Leu
50 55 60
Ile Asp Leu Asp Ser Pro Glu Ser Val Asp Arg Ala Ala Asp Glu Val
65 70 75 80
Ile Ala Leu Thr Asp Asn Cys Leu Tyr Gly Ile Phe Asn Asn Ala Gly
85 90 95
Phe Gly Met Tyr Gly Pro Leu Ser Thr Ile Ser Arg Ala Gln Met Glu
100 105 110
Gln Gln Phe Ser Ala Asn Phe Phe Gly Ala His Gln Leu Thr Met Arg
115 120 125
Leu Leu Pro Ala Met Leu Pro His Gly Glu Gly Arg Ile Val Met Thr
130 135 140
Ser Ser Val Met Gly Leu Ile Ser Thr Pro Gly Arg Gly Ala Tyr Ala
145 150 155 160
Ala Ser Lys Tyr Ala Leu Glu Ala Trp Ser Asp Ala Leu Arg Met Glu
165 170 175
Leu Arg His Ser Gly Ile Lys Val Ser Leu Ile Glu Pro Gly Pro Ile
180 185 190
Arg Thr Arg Phe Thr Asp Asn Val Asn Gln Thr Gln Ser Asp Lys Pro
195 200 205
Val Glu Asn Pro Gly Ile Ala Ala Arg Phe Thr Leu Gly Pro Glu Ala
210 215 220
Val Val Asp Lys Val Arg His Ala Phe Ile Ser Glu Lys Pro Lys Met
225 230 235 240
Arg Tyr Pro Val Thr Leu Val Thr Trp Ala Val Met Val Leu Lys Arg
245 250 255
Leu Leu Pro Gly Arg Val Met Asp Lys Ile Leu Gln Gly

