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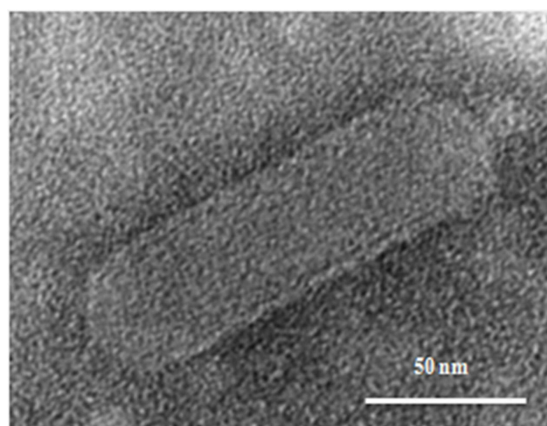
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(54) 발명의 명칭 **식중독을 유발하는 살모넬라에 특이적 사멸능을 갖는 박테리오파지**

(57) 요약

본 발명은 식중독을 유발하는 살모넬라에 특이적 사멸능을 갖는 신규 박테리오파지(bacteriophage)에 관한 것으로, 더욱 구체적으로는 살모넬라균이 서식할 수 있는 곳에서 시료를 채취하여 살모넬라균을 특이적으로 사멸시킬 수 있는 박테리오파지를 분리하고, 분리된 박테리오파지가 형태학적 분류 및 DNA 중합효소의 아미노산 서열상 포도비리테과 피에코32-유사 바이러스속에 속하는 신규한 박테리오파지임을 확인하여 기탁하였으며, 상기 수탁번호 KCTC18211P로 기탁된 박테리오파지 Salmonella phage SP(SalP)가 살모넬라균들만을 선택적으로 감염시켜 사멸시키는 것을 확인하였다. 따라서, 본 발명의 신규 박테리오파지 SalP는 살모넬라균에 의해 유발되는 질환의 예방 또는 치료용 약학적 조성물, 세균 사멸용 조성물, 미생물 제제, 항생제, 소독제, 식품 첨가제, 및 사료 첨가제로 유용하게 사용될 수 있다.

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특허청구의 범위

청구항 1

살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖고, 서열번호 1의 염기서열로 구성되는 유전체를 갖는 포도비리데(Podoviridae)과의 박테리오파지(Bacteriophage) SalP(*Salmonella* phage SP).

청구항 2

제 1항에 있어서, 상기 박테리오파지는 수탁번호 KCTC18211P로 기탁된 것을 특징으로 하는 박테리오파지 SalP.

청구항 3

제 1항에 있어서, 상기 살모넬라균은 살모넬라 본고리(*Salmonella bongori*), 살모넬라 콜레라수이스 아리조나이(*Salmonella choleraesuis arizonae*), 살모넬라 콜레라수이스 하운테나이(*Salmonella choleraesuis hountenae*), 살모넬라 티피뮤리움(*Salmonella typhimurium*) 및 살모넬라 엔테리티디스(*Salmonella enteritidis*)로 이루어진 군으로부터 선택되는 어느 하나인 것을 특징으로 하는 박테리오파지(Bacteriophage) SalP.

청구항 4

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는, 살모넬라균 유발성 질환의 예방 또는 치료용 약학적 조성물.

청구항 5

제 4항에 있어서, 상기 살모넬라균 유발성 질환은 유행성 또는 급성으로 발병하는 전염성 질환으로서, 급성 균혈증(Bacteremia), 가급티푸스, 살모넬라증, 장염, 또는 설사, 복통 및 발열을 동반하는 식중독 질환으로 이루어진 군으로부터 선택되는 어느 하나인 것을 특징으로 하는 살모넬라균 유발성 질환의 예방 또는 치료용 조성물.

청구항 6

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는 항생제.

청구항 7

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는 미생물 제제.

청구항 8

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는 소독제.

청구항 9

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는 항균용 식품 첨가제.

청구항 10

제 1항의 박테리오파지 SalP를 유효성분으로 함유하는 항균용 사료 첨가제.

명세서

기술분야

[0001] 본 발명은 식중독 원인균인 살모넬라에 특이적 사멸능을 가지는 신규 박테리오파지에 관한 것이다.

배경기술

[0002] 살모넬라(*Salmonella* spp.)는 일정한 유사 성상을 가지고 있는 균의 세균류에 붙여진 속명으로서 이 명칭은 1885년에 D. E. Salmon이 그의 공동연구자 Theobald Smith와 돼지 콜레라 바실러스균(*Cholera bacillus*)을 발견한 것을 기념하여 이와 유사한 세균에 대해 일괄적으로 명명한 것이다(1924년). 그 후 1888년 G가 독일에서 발생한 식중독에서 같은 균을 발견하게 되었는데, 이것이 살모넬라 식중독에 대한 최초의 보고가 되었다. 살모넬라는 물이나 토양, 곤충, 공장 표면, 부엌 표면, 동물 분변, 익히지 않은 고기, 가금육, 해산물 등의 환경에 널리 존재하는 흔한 균이다. 특히 가금류와 돼지에 널리 분포하며, 최근 미국에서는 달걀 중의 살모넬라 엔테라티디스(*Salmonella enteritidis*)의 분포에 대한 대대적인 연구가 진행된 바 있다.

