

Research Article



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Pseudomonas sp. EP-3에 의한 진딧물 살충성 rhamnolipid 생산을 위한 유자씨앗 부산물의 재활용

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¹ , ²LG ()

Reuse of Yuza Seed By-product for Production of Aphicidal Rhamnolipid by *Pseudomonas* sp. EP-3

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Abstract

BACKGROUND: Yuza seed by-product has been produced in a large amount from the agricultural farms in the southern area of Korea. It has been mostly abandoned after commercial process for the production of juice, jam and tea. The study on the reuse of the yuza seed by-product has received much attention as a bio-resource material for the production of active compound in agriculture.

METHODS AND RESULTS: Insecticidal rhamnolipid-producing *Pseudomonas* sp. EP-3 was grown in mineral salt media with the yuza seed by-product at 2, 20, 50 and 100 g/L. The growth of EP-3 was accompanied by an increase in insecticidal activity against green peach aphid. The highest insecticidal activity was observed when EP-3 was grown in the medium containing 50 g/L of the seed sample, producing approximately 996 mg/L of rhamnolipid at 96 h. Palmitic acid, stearic acid, oleic acid and linoleic acid were determined as the major fatty acids of the seed sample. The EP-3 cultures grown on the fatty acid mixture extracted

from the seed sample showed an aphid mortality similar to that of cultures grown on the seed sample. The EP-3 cultures grown on 50 g/L of the seed sample showed aphid mortality more than 90% under greenhouse conditions.

CONCLUSION: This study suggested that the yuza seed by-product may be used as a renewable material for microbial production of rhamnolipid against green peach aphid.

Key words: Aphid, Biopesticide, Biosurfactant, Rhamnolipid, Yuza

서론

(*Citrus junos*) 13,000~15,000 M/T
가
가
10%
(, 2013)
가

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가
가 가

가 (Grohmann and Baldwin, 1994; Gema *et al.*, 2002).

(Bouallagui *et al.*, 2005; George and Jayachadran, 2009; Jeong *et al.*, 2009).

(Choi *et al.*, 1984; Seo *et al.*, 2005).

가 (Silva *et al.*, 2012)
가
(Wilson and Otsuki, 2004; Carvalho, 2006).

가
(Isman, 2005; Goettel *et al.*, 2008).

가
(Nga and Kumar, 2008; Pardo-López *et al.*, 2012).

가
가

가

(*Myzus persicae*)

재료 및 방법

시약 및 용매

HPLC (Fisher Scientific, MA, USA)
EP (Juicei Chemical Co., Tokyo, Japan)

HPLC (Fisher Scientific, MA, USA)
EP (Juicei Chemical Co., Tokyo, Japan)

(>95%) Sigma-Aldrich (St. Louis, MO, USA)

유자씨앗 부산물

가

50°C 24

공시균주

rhamnolipid

Pseudomonas sp. EP-3 (Kim *et al.*, 2011) 2, 20, 50, 100 g/L 50 g/L glucose

Na₂HPO₄ 5.7 g/L, KH₂PO₄ 5.4 g/L, NaNO₃ 4.0 g/L, CaCl₂ 1.0 g/L EP-3

Luria Broth (LB)

25°C 24

살충물질의 추출 및 함량조사

Pseudomonas sp. EP-3 (EP-3)가 rhamnolipid
Kim (2011)

LC/MS/MS

(100 mL) 8,000 rpm 20

ethyl acetate 가 2

50°C

silica gel

SPE (Phenomenex, CA, USA) column

50% chloroform 6 mL ethyl acetate

SPE column 가 ethyl acetate

10% 가 6 mL

column 50% ethyl

acetate 6 mL methanol 가

40 °C methanol

HPLC MS/MS

MS/MS API 4000 triple quadrupole mass spectrometer (Applied Biosystems) HPLC Shimadzu LD 20AD

Phenomenex C18 stainless steel column (100×4.60 mm, 2.6 μm)

0.1% formic acid 5 mM ammonium formate가 가
acetonitrile (7:3, v/v) 0.3

mL/min rhamnolipid

MS/MS rhamnolipid (daughter ion) *m/z* 315.1(M+Na)⁺ dirhamnose

rhamnolipid (Pereira *et al.*, 2012). , rhamnolipid

(TLC,

Silica gel 60 F₂₅₄, 20×20 cm, Merck) . TLC
 chloroform : methanol : formic acid (80 : 20
 : 0.1, v/v/v) 10%
 100°C rhamnolipid

진딧물 살충효능 시험

Kim (2007)
 2 (10) 3
 (3.0×3.0 cm)
 (Whatman No. 6)가
 25°C 70%
 12
 EP-3
 Rhamnolipid
 10% (v/v) methanol
 (v/v) methanol 10%

유자씨앗의 성분 분석

EP-3가
 Kjeldahl
 Soxhlet
 105°C 가 , 600°C
 100 g
 (2011).