260

265

<210> 136

<211> 620

<212> PRT

<213> Escherichia coli dxs

<400> 136

Met Ser Phe Asp Ile Ala Lys Tyr Pro Thr Leu Ala Leu Val Asp Ser

1 5 10 15

Thr Gln Glu Leu Arg Leu Leu Pro Lys Glu Ser Leu Pro Lys Leu Cys

20 25 30

Asp Glu Leu Arg Arg Tyr Leu Leu Asp Ser Val Ser Arg Ser Ser Gly

35 40 45

His Phe Ala Ser Gly Leu Gly Thr Val Glu Leu Thr Val Ala Leu His

50 55 60

Tyr Val Tyr Asn Thr Pro Phe Asp Gln Leu Ile Trp Asp Val Gly His

65 70 75 80

Gln Ala Tyr Pro His Lys Ile Leu Thr Gly Arg Arg Asp Lys Ile Gly

85 90 95

Thr Ile Arg Gln Lys Gly Gly Leu His Pro Phe Pro Trp Arg Gly Glu

100 105 110

Ser Glu Tyr Asp Val Leu Ser Val Gly His Ser Ser Thr Ser Ile Ser

115 120 125

Ala Gly Ile Gly Ile Ala Val Ala Ala Glu Lys Glu Gly Lys Asn Arg

130 135 140

Arg Thr Val Cys Val Ile Gly Asp Gly Ala Ile Thr Ala Gly Met Ala

145 150 155 160

Phe Glu Ala Met Asn His Ala Gly Asp Ile Arg Pro Asp Met Leu Val

165 170 175

Ile Leu Asn Asp Asn Glu Met Ser Ile Ser Glu Asn Val Gly Ala Leu

180 185 190

Asn Asn His Leu Ala Gln Leu Leu Ser Gly Lys Leu Tyr Ser Ser Leu

195 200 205

Arg Glu Gly Gly Lys Lys Val Phe Ser Gly Val Pro Pro Ile Lys Glu

210 215 220

Leu Leu Lys Arg Thr Glu Glu His Ile Lys Gly Met Val Val Pro Gly

225 230 235 240

Thr Leu Phe Glu Glu Leu Gly Phe Asn Tyr Ile Gly Pro Val Asp Gly

245 250 255
His Asp Val Leu Gly Leu Ile Thr Thr Leu Lys Asn Met Arg Asp Leu

260 265 270
Lys Gly Pro Gln Phe Leu His Ile Met Thr Lys Lys Gly Arg Gly Tyr

275 280 285
Glu Pro Ala Glu Lys Asp Pro Ile Thr Phe His Ala Val Pro Lys Phe

290 295 300
Asp Pro Ser Ser Gly Cys Leu Pro Lys Ser Ser Gly Gly Leu Pro Ser

305 310 315 320
Tyr Ser Lys Ile Phe Gly Asp Trp Leu Cys Glu Thr Ala Ala Lys Asp

325 330 335

Asn Lys Leu Met Ala Ile Thr Pro Ala Met Arg Glu Gly Ser Gly Met

340 345 350

Val Glu Phe Ser Arg Lys Phe Pro Asp Arg Tyr Phe Asp Val Ala Ile

355 360 365

Ala Glu Gln His Ala Val Thr Phe Ala Ala Gly Leu Ala Ile Gly Gly

370 375 380

Tyr Lys Pro Ile Val Ala Ile Tyr Ser Thr Phe Leu Gln Arg Ala Tyr

385 390 395 400

Asp Gln Val Leu His Asp Val Ala Ile Gln Lys Leu Pro Val Leu Phe

405 410 415

Ala Ile Asp Arg Ala Gly Ile Val Gly Ala Asp Gly Gln Thr His Gln

420 425 430

Gly Ala Phe Asp Leu Ser Tyr Leu Arg Cys Ile Pro Glu Met Val Ile

435 440 445

Met Thr Pro Ser Asp Glu Asn Glu Cys Arg Gln Met Leu Tyr Thr Gly

450 455 460

Tyr His Tyr Asn Asp Gly Pro Ser Ala Val Arg Tyr Pro Arg Gly Asn

465 470 475 480

Ala Val Gly Val Glu Leu Thr Pro Leu Glu Lys Leu Pro Ile Gly Lys

485 490 495

Gly Ile Val Lys Arg Arg Gly Glu Lys Leu Ala Ile Leu Asn Phe Gly
500 505 510
Thr Leu Met Pro Glu Ala Ala Lys Val Ala Glu Ser Leu Asn Ala Thr
515 520 525
Leu Val Asp Met Arg Phe Val Lys Pro Leu Asp Glu Ala Leu Ile Leu
530 535 540
Glu Met Ala Ala Ser His Glu Ala Leu Val Thr Val Glu Glu Asn Ala
545 550 555 560
Ile Met Gly Gly Ala Gly Ser Gly Val Asn Glu Val Leu Met Ala His
565 570 575
Arg Lys Pro Val Pro Val Leu Asn Ile Gly Leu Pro Asp Phe Phe Ile
580 585 590
Pro Gln Gly Thr Gln Glu Glu Met Arg Ala Glu Leu Gly Leu Asp Ala
595 600 605
Ala Gly Met Glu Ala Lys Ile Lys Ala Trp Leu Ala
610 615 620

<210> 137

<211> 398

<212> PRT

<213> Escherichia coli dxr

<400> 137

Met Lys Gln Leu Thr Ile Leu Gly Ser Thr Gly Ser Ile Gly Cys Ser
1 5 10 15
Thr Leu Asp Val Val Arg His Asn Pro Glu His Phe Arg Val Val Ala
20 25 30
Leu Val Ala Gly Lys Asn Val Thr Arg Met Val Glu Gln Cys Leu Glu
35 40 45
Phe Ser Pro Arg Tyr Ala Val Met Asp Asp Glu Ala Ser Ala Lys Leu
50 55 60
Leu Lys Thr Met Leu Gln Gln Gln Gly Ser Arg Thr Glu Val Leu Ser
65 70 75 80
Gly Gln Gln Ala Ala Cys Asp Met Ala Ala Leu Glu Asp Val Asp Gln