[0003] 살모넬라는 식중독의 주요 원인균으로, 국내에서도 매우 빈번하게 발생하고 있으며, 단체 급식의 증가로 그 심각성이 계속 커지고 있다. 2000년 식중독 환자의 원인균을 조사한 보고에 의하면, 2,500건 이상이 살모넬라에 의한 식중독으로 조사되었는데, 이는 포도상구균(약 1,000여건), 비브리오(약 200여건) 등 다른 식중독 원인균에 비교하여서도 월등히 높은 비율을 차지하고 있는 것이다. WHO의 예측에 따르면 매년 살모넬라균에 감염된 사람이 약 1.5억 명에 달하는데, 육류나 야채가 오염된 물과 접촉되었을 때 감염 경로가 가능하다고 여겨져 왔다. 프랑스 연구팀 URGV에서 살모넬라 티피뮤리움(*Salmonella typhimurium*)이 식물 세포에 침입하여 증식한다는 것을 밝혔다. 실제로 2008년 미국의 대부분의 주에서 발생한 살모넬라 세인트폴(*Salmonella saintpaul*)에 의한 식중독의 경우 토마토로부터 감염이 확산된 것으로 알려졌다. 살모넬라에 감염되면 거친 피부, 식용결핍, 결막염, 침울, 옅은 변, 비장 증대, 사망 등의 증상을 보인다. 또한, 살모넬라는 사람이 음식으로 섭취하는 닭 등의 가금류 및 가축의 장내에 존재함으로써 쉽게 사람에게 전달될 수 있다. 미국의 경우 식중독에 의해 발생하는 경제 피해 규모가 20 ~ 40억 달러로 집계되고 있어 식중독 발생 예방은 경제적으로 매우 중요하다고 할 수 있다.

[0004] 항생제(또는 항균제)가 세균 감염에 의한 감염성 질환의 치료에 있어 여전히 주된 방법으로 널리 사용되고 있는 실정이지만, 1980년대 이후 과도한 항생제의 사용으로 더욱 많은 항생제 내성 균주가 발생하고 있으며, 1986년 최후의 항생제로 불리는 반코마이신(Vancomycin)에 내성을 지닌 황색포도상구균 및 다제 내성을 가진 균주가 다수 발견됨으로써 의학계에 큰 충격을 주었다. 반코마이신에 내성을 보이는 반코마이신 내성 장구균(Vancomycin Resistant Enterococci; VRE)은 1986년 프랑스에서 처음 보고되었고, 1988년 미국에서 분리된 이래, 치료가 곤란한 병원감염(nosocomial infections)의 원인균으로, 그 분리 빈도가 해마다 증가하는 추세이다. 국내에서는 1992년에 VRE가 처음으로 분리되었다.

[0005] 따라서 기존 항생제에 내성을 갖는 세균에 의한 질환까지도 치료할 수 있어 국민 건강 증진에 기여할 수 있고, 의학 기술을 선도할 수 있는 기존 항생제와는 근본적으로 다른 방법에 의한 새로운 개념의 항생물질의 개발이 시급한 상황이다.

[0006] 식물바이러스와 동물바이러스 발견 후, 1915년 트윙트가 포도상구균의 용균현상을 관찰하였고, 1917년 파스퇴르 연구소의 헤렐트가 적리균에서도 같은 현상을 관찰하게 되면서 이것이 세균에 감염하는 바이러스(세균바이러스

스)에 의한 것임을 알아내었다. 이후, 세균에 감염하는 바이러스는 "세균(Bacteria)"을 "먹는다(Phage)"의 미에서 박테리오파지(bacteriophage)로 명명하였으며 간단히는 "파지"로 불리고 있다. 파지는 크게 용균성 파지와 용원성 파지로 분류하며, 용균 사이클만 반복하는 것을 용균성(virulent) 파지, 용원 사이클만 반복하는 것을 약독성(temperate) 파지로 정의한다. 약독성 파지는 숙주세균의 유전자에 파지의 유전자정보를 넣어 파지의 모양은 사라지지만 숙주세균이 파괴되지도 않으며, 이런 상태는 숙주세균이 좋은 환경조건에 있는 한 지속되는데, 숙주세균의 환경이 악화되면 숙주세균의 유전자에 있던 일부의 파지 유전자는 용균사이클로 바뀌어 새로운 파지를 만들어 숙주세균을 용균시켜 숙주세포 밖으로 방출된다. 용균 사이클에 있는 파지의 경우, 세균 감염 약 30분 후에 100~200 개의 새로운 파지를 만들어 세균의 막을 파괴하며 밖으로 나와 용균 시키는데 세균의 증식속도보다 파지의 증식속도가 훨씬 높으므로 따라서 세균의 증식을 제한하는 중요한 요인이 될 수 있다. 이러한 파지는 발견된 1910년부터 1930년대에 걸쳐서 파지의 숙주특이성 문제 및 파지의 치료목적으로의 제조에 있어 숙주세균의 내독소, 외독소 문제 등 그 당시 과학적으로 풀지 못하는 문제가 있어 이용에 제한이 있었다. 이즈음 1929년 플레밍이 발견한 페니실린 및 1943년 위크스만이 발견한 스트렙토마이신은 파지와 대조적으로 항균범위가 넓어 하나의 약으로 복수의 감염증에 효과를 나타냈으며 처음에는 내성균 문제도 없었기 때문에 이 항생제를 이용한 화학요법제가 널리 사용되게 되었다. 이에 반해 효과가 불확실한 파지요법은 점점 사라지게 되었으며, 동구권과 구소련연방에서만 명맥이 유지되어 왔다.