5 g 100 mL chloroform : methanol (2 :
 1,v/v) 2
 200 mL
 chloroform : methanol : 0.9% NaCl (3 : 50 : 47, v/v)
 chloroform
 (50 mg)
 5 mL NaOH : methanol : H₂O (3 : 10 : 10, v/v/v)
 80°C 25
 30%
 (v/v) 6N HCl 10 mL methanol 가
 80°C 1
 hexane : methyl
tert-butyl ether (1 : 1, v/v)
 hexane
 (Kim *et al.*, 2011).

Shimadzu QP2010 (Tohyo, Japan)
 DB5-MS (30 m×0.25 mm i.d., 0.25 μm film
 thickness) . 1 mL/min
 split mode 20:1
 50°C 2 5°C
 280°C 280°C 10
 70 eV electronic ionization
 mode

유자씨앗 지방추출물을 이용한 EP-3의 배양

EP-3가 rhamnolipid
 TLC
시설재배 조건에서 진딧물 살충효능 시험
 가 EP-3
 가
 50
 EP-3
 24
 5
 EP-3

통계처리

IBM SPSS
 statistics 21.0 software
 ±
P<0.05 Duncan’s multiple range test

결과 및 고찰

EP-3의 생장과 살충물질 생산

EP-3
 18 가
 (Fig. 1). 가
 가 50 g/L 가 glucose
 가
 glucose EP-3 가
 50 g/L 가
 50 g/L
 가 가
 EP-3

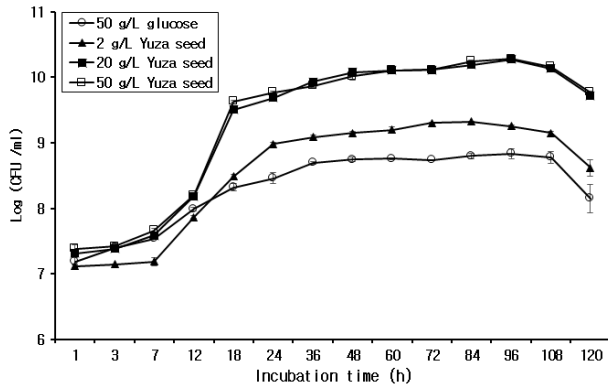


Fig. 1. Growth of *Pseudomonas* sp. EP-3 on the yuza seed by-product or glucose.

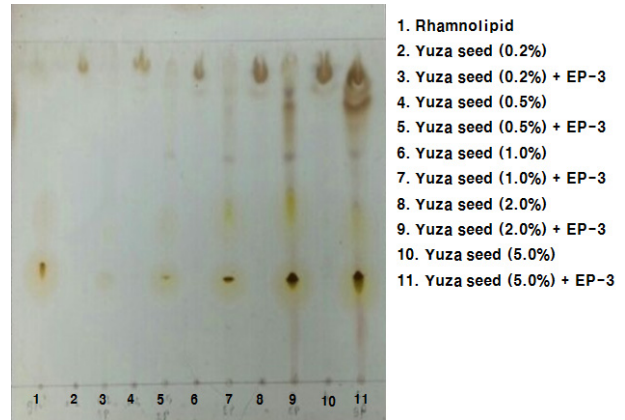


Fig. 3. Thin layer chromatograms of the extracts of yuza seed by-product or cultures grown on yuza seed by-products.

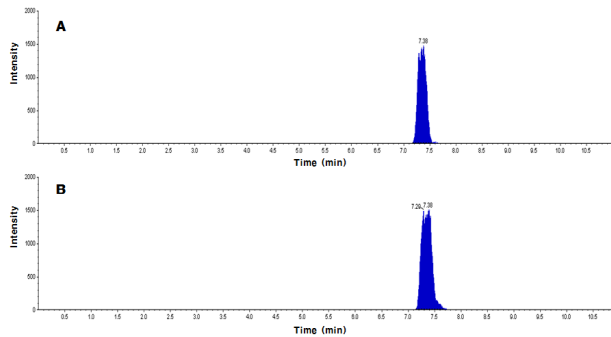


Fig. 2. LC/MS/MS total ion chromatograms of dirhamnose from the extracts of cultures grown on 5% (w/v) glucose (A) and 5% (w/v) yuza seed by-product. The total ion chromatograms were obtained from the fragmented ion at $(M+Na)^+ m/z$ 315.1 generated from rhamnolipid.