	85	90	95
Val Met Ala Ala Ile Val Gly Ala Ala Gly Leu Leu Pro Thr Leu Ala			
	100	105	110
Ala Ile Arg Ala Gly Lys Thr Ile Leu Leu Ala Asn Lys Glu Ser Leu			
	115	120	125
Val Thr Cys Gly Arg Leu Phe Met Asp Ala Val Lys Gln Ser Lys Ala			
	130	135	140
Gln Leu Leu Pro Val Asp Ser Glu His Asn Ala Ile Phe Gln Ser Leu			
145	150	155	160
Pro Gln Pro Ile Gln His Asn Leu Gly Tyr Ala Asp Leu Glu Gln Asn			
	165	170	175
Gly Val Val Ser Ile Leu Leu Thr Gly Ser Gly Gly Pro Phe Arg Glu			
	180	185	190
Thr Pro Leu Arg Asp Leu Ala Thr Met Thr Pro Asp Gln Ala Cys Arg			
	195	200	205
His Pro Asn Trp Ser Met Gly Arg Lys Ile Ser Val Asp Ser Ala Thr			
	210	215	220
Met Met Asn Lys Gly Leu Glu Tyr Ile Glu Ala Arg Trp Leu Phe Asn			
225	230	235	240
Ala Ser Ala Ser Gln Met Glu Val Leu Ile His Pro Gln Ser Val Ile			
	245	250	255
His Ser Met Val Arg Tyr Gln Asp Gly Ser Val Leu Ala Gln Leu Gly			
	260	265	270
Glu Pro Asp Met Arg Thr Pro Ile Ala His Thr Met Ala Trp Pro Asn			
	275	280	285
Arg Val Asn Ser Gly Val Lys Pro Leu Asp Phe Cys Lys Leu Ser Ala			
	290	295	300
Leu Thr Phe Ala Ala Pro Asp Tyr Asp Arg Tyr Pro Cys Leu Lys Leu			
305	310	315	320
Ala Met Glu Ala Phe Glu Gln Gly Gln Ala Ala Thr Thr Ala Leu Asn			
	325	330	335