[0007] 1980년대에 들어 파지요법에 대한 생각이 바뀌게 되는데, 1982년 영국의 연구자그룹인 스미스와 휴긴스의 뇌막염 치료 모델실험에서 마우스의 피하 근육 내에 균을 주사하고 반대쪽의 피하에는 균의 특이적인 용균파지를 주사한 결과, 파지를 주사하지 않은 대조군은 100% 폐사한 것에 비해 파지를 주사한 실험군의 경우 100% 생존하였으며, 항생제(스트렙토마이신 등)의 효과보다도 우월한 결과를 나타내었다. 특히, 놀라운 것은 균을 직접 뇌 내에 주사한 경우와 파지를 근육주사 하는 경우 모두에서 파지가 뇌에서 회수됨으로써, 근육 주사한 파지가 혈류를 통해 뇌조직으로 이동하여 혈액뇌관문(blood-brain barrier)을 통과한다는 사실을 확인할 수 있었다(Smith HW, Huggins MB, J.Gen.Microbiol, 1982, Feb;128(2); 307-18).

[0008] 그 후 용균성 파지를 이용하여 송아지의 대장균성 설사증, 어류의 다양한 질병, 반코마이신 내성 장구균 패혈증, 결핵균, 닭의 파라티푸스 및 대장균 0157:H7 등 다양한 질병에 대해 성공적인 예방 및 치료사례가 보고되었으며, 최근 항생제에 내성을 가진 세균들의 출현으로 박테리오파지를 사용한 치료법, 즉, 박테리오파지(또는 파지) 요법에 대한 관심이 급부상하고 있다. 박테리오파지 요법은 파지 자체가 세균에 침투하거나 용균시키는 기능이 있기 때문에 항생제의 내성에는 영향을 받지 않아 항생제에 다양한 면역이 생긴 세균에도 효과적이며, 세균이 파지에 내성이 생긴다고 하더라도 서로 공존해 왔기 때문에 파지도 그 내성에 금방 익숙해져 다시 그 세균에 영향을 미칠 수 있어, 그만큼 내성에 대한 반응도 빠르다고 할 수 있다. 또한, 현대 과학의 발달로 파지 요법에 사용될 파지 개발이 항생제 개발에 비해 비용도 적게 들고 시간도 그만큼 단축되어 손쉽게 사용될 수 있으며, 발견된 부작용도 많지 않아 인체에 대한 치료법으로서의 잠재적 가능성을 보이고 있다.

[0009] 이에, 본 발명자들은 인체를 포함한 동물성 살모넬라증(salmonellosis) 중 식중독의 주된 원인균인 살모넬라균(*Salmonella* spp.)을 특이적으로 사멸시킬 수 있는 능력을 갖는 새로운 박테리오파지를 선별하기 위해, 다양한 시료를 채취하여 파지를 분리한 후, 살모넬라균(*Salmonella* spp.)을 특이적으로 사멸시킬 수 있는 파지를 선별하고, 분리 및 선별한 파지 *Salmonella* phage SP를 DNA중합효소 아미노산 서열 분석을 통해 포도비리데(Podoviridae)과에 속하는 신규한 파지임을 확인함으로써 본 발명을 완성하였다.

발명의 내용

해결하려는 과제

[0010] 본 발명의 목적은 살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖고, 서열번호 1의 염기서열로 구성되는 유전체를 갖는 포도비리데(Podoviridae)과의 박테리오파지(Bacteriophage) SalP(*Salmonella* phage SP)(수탁번호 KCTC18211P)를 제공하기 위한 것이다.

[0011] 또한, 본 발명의 또 다른 목적은, 박테리오파지 SalP(수탁번호 KCTC18211P)를 유효성분으로 함유하는, 살모넬라균 유발성 질환의 예방 또는 치료용 약학적 조성물을 제공하기 위한 것이다.

[0012] 또한, 본 발명의 또 다른 목적은, 박테리오파지 SalP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항생제를

제공하기 위한 것이다.

- [0013] 또한, 본 발명의 또 다른 목적은, 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 미생물 제제를 제공하기 위한 것이다.
- [0014] 또한, 본 발명의 또 다른 목적은, 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 소독제를 제공하기 위한 것이다.
- [0015] 또한, 본 발명의 또 다른 목적은, 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 식품 첨가제를 제공하기 위한 것이다.
- [0016] 아울러, 본 발명의 또 다른 목적은, 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 사료 첨가제를 제공하기 위한 것이다.

과제의 해결 수단

- [0017] 상기 목적을 달성하기 위하여, 본 발명은 살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖고, 서열번호 1의 염기서열로 구성된 유전체를 갖는 포도비리데(Podoviridae)과의 박테리오파지(Bacteriophage) SaIP(수탁번호 KCTC18211P)를 제공한다.
- [0018] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는, 살모넬라균 유발성 질환의 예방 또는 치료용 약학적 조성물을 제공한다.
- [0019] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항생제를 제공한다.
- [0020] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 미생물 제제를 제공한다.
- [0021] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 소독제를 제공한다.
- [0022] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 식품 첨가제를 제공한다.
- [0023] 아울러, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 사료 첨가제를 제공한다.

발명의 효과

- [0024] 본 발명의 서열번호 1의 염기서열로 구성된 유전체를 갖는 포도비리데(Podoviridae)과의 신규한 박테리오파지(Bacteriophage) SaIP(수탁번호 KCTC18211P)는 살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖음을 확인함으로써, 살모넬라균에 의해 유발되는 질환의 예방 또는 치료용 약학적 조성물, 항생제, 미생물 제제, 소독제, 항균용 식품 첨가제, 및 항균용 사료 첨가제로 유용하게 사용될 수 있다.

