# Efficacy of Syringomycin E in a Murine Model of Vaginal Candidiasis

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(Received for publication April 6, 1998)

Syringomycin E (SR-E), a new antifungal produced by the bacterium *Pseudomonas syringae* pv. *syringae*, was evaluated in a murine vaginal candidiasis model. In one study, mice were treated intravaginally b.i.d. for 4 days with drug carrier, SR-E 2% in either PEG-400 or PEG-ointment, or 1% clotrimazole as a positive control. Quantitative vaginal cultures were taken prior to treatment on day 1 and on days 5, 6, and 7. Both formulations showed a reduction of yeast colonization in the vaginas on day 5 ($P \leq 0.06$ and $P \leq 0.03$ for SR-E/PEG-400 and SR-E/PEG ointment, respectively) and SR-E/PEG ointment reduced the colonization on day 7 ($P \leq 0.06$) when compared to carrier treated controls. In a second study, SR-E was formulated in Aquaphor at three higher concentrations of SR-E [3%, 6%, or 12% (w/v)]. SR-E showed dose-dependent efficacy. The 3% dose showed no effect while the 6% and 12% doses reduced the number of yeasts. The 12% dose showed a significant reduction on days 5 ($P \leq 0.01$), 6 ($P \leq 0.06$), and 7 ($P \leq 0.03$) when compared with the drug carrier controls and on day 5 was more effective than clotrimazole ($P \leq 0.03$). Clotrimazole did not significantly reduce the yeasts in the vagina until days 6 ($P \leq 0.01$) and 7 ($P \leq 0.01$) when compared to the drug carrier controls. No vaginal inflammatory response was evident by histological examination in uninfected animals treated with SR-E. No SR-E could be detected in plasma, kidney, or liver. SR-E (12%) was an effective treatment when compared to 1% clotrimazole.

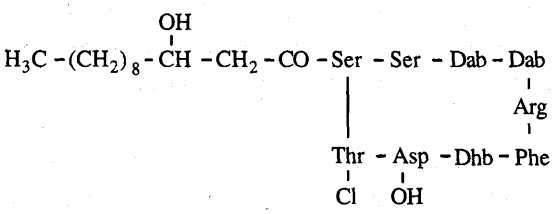
Syringomycin E (SR-E) is a cyclic lipodepsinonapeptide (CLP) produced by the plant bacterium *Pseudomonas syringae* pv. *syringae*. SR-E is a nonapeptide moiety (Fig. 1) with an unusual *C*-terminal sequence dehydroaminobutanoic acid-Asp(3-OH)-Thr(4-Cl) and an *N*-terminal Ser *N*-acylated by a long-chain unbranched 3-hydroxy fatty acid and *O*-acylated by the *C*-terminal carboxyl to form a macrolactone ring.

SR-E has *in vitro* fungicidal activities against a broad spectrum of pathogenic fungi[1]. Its target is the fungal plasma membrane. SR-E alters several membrane functions such as membrane potential, protein phosphorylation, $H^+$-ATPase activity, and cation-transport fluxes[2~6]. These effects are likely related to channel formation in model membranes[7,8]. Recent molecular genetic studies with *Saccharomyces cerevisiae* indicate that lipids are involved in the action of SR-E[9,10].

This paper describes studies that evaluate the efficacy of SR-E in an experimentally induced vaginal candidiasis in mice by means of a simple and non-lethal model[11~14]. In one study, SR-E at 2% was formulated in two PEG-based carriers, and in another study, SR-E was formulated in an Aquaphor-based carrier at three con-

Fig. 1. Structure of syringomycin-E.

Dab, diaminobutanoic acid; Dhb, dehydroaminobutanoic acid.

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centrations (3%, 6%, and 12%).