Ala Ala Asn Glu Ile Thr Val Ala Ala Phe Leu Ala Gln Gln Ile Arg

340 345 350
Phe Thr Asp Ile Ala Ala Leu Asn Leu Ser Val Leu Glu Lys Met Asp

355 360 365
Met Arg Glu Pro Gln Cys Val Asp Asp Val Leu Ser Val Asp Ala Asn

370 375 380
Ala Arg Glu Val Ala Arg Lys Glu Val Met Arg Leu Ala Ser

385 390 395

<210> 138

<211> 299

<212> PRT

<213> Escherichia coli ispA

<400> 138

Met Asp Phe Pro Gln Gln Leu Glu Ala Cys Val Lys Gln Ala Asn Gln

1 5 10 15
Ala Leu Ser Arg Phe Ile Ala Pro Leu Pro Phe Gln Asn Thr Pro Val

20 25 30
Val Glu Thr Met Gln Tyr Gly Ala Leu Leu Gly Gly Lys Arg Leu Arg

35 40 45
Pro Phe Leu Val Tyr Ala Thr Gly His Met Phe Gly Val Ser Thr Asn

50 55 60
Thr Leu Asp Ala Pro Ala Ala Ala Val Glu Cys Ile His Ala Tyr Ser

65 70 75 80

Leu Ile His Asp Asp Leu Pro Ala Met Asp Asp Asp Asp Leu Arg Arg

85 90 95
Gly Leu Pro Thr Cys His Val Lys Phe Gly Glu Ala Asn Ala Ile Leu

100 105 110
Ala Gly Asp Ala Leu Gln Thr Leu Ala Phe Ser Ile Leu Ser Asp Ala

115 120 125
Asp Met Pro Glu Val Ser Asp Arg Asp Arg Ile Ser Met Ile Ser Glu

130 135 140

Leu Ala Ser Ala Ser Gly Ile Ala Gly Met Cys Gly Gly Gln Ala Leu

145 150 155 160

Asp Leu Asp Ala Glu Gly Lys His Val Pro Leu Asp Ala Leu Glu Arg

165 170 175

Ile His Arg His Lys Thr Gly Ala Leu Ile Arg Ala Ala Val Arg Leu

180 185 190

Gly Ala Leu Ser Ala Gly Asp Lys Gly Arg Arg Ala Leu Pro Val Leu

195 200 205

Asp Lys Tyr Ala Glu Ser Ile Gly Leu Ala Phe Gln Val Gln Asp Asp

210 215 220

Ile Leu Asp Val Val Gly Asp Thr Ala Thr Leu Gly Lys Arg Gln Gly

225 230 235 240

Ala Asp Gln Gln Leu Gly Lys Ser Thr Tyr Pro Ala Leu Leu Gly Leu

245 250 255

Glu Gln Ala Arg Lys Lys Ala Arg Asp Leu Ile Asp Asp Ala Arg Gln

260 265 270

Ser Leu Lys Gln Leu Ala Glu Gln Ser Leu Asp Thr Ser Ala Leu Glu

275 280 285

Ala Leu Ala Asp Tyr Ile Ile Gln Arg Asn Lys

290 295

<210> 139

<211> 551

<212> PRT

<213> Clausena lansium STS

<400> 139

Met Ser Thr Gln Gln Val Ser Ser Glu Asn Ile Val Arg Asn Ala Ala

1 5 10 15

Asn Phe His Pro Asn Ile Trp Gly Asn His Phe Leu Thr Cys Pro Ser

20 25 30

Gln Thr Ile Asp Ser Trp Thr Gln Gln His His Lys Glu Leu Lys Glu

35 40 45

Glu Val Arg Lys Met Met Val Ser Asp Ala Asn Lys Pro Ala Gln Arg

50

55

60

Leu Arg Leu Ile Asp Thr Val Gln Arg Leu Gly Val Ala Tyr His Phe

65

70

75

80

Glu Lys Glu Ile Asp Asp Ala Leu Glu Lys Ile Gly His Asp Pro Phe

85

90

95

Asp Asp Lys Asp Asp Leu Tyr Ile Val Ser Leu Cys Phe Arg Leu Leu

100

105

110

Arg Gln His Gly Ile Lys Ile Ser Cys Asp Val Phe Glu Lys Phe Lys

115

120

125

Asp Asp Asp Gly Lys Phe Lys Ala Ser Leu Met Asn Asp Val Gln Gly

130

135

140

Met Leu Ser Leu Tyr Glu Ala Ala His Leu Ala Ile His Gly Glu Asp

145

150

155

160

Ile Leu Asp Glu Ala Ile Val Phe Thr Thr Thr His Leu Lys Ser Thr

165

170

175

Val Ser Asn Ser Pro Val Asn Ser Thr Phe Ala Glu Gln Ile Arg His

180

185

190

Ser Leu Arg Val Pro Leu Arg Lys Ala Val Pro Arg Leu Glu Ser Arg

195

200

205

Tyr Phe Leu Asp Ile Tyr Ser Arg Asp Asp Leu His Asp Lys Thr Leu

210

215

220

Leu Asn Phe Ala Lys Leu Asp Phe Asn Ile Leu Gln Ala Met His Gln

225

230

235

240

Lys Glu Ala Ser Glu Met Thr Arg Trp Trp Arg Asp Phe Asp Phe Leu

245

250

255

Lys Lys Leu Pro Tyr Ile Arg Asp Arg Val Val Glu Leu Tyr Phe Trp

260

265

270

Ile Leu Val Gly Val Ser Tyr Gln Pro Lys Phe Ser Thr Gly Arg Ile

275

280

285

Phe Leu Ser Lys Ile Ile Cys Leu Glu Thr Leu Val Asp Asp Thr Phe

290 295 300
 Asp Ala Tyr Gly Thr Phe Asp Glu Leu Ala Ile Phe Thr Glu Ala Val
 305 310 315 320
 Thr Arg Trp Asp Leu Gly His Arg Asp Ala Leu Pro Glu Tyr Met Lys
 325 330 335
 Phe Ile Phe Lys Thr Leu Ile Asp Val Tyr Ser Glu Ala Glu Gln Glu