도면의 간단한 설명

- [0025] 도 1은 살모넬라균을 특이적으로 사멸시킬 수 있는 신규한 박테리오파지(Bacteriophage)인 *Salmonella* phage SP(SaIP)를 전자현미경으로 촬영한 사진이다.
- 도 2는 포도비리데(Podoviridae)과 *Salmonella* phage SP의 DNA polymerase의 계통도 분석을 나타낸 그림이다. DNA polymerase 유전자에 따른 인접한 계통도에서 기타 다른 관련된 분류군들 사이에서 *Salmonella* phage SP의 위치를 보여준다(Scale bar, 0.2 substitutions per nucleotide position).

발명을 실시하기 위한 구체적인 내용

- [0026] 이하, 본 발명을 상세히 설명한다.

- [0027] 본 발명은 살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖고, 서열번호 1의 염기서열로 구성된 유전체를 갖는 포도비리데(Podoviridae)과의 박테리오파지(Bacteriophage) SalP(*Salmonella* phage SP)를 제공한다.
- [0028] 상기 SalP는 수탁번호 KCTC18211P로 기탁된 것이 바람직하나, 이에 한정되지 않는다.
- [0029] 상기 살모넬라균은 살모넬라 본고리(*Salmonella bongori*), 살모넬라 콜레라수이스 아리조나이(*Salmonella choleraesuis arizonae*), 살모넬라 콜레라수이스 하운테나이(*Salmonella choleraesuis hountenae*), 살모넬라 티피뮤리움(*Salmonella typhimurium*) 및 살모넬라 엔테리티디스(*Salmonella enteritidis*)로 이루어진 군으로부터 선택되는 어느 하나인 것이 바람직하나 이에 한정되지 않는다.
- [0030] 본 발명의 구체적인 실시예에서, 본 발명자들은 살모넬라균이 서식할 수 있는 곳에서 시료를 채취한 다음, 원심 분리하여 상등액을 회수하고 상기 상등액을 이용하여 용균반 검사를 실시함으로써, 살모넬라균을 특이적으로 사멸시킬 수 있는 박테리오파지를 분리하였다. 또한, 분리한 박테리오파지를 분류하기 위하여 투과전자현미경을 통하여 그 형태를 촬영하였으며, 그 결과, 분리된 박테리오파지가 형태학적 분류상 포도비리데(Podoviridae)과 피에코32-유사 바이러스(Phieco32-like viruses)속에 속하는 것을 확인하였다(도 1 참조). 또한, 상기 분리된 살모넬라균에 특이적으로 감염하는 박테리오파지의 유전적 특성을 분석하기 위해 박테리오파지의 전체 유전체 서열을 밝혔다(서열번호 1 참조). 상기 분리된 박테리오파지의 90,127개의 DNA 서열 중에서 DNA 중합효소의 아미노산 서열을 포도비리데과의 박테리오파지의 DNA 중합효소 아미노산 서열과 비교하였고, 그 결과, 다른 포도비리데과의 박테리오파지들과 차이가 있는 신규한 박테리오파지임을 확인하였으며(도 2 참조), 이렇게 분리한 신규한 박테리오파지를 한국생명공학연구원 생물자원센터에 2011년 5월 12일자로 기탁하였다(수탁번호 KCTC18211P). 아울러, 분리한 살모넬라균에 특이적 사멸능을 갖는 박테리오파지의 숙주에 대한 감염 특이성을 조사하기 위해, 살모넬라균과 분자계통적으로 가장 가까운 10개의 균들을 선택하여 박테리오파지의 용균활성을 조사하였고, 그 결과, 살모넬라균들만을 선택적으로 감염시켜 사멸시키는 것을 확인하였다(표 1 참조).
- [0031] 이를 통해, 본 발명은 살모넬라균(*Salmonella* spp.)에 특이적 사멸능을 갖는 포도비리데(Podoviridae)과의 신규한 박테리오파지(Bacteriophage) SalP(수탁번호 KCTC18211P)를 분리하였다.
- [0032] 또한, 본 발명은 상기의 박테리오파지 SalP(수탁번호 KCTC18211P)를 유효성분으로 함유하는, 살모넬라균 유발성 질환의 예방 또는 치료용 약학적 조성물을 제공한다.
- [0033] 상기 살모넬라균 유발성 질환은 유행성 또는 급성으로 발병하는 전염성 질환으로서, 급성 균혈증(Bacteremia), 가급티푸스, 살모넬라증, 장염, 또는 설사, 복통 및 발열을 동반하는 식중독 질환으로 이루어지는 군으로부터 선택되는 어느 하나인 것이 바람직하나, 이에 한정되지 않는다.
- [0034] 본 발명의 구체적인 실시예에서, 본 발명자들은 본 발명의 박테리오파지를 살모넬라균을 숙주로 함께 배양하여 분리하였으며, 살모넬라균을 특이적으로 감염시켜 사멸시키는지 확인하기 위해 살모넬라균과 분자계통적으로 가장 가까운 10개의 균들을 선택하여 TSA 배지에 도말하고, 이후, 10개의 균들이 배양된 배지에 정제한 박테리오파지를 주입하여 환 모양의 용균반이 생기는 것을 확인하였다. 그 결과, 본 발명의 신규한 박테리오파지 SalP는 살모넬라균만을 선택적으로 사멸시킴을 확인하였다.
- [0035] 따라서 상기 분리된 박테리오파지 SalP(수탁번호 KCTC18211P)는 살모넬라균 유발성 질환의 예방 또는 치료용 약학적 조성물로 유용하게 사용할 수 있다.
- [0036] 본 발명의 조성물의 치료적으로 유효한 양은 여러 요소, 예를 들면 투여방법, 목적부위, 환자의 상태 등에 따라 달라질 수 있다. 따라서, 인체에 사용 시 투여량은 안전성 및 효율성을 함께 고려하여 적정량으로 결정되어야 한다. 동물실험을 통해 결정된 유효량으로부터 인간에 사용되는 양을 추정하는 것도 가능하다. 유효한 양의 결정시 고려할 이러한 사항은, 예를 들면 Hardman and Limbird, eds., Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed.(2001), Pergamon Press; 및 E.W. Martin ed., Remington's Pharmaceutical Sciences, 18th ed.(1990), Mack Publishing Co.에 기술되어있다.
- [0037] 본 발명의 조성물은 또한 생물학적 제제에 통상적으로 사용되는 담체, 희석제, 부형제 또는 둘 이상의 이들의