## Materials and Methods

### Drug Vehicle and Compound Preparation

In study I, SR-E was suspended in acidified (0.0001 N HCl) solutions of PEG 400 or PEG ointment (PEG 400/3350 at a 3:2 ratio, respectively) at 65°C. In study II, an ointment was made by mixing Aquaphor original formula (58 to 70%, w/v; Beiersdorf, Inc., Norwalk, CT), glycerin (15%, v/v), double distilled water (15%, v/v), 2-[*N*-morpholino]ethanesulfonic acid (MES, 5 mM; pH 6.0) and SR-E (0, 3, 6, or 12%, w/v). The Aquaphor concentration was decreased as the SR-E concentration was increased. Each formulation was mixed by warming the measured amount of Aquaphor in a water bath at 65°C. The other ingredients were added and immediately mixed. Each SR-E formulation was administered intravaginally using a 20 gauge ball-ended dosing needle.

Clotrimazole 1% (Gyne-Lotrimin; Schering-Plough Health Care Products, Inc., Memphis, TN), which is a commercially available cream, was used as a positive control in both studies. All compounds were stored at 4°C in the dark.

Activities of carrier (0% SR-E), all SR-E-formulations, and clotrimazole were verified before treatment (day 0) and after treatment (day 5). This was done by placing 20 $\mu$l of each drug formulation on blank sensitivity disks and placing them on the surface of Sabouraud dextrose agar (SDA) plates overlaid with a lawn of *Candida albicans* SC-9172. The compounds were applied at room temperature on day 0 and at 4°C post-treatment on day 5. The plates were incubated for 24 hours at 35°C. After incubation, zones of inhibition for each formulation and clotrimazole were determined.

### Animals

Twenty-four to twenty-five gram unaltered (ovaries and uterus intact) female ICR mice were obtained (Simonsen Laboratories, Gilroy, CA). The animals were randomly divided into groups of five mice each. Each group was housed in a polycarbonate shoebox (48 × 27 × 15 cm) cage. All animals were given *ad libitum* access to a standard laboratory rodent diet and water.

### Induction and Maintenance of Pseudoestrus

An injection solution was prepared using commercially available estradiol valerate (Schein, Pharmaceutical, Inc., Phoenix, AZ) which had an estradiol valerate concentration of 20 mg/ml. The stock solution was diluted using sterile sesame oil (Sigma Chemical Co., St. Louis, MO) to a final concentration of 5 mg/ml. Three days prior to challenge with *C. albicans*, the mice were each given 0.1 ml (0.5 mg estradiol valerate) subcutaneously. Animals were maintained in pseudoestrus by weekly injections.

### Preparation of Challenge Inoculum and Challenge

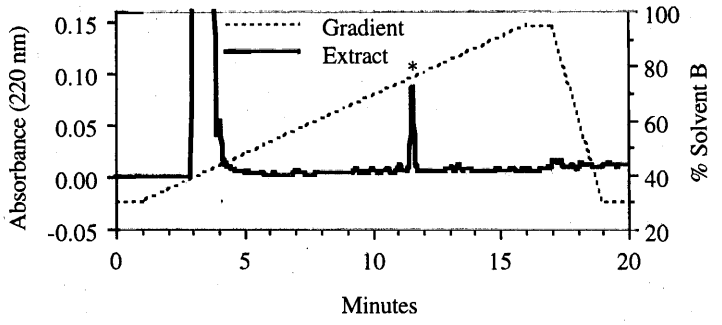
The *C. albicans* SC-9172 clinical isolate was obtained from the Squibb Culture Collection (Squibb Corporation, Princeton, NJ). Isolated colonies were picked from the surface of an SDA plate and grown for 24 hours at 30°C in Sabouraud dextrose broth. One (1.0) ml aliquots of the turbid broth culture were transferred to 20 ml of phytone-peptone (1%) and glucose (1%) broth in 50 ml Erlenmeyer flasks. The cultures were incubated with shaking for 36 hours at room temperature[15]. The cultures were then placed into conical centrifuge tubes and centrifuged at 500 × *g* for 10 minutes. After removing the supernatant fluids, the pellets were resuspended and pooled together in RPMI 1640 (with L-glutamine) tissue culture media with 1000 $\mu$g/ml of streptomycin sulfate and 1000 units/ml penicillin (designated RPMI+). The concentration of cells was determined by hemacytometer count, and the suspension was diluted with RPMI+ to make a challenge inoculum with $2.5 \times 10^7$ organisms/ml. The concentration of the challenge inoculum was verified by hemacytometer counting and plating of tenfold serial dilutions on BiGGY agar (BBL; Becton Dickinson Microbiology Systems, Cockeysville, MD). Mice were anesthetized with 80 mg/kg ketamine hydrochloride (Ketaset; Fort Dodge Laboratories, Inc., Fort Dodge, IA) given intraperitoneally and 20 $\mu$l of the inoculum was placed in the vagina using a 22 gauge ball-ended stainless steel dosing needle.