 340 345 350
 Leu Ala Lys Glu Gly Arg Ser Tyr Ser Ile His Tyr Ala Ile Arg Ser
 355 360 365
 Phe Gln Glu Leu Val Met Lys Tyr Phe Cys Glu Ala Lys Trp Leu Asn
 370 375 380
 Lys Gly Tyr Val Pro Ser Leu Asp Asp Tyr Lys Ser Val Ser Leu Arg
 385 390 395 400
 Ser Ile Gly Phe Leu Pro Ile Ala Val Ala Ser Phe Val Phe Met Gly
 405 410 415

 Asp Ile Ala Thr Lys Glu Val Phe Glu Trp Glu Met Asn Asn Pro Lys
 420 425 430
 Ile Ile Ile Ala Ala Glu Thr Ile Phe Arg Phe Leu Asp Asp Ile Ala
 435 440 445
 Gly His Arg Phe Glu Gln Lys Arg Glu His Ser Pro Ser Ala Ile Glu
 450 455 460
 Cys Tyr Lys Asn Gln His Gly Val Ser Glu Glu Glu Ala Val Lys Ala
 465 470 475 480
 Leu Ser Leu Glu Val Ala Asn Ser Trp Lys Asp Ile Asn Glu Glu Leu

 485 490 495
 Leu Leu Asn Pro Met Ala Ile Pro Leu Pro Leu Leu Gln Val Ile Leu
 500 505 510
 Asp Leu Ser Arg Ser Ala Asp Phe Met Tyr Gly Asn Ala Gln Asp Arg
 515 520 525
 Phe Thr His Ser Thr Met Met Lys Asp Gln Val Asp Leu Val Leu Lys
 530 535 540
 Asp Pro Val Lys Leu Asp Asp

545	550																		
<210>	140																		
<211>	572																		
<																			
212>	PRT																		
<213>	Artificial Sequence																		
<220><223>	Matricaria recutita MrBBS																		
<400>	140																		
Met	Ser	Thr	Leu	Ser	Val	Ser	Thr	Pro	Ser	Phe	Ser	Ser	Ser	Pro	Leu				
1					5					10					15				
Ser	Ser	Val	Asn	Lys	Asn	Ser	Thr	Lys	Gln	His	Val	Thr	Arg	Asn	Ser				
				20					25					30					
Val	Ile	Phe	His	Asp	Ser	Ile	Trp	Gly	Asp	Gln	Phe	Leu	Glu	Tyr	Lys				
				35					40					45					
Glu	Lys	Phe	Asn	Val	Ala	Thr	Glu	Lys	Gln	Leu	Ile	Glu	Glu	Leu	Lys				
				50					55					60					
Glu	Glu	Val	Arg	Asn	Glu	Leu	Met	Ile	Arg	Ala	Cys	Asn	Glu	Ala	Ser				
65						70					75				80				
Arg	Tyr	Ile	Lys	Leu	Ile	Gln	Leu	Ile	Asp	Val	Val	Glu	Arg	Leu	Gly				
					85					90					95				
Leu	Ala	Tyr	His	Phe	Glu	Lys	Glu	Ile	Glu	Glu	Ser	Leu	Gln	His	Ile				
				100					105					110					
Tyr	Val	Thr	Tyr	Gly	His	Lys	Trp	Thr	Asn	Tyr	Asn	Asn	Ile	Glu	Ser				
				115					120					125					
Leu	Ser	Leu	Trp	Phe	Arg	Leu	Leu	Arg	Gln	Asn	Gly	Phe	Asn	Val	Ser				
				130					135					140					
Ser	Asp	Ile	Phe	Glu	Asn	His	Ile	Asp	Glu	Lys	Gly	Asn	Phe	Gln	Glu				
145						150					155				160				
Ser	Leu	Cys	Asn	Asp	Pro	Gln	Gly	Met	Leu	Ala	Leu	Tyr	Glu	Ala	Ala				
					165					170					175				
Tyr	Met	Arg	Val	Glu	Gly	Glu	Ile	Ile	Leu	Asp	Lys	Ala	Leu	Glu	Phe				
				180					185						190				