Table 1. Rhamnolipid concentration in *Pseudomonas* sp. EP-3 cultures grown on glucose or the yuza seed by-product

Medium (50 g/L)	Rhamnolipid (mg/L)*
Yuza seed	996.0±40.7
Glucose	123.5±4.3

*The data are means ± SD of triplicate.

50 g/L
EP-3가
MS TLC
Fig. 2 3
50 g/L
glucose
8
rhamnolipid
LC/MS/
EP-3
rhamnolipid
996 mg/L
124 mg/L
EP-3
(Table 1).

10
~81.82%
(Table 2).
50 g/L
glucose
EP-3
rhamnolipid
13.64
2.7
rhamnolipid
14.9%,
27.5%,
(Lee *et al.*, 1987)
52.4%
48

Table 2. Aphid mortality of *Pseudomonas* sp. EP-3 cultures grown on glucose or the yuza seed by-product

Medium	Aphid mortality (%) [*]		
	Without cultures	With cultures	
		Not diluted	10X diluted
Yuza seed (2 g/L)	4.6±13.6f	61.4±7.9c	13.6±3.9ef
Yuza seed (20 g/L)	22.7±10.4de	100.0±0.0a	59.2±6.8c
Yuza seed (50 g/L)	13.6±10.4ef	100.0±0.0a	81.8±7.9b
Glucose (50 g/L)	3.6±11.2f	100.0±0.0a	29.6±10.4d

*The data are means±SD of triplicate. Means with different superscripts within the column are significantly different at $P<0.05$ by Duncan's multiple range test.

Table 3. Aphid mortality of *Pseudomonas* sp. EP-3 cultures grown on the yuza seed by-product at 50 g/L or the yuza seed lipid at 1.2 g/L

Fraction of culture	Aphid mortality (%)*	
	Yuza seed	Yuza seed lipid
1/8	100.0±0.0a	93.0±0.0a
1/24	86.1±14.0a	55.8±10.7b
1/72	51.2±7.0b	48.8±21.3bc

*The data are means±SD of triplicate. Means with different superscripts within a column are significantly different at P<0.05 by Duncan's multiple range test.

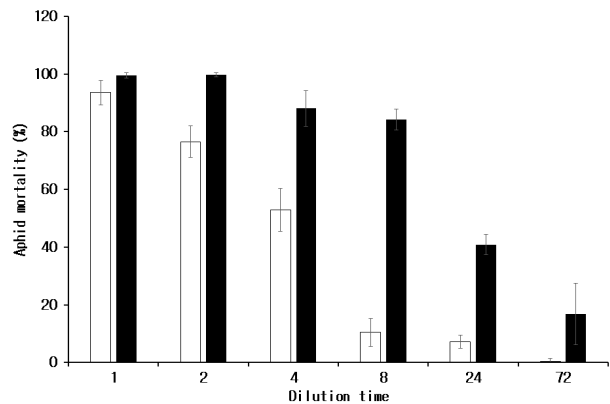


Fig. 4. Aphid mortality under greenhouse conditions of *Pseudomonas* sp. EP-3 cultures grown on glucose () or the yuza seed by-product () at 50 g/L.

Fig. 1

EP-3

EP-3

EP-3가

rhamnolipid (Kim *et al.*, 2011)

EP-3가

palmitic acid, stearic acid, oleic acid, linoleic acid

21.2%, 1.9%, 29.3%, 47.6%

EP-3

50 g/L

가

Table 3

8, 24, 72

100%, 86%, 51%

8, 24, 72

93%, 55%, 48%

Rhamnolipid

(Ron and Rosenberg, 2002; Thavasi *et al.*, 2011).

(Abouseoud *et al.*, 2008; George and Jayachandran, 2012).

EP-3 ()

rhamnolipid

시설재배 조건에서 EP-3 배양액의 진딧물 살충효능 시험

EP-3 50 g/L glucose 50 g/L

24

Fig. 4

80%

glucose 7

Glucose 80%

2

가 EP-3

rhamnolipid

가

(Chayabutra *et al.*, 2001; Mata-Sanddoval *et al.*, 2001; Deepika *et al.*, 2016).

EP-3 가

rhamnolipid

가

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