조합을 포함할 수 있다. 약제학적으로 허용 가능한 담체는 조성물을 생체 내 전달에 적합한 것이면 특별히 제한되지 않으며, 예를 들면, Merck Index, 13th ed., Merck & Co. Inc. 에 기재된 화합물, 식염수, 멸균수, 링거액, 완충 식염수, 텍스트로스 용액, 말토 텍스트린 용액, 글리세롤, 에탄올 및 이들 성분 중 1 성분 이상을 혼합하여 이용할 수 있으며, 필요에 따라 항산화제, 완충액, 정균제 등 다른 통상의 첨가제를 첨가할 수 있다. 또한, 희석제, 분산제, 계면활성제, 결합제 및 윤활제를 부가적으로 첨가하여 수용액, 현탁액, 유탁액 등과 같은 주이용 제형, 환약, 캡슐, 과립 또는 정제로 제제화할 수 있다. 더 나아가 당 분야의 적절한 방법으로 또는 Remington's Pharmaceutical Science(Mack Publishing Company, Easton PA, 18th, 1990)에 개시되어 있는 방법을 이용하여 각 질환에 따라 또는 성분에 따라 바람직하게 제제화할 수 있다.

[0038] 본 발명의 조성물에 추가로 동일 또는 유사한 기능을 나타내는 유효성분을 1종 이상 함유할 수 있다. 본 발명의 조성물은, 조성물 총 중량에 대하여 상기 파지를 0.0001 내지 10 중량 %로, 바람직하게는 0.001 내지 1 중량 %를 포함한다.

[0039] 본 발명의 조성물은 목적하는 방법에 따라 질환 부위의 도포 또는 분무하는 방법으로 이용할 수 있으며, 그 밖에 비 경구 투여(예를 들어 정맥 내, 피하, 복강 내 또는 국소에 적용)하거나 경구 투여할 수 있다. 본 발명의 약학적 조성물의 적합한 도포, 분무, 및 투여량은 환자의 체중, 연령, 성별, 건강상태, 식이, 제제화 방법, 투여시간, 투여방법, 배설물 및 질환의 중증도 등에 따라 그 범위가 다양하며, 보통으로 숙련된 의사는 소망하는 치료에 효과적인 투여량을 용이하게 결정 및 처방할 수 있다. 본 발명에 따른 약학적 조성물은 일반적으로, 1×10^3 내지 1×10^{10} pfu/ml의 박테리오파지를 함유하며, 하루 일 회 내지 수회에 나누어 투여하는 것이 더욱 바람직하다.

[0040] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 세균 사멸용 조성물을 제공한다.

[0041] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 미생물 제제를 제공한다.

[0042] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항생제를 제공한다.

[0043] 또한, 본 발명은 박테리오파지 SaIP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 세균 사멸용 소독제를 제공한다.

[0044] 상기 세균은 살모넬라균인 것을 특징으로 하는 세균 사멸용 조성물을 제공한다.

[0045] 상기 살모넬라균은 살모넬라 본고리(*Salmonella bongori*), 살모넬라 콜레라수이스 아리조나이(*Salmonella choleraesuis arizonae*), 살모넬라 콜레라수이스 하운테나이(*Salmonella choleraesuis hountenae*), 살모넬라 티피뮤리움(*Salmonella typhimurium*) 및 살모넬라 엔테리티디스(*Salmonella enteritidis*)로 이루어진 군으로부터 선택되는 어느 하나인 것이 바람직하나 이에 한정되지 않는다.

[0046] 본 발명의 구체적인 실시예에서, 본 발명자들은 본 발명의 박테리오파지를 살모넬라균을 숙주로 함께 배양하여 분리하였으며, 살모넬라균을 특이적으로 감염시켜 사멸시키는지 확인하기 위해 살모넬라균과 분자계통적으로 가장 가까운 10개의 균들을 선택하여 TSA 배지에 도말하고, 이후, 10개의 균들이 배양된 배지에 정제한 박테리오파지를 주입하여 환 모양의 용균반이 생기는 것을 확인하였다. 그 결과, 본 발명의 신규한 박테리오파지 SaIP는 살모넬라균만을 선택적으로 사멸시킴을 확인하였다.

[0047] 따라서 상기 분리된 박테리오파지 SaIP(수탁번호 KCTC18211P)는 살모넬라균 세균 사멸용 조성물, 세균 사멸용 미생물제제, 항생제 및 소독제로 사용할 수 있다.