Thr Lys Leu His Leu Gly Ile Ile Ser Asn Asp Pro Ser Cys Asp Ser

195 200 205

Ser Leu Arg Thr Glu Ile Lys Gln Ala Leu Lys Gln Pro Leu Arg Arg

210 215 220

Arg Leu Pro Arg Leu Glu Ala Val Arg Tyr Ile Ala Ile Tyr Gln Gln

225 230 235 240

Lys Ala Ser His Ser Glu Val Leu Leu Lys Leu Ala Lys Leu Asp Phe

245 250 255

Asn Val Leu Gln Glu Met His Lys Asp Glu Leu Ser Gln Ile Cys Lys

260 265 270

Trp Trp Lys Asp Leu Asp Ile Arg Asn Lys Leu Pro Tyr Val Arg Asp

275 280 285

Arg Leu Ile Glu Gly Tyr Phe Trp Ile Leu Gly Ile Tyr Phe Glu Pro

290 295 300

Gln His Ser Arg Thr Arg Met Phe Leu Met Lys Thr Cys Met Trp Leu

305 310 315 320

Ile Val Leu Asp Asp Thr Phe Asp Asn Tyr Gly Thr Tyr Glu Glu Leu

325 330 335

Glu Ile Phe Thr Gln Ala Val Glu Arg Trp Ser Ile Thr Cys Leu Asp

340 345 350

Glu Leu Pro Glu Tyr Met Lys Leu Ile Tyr His Glu Gln Phe Arg Val

355 360 365

His Gln Glu Met Glu Glu Ser Leu Glu Lys Glu Gly Lys Ala Tyr Gln

370 375 380

Ile His Tyr Ile Lys Glu Met Ala Lys Glu Gly Thr Arg Ser Leu Leu

385 390 395 400

Leu Glu Ala Lys Trp Leu Lys Glu Gly Tyr Met Pro Thr Leu Asp Glu

405 410 415

Tyr Leu Ser Asn Ser Leu Val Thr Cys Gly Tyr Ala Leu Met Thr Ala

420 425 430

Arg Ser Tyr Val Ala Arg Asp Asp Gly Ile Val Thr Glu Asp Ala Phe

435 440 445
 Lys Trp Val Ala Thr His Pro Pro Ile Val Lys Ala Ala Cys Lys Ile
 450 455 460
 Leu Arg Leu Met Asp Asp Ile Ala Thr His Lys Glu Glu Gln Glu Arg
 465 470 475 480
 Gly His Ile Ala Ser Ser Ile Glu Cys Tyr Arg Lys Glu Thr Gly Ala

485 490 495
 Ser Glu Glu Glu Ala Cys Met Asp Phe Leu Lys Gln Val Glu Asp Gly
 500 505 510
 Trp Lys Val Ile Asn Gln Glu Ser Leu Met Pro Thr Asp Val Pro Phe
 515 520 525
 Pro Leu Leu Ile Pro Ala Ile Asn Leu Ala Arg Val Ser Asp Thr Leu
 530 535 540
 Tyr Lys Asp Asn Asp Gly Tyr Asn His Ala Asp Lys Glu Val Ile Gly
 545 550 555 560

Tyr Ile Lys Ser Leu Phe Val His Pro Met Ile Val

565 570

<210> 141

<211> 391

<212> PRT

<213> Artificial Sequence

<220><223> EGFP

<400> 141

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile

35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys

65	70	75	80
Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu			
	85	90	95
Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu			
	100	105	110
Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly			
	115	120	125
Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr			
	130	135	140
Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn			
145	150	155	160
Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser			
	165	170	175
Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly			
	180	185	190
Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu			
	195	200	205
Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe			
	210	215	220
Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys Gly			
225	230	235	240
Tyr Pro Ala Gly Trp Arg Pro Leu Glu Met Val Glu Arg Lys Lys Leu			
	245	250	255
Gly Val Leu Gln Pro Thr Gln Gln Cys Arg Leu Thr Val His Gln Ser			
	260	265	270
Lys Ile Met Val Asp Ser Pro Lys Ala Leu Lys Arg Lys Ala Phe Gly			
	275	280	285
Asp Glu Asn Phe Ser Pro Val Pro Thr Leu Ser Arg Lys Ser Ile Phe			
	290	295	300
Glu Asp Pro Pro Thr Pro Ala Ser Lys Arg Ser Lys Leu Ser Asp Lys			
305	310	315	320

Val Asp Ser Ala Ile Gln Thr Asp Asn Leu Cys Cys Gly Asn Gly Gly

325 330 335

Thr Glu Ile Ser Arg Gly Ser Ser Ser Ser Lys Ser Ser Ser Glu Ala

340 345 350

Ser Thr Lys Ala Cys Ser Gln Lys Ser Gln Ser Gln Ser Val Leu Asp

355 360 365

Met Leu Thr Ser Ala Glu Pro Asn Glu Gln Tyr Trp Gln Met Met Ala

370 375 380

Ala Glu Arg Lys Ala Ala Leu

385 390

<210> 142

<211> 410

<

212> PRT

<213> Lactococcus lactis subsp. cremoris NZ9000 mvaA

<400> 142

Met Arg Lys Lys Phe Tyr Gln Met Ser Pro Gln Glu Arg Leu Asn Ser

1 5 10 15

Leu Asn Leu Ser Glu Lys Ser Gln Glu Ile Leu Ser Glu Met Ala Leu

20 25 30

Asp Thr Thr Ile Leu Asp Asn Leu Ile Glu Asn Gln Ile Ser Glu Phe

35 40 45

Glu Leu Pro Met Gly Ile Ala Gln Asn Phe Val Ile Asn Gly Gln Ser

50 55 60

Phe Leu Ile Pro Met Val Thr Glu Glu Pro Ser Val Ile Ala Ala Ala

65 70 75 80

Ser Asn Gly Ala Lys Ile Ala Gly Asn Phe Val Ala Glu Ile Lys Glu

85 90 95

Arg Leu Met Arg Gly Gln Ile Val Phe Tyr Asp Val Lys Asn Ser Asp

100 105 110

Lys Ile Ala Asn Glu Ile Leu Glu Lys Gln Glu Lys Ile Phe Glu Gln

115 120 125

Ala Glu Leu Ser Tyr Pro Ser Ile Val Lys Arg Gly Gly Gly Leu Arg
130 135 140

Glu Val Ser Ser Arg Ile Phe Ser Ser Gln Lys Phe Leu Ser Val Asp
145 150 155 160

Val Lys Val Asp Val Lys Asp Ala Met Gly Ala Asn Ile Ile Asn Ser
165 170 175

Ile Leu Glu Gly Ile Ala Glu Leu Phe Arg Arg Trp Phe Pro Asp Glu
180 185 190

Lys Ile Leu Phe Ser Ile Leu Ser Asn Tyr Ala Thr Glu Ser Leu Val
195 200 205

Lys Val Thr Cys Glu Ile Pro Val Glu Arg Leu Ser Lys Lys Ala Asp
210 215 220

Gly Tyr Glu Ile Gly Gln Lys Ile Met Ala Ala Ser Gln Tyr Ser Lys
225 230 235 240

Ile Asp Pro Tyr Arg Ala Ser Thr His Asn Lys Gly Ile Met Asn Gly
245 250 255

Ile Asn Ala Val Ile Leu Ala Thr Gly Asn Asp Thr Arg Ala Ile Ser
260 265 270

Ala Ala Ile His Ala Tyr Ala Ala Lys Asp Gly Ala Tyr Gln Gly Leu
275 280 285

Ala Asn Trp Glu Leu Gln Glu Lys Met Leu Val Gly Glu Leu Glu Phe
290 295 300

Pro Leu Pro Val Ala Thr Val Gly Gly Gly Val Lys Val Leu Pro Lys
305 310 315 320

Ala Gln Ala Ala Met Glu Ile Leu Gly Ile Ser Asp Ala Lys Glu Leu
325 330 335

Ala Lys Val Ile Ala Ala Val Gly Leu Ala Gln Asn Leu Ala Ala Leu
340 345 350

Arg Ala Leu Val Ser Glu Gly Ile Gln Gln Gly His Met Ser Leu Gln
355 360 365

Ala Arg Ser Leu Ala Leu Ser Val Gly Ala Gln Ala Asp Glu Ile Ala
370 375 380