[0048] 본 발명의 세균 사멸용 조성물, 세균 사멸용 미생물제제, 항생제 및 소독제는 통상적인 세균 사멸용 조성물, 세

균 사멸용 미생물제제, 및 소독제 제조방법으로 제조될 수 있으며, 유효성분인 본 발명의 박테리오파지 또는 이의 배양물 이외 필요에 따라, 담체, 기타 보조제 등을 추가적으로 포함할 수 있는데, 상기 담체는 생리식염수, 증류수, 사료, 박테리아용 배지 등일 수 있으며, 보조제는 pH 교정 등의 목적으로 완충액 등일 수 있으나 이에 한정되는 것은 아니다. 또한, 본 발명의 세균 사멸용 조성물, 세균 사멸용 미생물제제, 및 소독제는 액상, 반고체상 또는 고체상으로 제제 형태는 분말제, 과립제, 정제 등의 고체상 제제, 현탁액 또는 주사액제 등의 액상 제제 및 크림, 로션, 젤, 페이스트 등의 반고체상 제제 등으로 제형화할 수 있다.

[0049] 또한, 본 발명은 박테리오파지 SalP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 식품 첨가제를 제공한다.

[0050] 아울러, 본 발명은 박테리오파지 SalP(수탁번호 KCTC18211P)를 유효성분으로 함유하는 항균용 사료 첨가제를 제공한다.

[0051] 본 발명의 구체적인 실시예에서, 본 발명자들은 본 발명의 박테리오파지를 살모넬라균을 숙주로 함께 배양하여 분리하였으며, 살모넬라균을 특이적으로 감염시켜 사멸시키는지 확인하기 위해 살모넬라균과 분자계통적으로 가장 가까운 10개의 균들을 선택하여 TSA 배지에 도말하고, 이후, 10개의 균들이 배양된 배지에 정제한 박테리오파지를 주입하여 환 모양의 용균반이 생기는 것을 확인하였다. 그 결과, 본 발명의 신규한 박테리오파지 SalP는 살모넬라균만을 선택적으로 사멸시킴을 확인하였다.

[0052] 따라서 상기 분리된 박테리오파지 SalP(수탁번호 KCTC18211P)는 식품 첨가제 및 사료 첨가제로 사용할 수 있다.

[0053] 상기 식품 첨가제가 사용될 수 있는 식품에는 제한이 없으며, 상기 식품 첨가제는 살모넬라균에 감염되기 쉬운 식품에 당업계에 공지된 방법에 따라 첨가제의 형태로 제조될 수 있다.

[0054] 상기 사료 첨가제는 가축용 및 어류용 사료에 첨가제의 형태로 제조될 수 있으며, 살모넬라균의 감염 용이성, 감염 정도에 따라 달리 정할 수 있다.

[0055] 이하, 본 발명을 실시예, 실험예, 및 제조예에 의해 상세히 설명한다.

[0056] 단, 하기 실시예, 실험예, 및 제조예는 본 발명을 예시하는 것일 뿐, 본 발명의 내용이 하기 실시예, 실험예, 및 제조예에 한정되는 것은 아니다.

[0057] <실시예 1> 살모넬라균을 특이적으로 사멸시킬 수 있는 박테리오파지의 분리

[0058] 본 발명자들은 살모넬라균(*Salmonella* spp.)에 특이적으로 감염하여 선택적으로 살모넬라균을 사멸시킬 수 있는 박테리오파지(Bacteriophage)를 분리하기 위하여, 채집하여 얻은 시료에서 박테리오파지를 분리하였다.

[0059] 구체적으로, 박테리오파지가 존재할 것이라 예상되는 메뚜기, 노린재, 잠자리, 초파리, 딱정벌레 5종을 채집하여 믹서기로 갈아서 섞은 다음 8,000 rpm에서 20분간 원심분리하여 상등액을 회수하였다. 이 회수된 상등액을 0.22 μ m의 필터를 이용하여 여과를 하였고, 얻어진 여과액을 숙주인 살모넬라균과 함께 30 °C에서 2일 동안 박테리아 배양용 아가(agar) 배지인 TSA(Tryptic Soy Agar, BD) 배지에서 배양한 후, 8,000 rpm에서 20분간 원심분리하여 상등액을 회수하였다. 이 회수한 상등액을 0.22 μ m의 필터를 이용하여 여과하여 살모넬라균에 특이적으로 감염하는 박테리오파지를 분리하였다. 또한, 이렇게 얻어진 분리된 박테리오파지에 의한 살모넬라균의 사멸 여부를 용균반 검사(plaque assay)로 확인하였다.

[0060] <실시예 2> 살모넬라균을 특이적으로 사멸시킬 수 있는 박테리오파지의 동정

[0061] <2-1> 박테리오파지의 형태학적 분류

[0062] 본 발명자들은 상기 <실시예 1>에서 분리한 살모넬라균을 특이적으로 사멸시키는 박테리오파지의 형태학적 분류를 위하여 투과전자현미경으로 그 형태를 촬영하였다.

[0063] 구체적으로, 상기 분리한 살모넬라균에 특이적으로 감염하여 사멸시킬 수 있는 박테리오파지의 형태 및 특성화를 관찰하기 위해 염화세슘(CsCl) 밀도 구배법(밀도: 1.15 g/ml, 1.45 g/ml, 1.50 g/ml 및 1.70 g/ml)을 이용한 원심분리(20,000 rpm, 2시간, 4 ℃)를 통하여 박테리오파지를 정제하였다. 이렇게 정제한 박테리오파지는 형태를 관찰하기 위해 박테리오파지를 구리 격자(copper grid)에 묻히고, 2% 유라닐 아세테이트(uranyl acetate)로 negative staining과 건조를 수행한 후, 투과전자현미경을 통하여 그 형태를 촬영하였다.