Ile Leu Ser Gln Gln Leu Arg Lys Glu Lys Val Met Asn Gln Glu Val
385 390 395 400

Ala Gln Asn Leu Leu Lys Asn Leu Arg Lys
405 410

<210

> 143

<211> 410

<212> PRT

<213> Artificial Sequence

<220><223> Lactococcus lactis subsp. cremoris MG1363

Hydroxymethylglutaryl-CoA reductase

<400> 143

Met Arg Lys Lys Phe Tyr Gln Met Ser Pro Gln Glu Arg Leu Asn Ser

1 5 10 15

Leu Asn Leu Ser Glu Lys Ser Gln Glu Ile Leu Ser Glu Met Ala Leu

20 25 30

Asp Thr Thr Ile Leu Asp Asn Leu Ile Glu Asn Gln Ile Ser Glu Phe

35 40 45

Glu Leu Pro Met Gly Ile Ala Gln Asn Phe Val Ile Asn Gly Gln Ser

50 55 60

Phe Leu Ile Pro Met Val Thr Glu Glu Pro Ser Val Ile Ala Ala Ala

65 70 75 80

Ser Asn Gly Ala Lys Ile Ala Gly Asn Phe Val Ala Glu Ile Lys Glu

85 90 95

Arg Leu Met Arg Gly Gln Ile Val Phe Tyr Asp Val Lys Asn Ser Asp

100 105 110

Lys Ile Ala Asn Glu Ile Leu Glu Lys Gln Glu Lys Ile Phe Glu Gln

115 120 125

Ala Glu Leu Ser Tyr Pro Ser Ile Val Lys Arg Gly Gly Gly Leu Arg

130 135 140

Glu Val Ser Ser Arg Ile Phe Ser Ser Gln Lys Phe Leu Ser Val Asp

145 150 155 160

Val Lys Val Asp Val Lys Asp Ala Met Gly Ala Asn Ile Ile Asn Ser

165 170 175
 Ile Leu Glu Gly Ile Ala Glu Leu Phe Arg Arg Trp Phe Pro Asp Glu
 180 185 190

 Lys Ile Leu Phe Ser Ile Leu Ser Asn Tyr Ala Thr Glu Ser Leu Val
 195 200 205
 Lys Val Thr Cys Glu Ile Pro Val Glu Arg Leu Ser Lys Lys Ala Asp
 210 215 220
 Gly Tyr Glu Ile Gly Gln Lys Ile Met Ala Ala Ser Gln Tyr Ser Lys
 225 230 235 240
 Ile Asp Pro Tyr Arg Ala Ser Thr His Asn Lys Gly Ile Met Asn Gly
 245 250 255
 Ile Asn Ala Val Ile Leu Ala Thr Gly Asn Asp Thr Arg Ala Ile Ser

 260 265 270
 Ala Ala Ile His Ala Tyr Ala Ala Lys Asp Gly Ala Tyr Gln Gly Leu
 275 280 285
 Ala Asn Trp Glu Leu Gln Glu Lys Met Leu Val Gly Glu Leu Glu Phe
 290 295 300
 Pro Leu Pro Val Ala Thr Val Gly Gly Gly Val Lys Val Leu Pro Lys
 305 310 315 320
 Ala Gln Ala Ala Met Glu Ile Leu Gly Ile Ser Asp Ala Lys Glu Leu
 325 330 335

 Ala Lys Val Ile Ala Ala Val Gly Leu Ala Gln Asn Leu Ala Ala Leu
 340 345 350
 Arg Ala Leu Val Ser Glu Gly Ile Gln Gln Gly His Met Ser Leu Gln
 355 360 365
 Ala Arg Ser Leu Ala Leu Ser Val Gly Ala Gln Ala Asp Glu Ile Ala
 370 375 380
 Ile Leu Ser Gln Gln Leu Arg Lys Glu Lys Val Met Asn Gln Glu Val
 385 390 395 400
 Ala Gln Asn Leu Leu Lys Asn Leu Arg Lys

 405 410