[0064] 그 결과 분리된 박테리오파지가 형태학적 분류상 포도비리대(Podoviridae)과 PhiEco32-like viruses속에 속하는 것을 확인할 수 있었다(도 1).

[0065] <2-2> 살모넬라균에 특이적인 분리된 박테리오파지의 유전적 특성 분석

[0066] 본 발명자들은 상기 <실시예 1>에서 분리한 살모넬라균에 특이적으로 감염하여 사멸시키는 박테리오파지의 계통학적 위치를 확인하기 위하여 박테리오파지의 유전적 특성을 분석하였다.

[0067] 구체적으로, 박테리오파지를 박테리아 배양용 아가(agar) 배지인 TSA(Tryptic Soy Agar) 배지에서 대량 배양하고 DNA를 분리하여(Sambrook, J. & Russell, D. W. (2001). Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, New York.), 상기 분리된 신규한 박테리오파지의 90,127개의 전체 유전체 서열(서열 번호 1)을 밝혔다. 이 중, DNA 중합 효소 단백질의 DNA 서열(서열 번호 2)을 포도비리대(podoviridae)과의 다른 박테리오파지의 DNA 중합 효소 단백질의 DNA 서열(서열 번호 3)과 비교하여, 분자 계통도를 작성하였다(도 2).

[0068] 그 결과, 본 발명의 박테리오파지는 다른 박테리오파지와 DNA 중합 효소 단백질의 DNA 서열에서 차이가 있음을 확인하였고, 분자 계통학적으로 가장 가까운 엔테로파지 피에코32(Enterobacteria phage phiEco32)의 Type 1 DNA 중합효소의 DNA 서열과 49% 상동성을 가지는 것에 불과함을 확인하여, 상기 분리된 본 발명의 박테리오파지가 신규한 것임을 확인하였다.

[0069] 아울러, 이렇게 확인된 본 발명의 신규한 박테리오파지를 Salmonella phage SP(SalP)라 명명하였다.

[0070] <실험예 1> Salmonella phage SP의 살모넬라속 균주에 대한 특이적 사멸 효과 확인

[0071] 본 발명자들은 상기 <실시예 1>에서 분리한 박테리오파지의 다양한 숙주에 대한 감염성을 용균반 검사(plaque assay)로 조사하였다.

[0072] 구체적으로, 분리된 박테리오파지 Salmonella phage SP의 다양한 숙주에 대한 감염성에 대해 조사하기 위하여, 살모넬라균과 분자계통적으로 가장 가까운 Escherichia coli(KCTC2293), Escherichia coli(KCTC2441), Escherichia coli(KCTC2790), Shigella sonnei(KCTC2009), Shigella sonnei(KCTC2518), Salmonella bongori(DSM13772), Salmonella choleraesuis arizonae(DSM9386), Salmonella choleraesuis hountenae(DSM9221), Salmonella typhimurium(ATCC13311), 및 Salmonella enteritidis(ATCC13076)의 10개의 균들을 선택하여 이들을 TSA(trytic soy agar) 배지에 도말하였다. 10개의 숙주 균주가 도말된 TSA 배지에 페이퍼 디스크(paper disc)를 가운데 놓은 후, 상기 <실시예 2-1>에서 정제한 본 발명의 박테리오파지 Salmonella phage SP 5 ul를 디스크 위에 접종하였다. 상기 접종한 배지를 30 ℃에서 24시간 동안 배양한 후, 디스크 주변으로 환이 생기면 박테리오파지에 대한 용균(lysis) 활성이 있는 것으로 나타내었다(표 1).

표 1

[0073]

No.	Culture collection number*	Species	Lysis by Salmonella phage SP
1	KCTC2293	Escherichia coli	-
2	KCTC2441	Escherichia coli	-
3	KCTC2790	Escherichia coli	-

4	KCTC2009	<i>higella sonnei</i>	-
5	KCTC2518	<i>higella sonnei</i>	-
6	DSM 13772	<i>almonella bongori</i>	+
7	DSM 9386	<i>almonella choleraesuis arizonae</i>	+
8	DSM 9221	<i>almonella choleraesuis hountenae</i>	+
9	ATCC 13311	<i>almonella typhimurium</i>	+
10	ATCC 13076	<i>almonella enteritidis</i>	+

[0074] Lysis patterns of phage, + plaques; - no plaques

[0075] * KCTC: the Korean Collection for Type Cultures

[0076] DSM: the German Collection of Microorganisms and Cell Cultures

[0077] ATCC: the American Type Culture Collection

[0078] 그 결과, 본 발명에서 분리한 박테리오파지 *Salmonella* phage SP가 살모넬라균을 특이적으로 사멸시킴을 확인할 수 있었다.

[0079] <제조예 1> 약학적 제제의 제조

[0080] <1-1> 산제의 제조

[0081] 박테리오파지 *Salmonella* phage SP 2 g

[0082] 유당 1 g

[0083] 상기의 성분을 혼합하고 기밀포에 충전하여 산제를 제조하였다.

[0084] <1-2> 정제의 제조

[0085] 박테리오파지 *Salmonella* phage SP 100 mg

[0086] 옥수수전분 100 mg

[0087] 유 당 100 mg

[0088] 스테아린산 마그네 2 mg

[0089] 상기의 성분을 혼합한 후, 통상의 정제의 제조방법에 따라서 타정하여 정제를 제조하였다.

[0090] <1-3> 캡슐제의 제조

[0091] 박테리오파지 *Salmonella* phage SP 100 mg

[0092] 옥수수전분 100 mg

[0093] 유 당 100 mg

[0094] 스테아린산 마그네슘 2 mg

[0095] 상기의 성분을 혼합한 후, 통상의 캡슐제의 제조방법에 따라서 젤라틴 캡슐에 충전하여 캡슐제를 제조하였다.

[0096] <1-4> 환의 제조

[0097] 박테리오파지 *Salmonella* phage SP 1 g

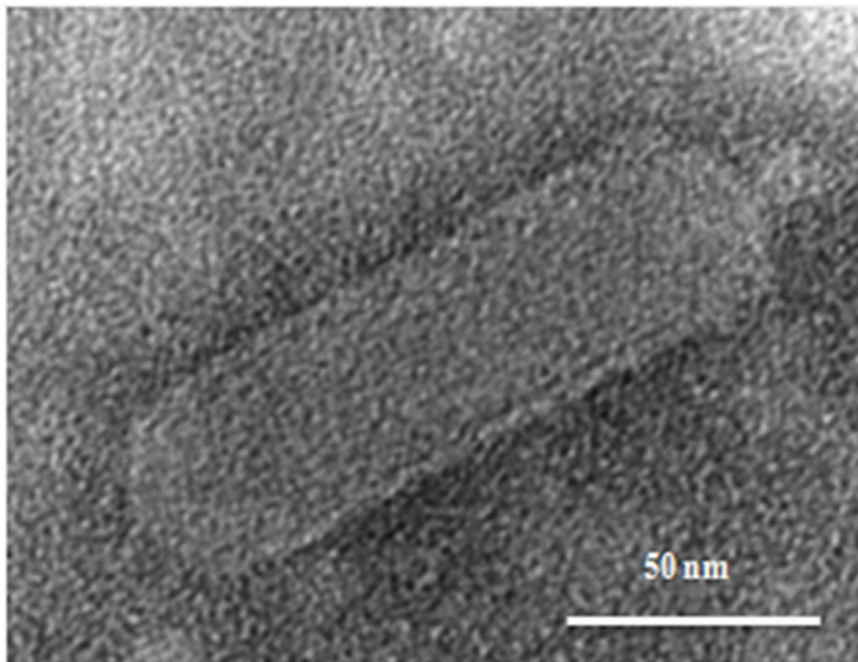
[0098]	유당	1.5 g
[0099]	글리세린	1 g
[0100]	자일리톨	0.5 g
[0101]	상기의 성분을 혼합한 후, 통상의 방법에 따라 1환 당 4 g이 되도록 제조하였다.	
[0102]	<1-5> 과립의 제조	
[0103]	박테리오파지 Salmonella phage SP	150 mg
[0104]	대두추출물	50 mg
[0105]	포도당	200 mg
[0106]	전분	600 mg
[0107]	상기의 성분을 혼합한 후, 30 % 에탄올 100 mg을 첨가하여 섞씨 60 ℃에서 건조하여 과립을 형성한 후 포에 충전 하였다.	
[0108]	<1-6> 주사제의 제조	
[0109]	활성성분으로서 박테리오파지를 100 mg을 함유시키고, 그 밖에도 만니톨 180 mg, Na ₂ HPO ₄ ·12H ₂ O 26 mg 및 증류수 2974 mg를 함유시켜 주사제를 제조하였다.	

수탁번호

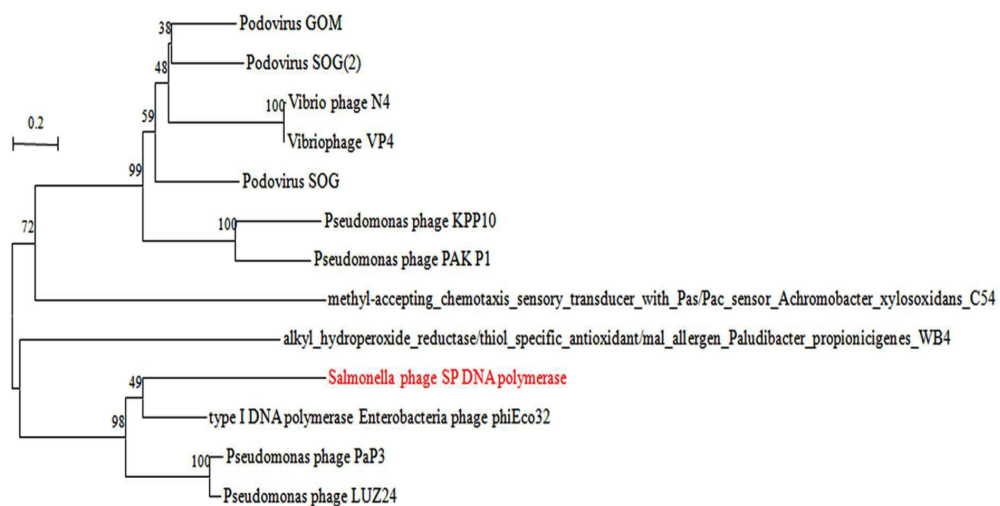
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	수탁번호 : KCTC18211
	수탁일자 : 20110512

도면

도면1



도면2



서열 목록

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- <120> Bacteriophage having bacteriocidal effect on Salmonella spp., the cause of sitotoxism
- <130> 11P-05-15
- <160> 3
- <170> Kopatent In 1.71

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