### Culturing

The vagina of each mouse was cultured immediately before being challenged by inserting a sterile urethro-genital calcium alginate applicator (Baxter Scientific Products, McGaw Park, IL) dampened with sterile phosphate buffered saline (PBS) into the vagina and twisting it several times before removal[13]. The applicator was smeared on BiGGY agar and incubated at 35°C for 48 hours. The cultures were read as positive (+) or negative (−).

For quantitative cultures, a sterile urethro-genital calcium alginate applicator was dampened with sterile PBS and inserted into the vagina and twisted several times. The applicator was removed and then swirled in 0.4 ml sterile PBS to free yeast and cellular debris from

Fig. 2. HPLC profile of mouse liver spiked with 12.5 $\mu$g SR-E.




SR-E was added to the liver tissue before homogenization. The asterisk denotes the SR-E peak. The dotted line indicates the elution gradient of solvent B (0.05% trifluoroacetic acid in acetonitrile/2-propanol 4:1, v/v).

the swab. Serial tenfold dilutions were made using sterile PBS and plated on BiGGY agar. The plates were incubated at 35°C for 48 hours. Following incubation, those plates with 30~300 colonies were counted and recorded[13,14,16].

Treatment

Animals were treated intravaginally b.i.d. with 20 $\mu$l of drug carrier (negative control), one of the formulations of SR-E, or clotrimazole 1% (comparison control) for 4 consecutive days starting 24 hours after challenge (day 1). Treatments with various formulations in non-challenged animals were done for tissue drug levels and histopathology studies.

Plasma and Tissue Drug Levels and Histopathology

Four groups of unchallenged mice treated with Aquaphor (drug carrier) or SR-E (3%, 6% and 12%) in Aquaphor were sacrificed 24 hours after the last treatment on day 5 for tissue collection. Plasma, kidney and liver were collected and frozen immediately. Kidney and liver tissues were diluted 4-fold with acidified methanol (0.01 N HCl), homogenized 3 times for 30 seconds on ice using a mechanical homogenizer (Tissue-Tearor; Biospec Products, Inc., Bartlesville, OK). The homogenized extracts were incubated in a 65°C water bath for 10 minutes and centrifuged at 12,000 $\times g$ for 10 minutes. The supernatant was collected and filtered through a 0.22 $\mu$m syringe filter[17]. Plasma samples were prepared as above without homogenizing or heating[17].

The filtrate (50 $\mu$l) was analyzed for SR-E using a Beckman Nouveau Gold HPLC system (Beckman Instruments, Inc., Palo Alto, CA) equipped with an Atlantis C18 column (4.6 × 250 mm i.d., 5 $\mu$m average particle size, and 300 Å pore size; Phenomenex, Torrance, CA) at a flow rate of 1 ml/minute. Elution was performed by a linear solvent gradient (30 to 95% solvent B; Fig. 2) obtained by mixing solvent A (0.05% trifluoroacetic acid in water) with solvent B (0.05% trifluoroacetic acid in acetonitrile/2-propanol 4:1, v/v). The effluent was monitored at 220 nm. Tissue standards were made by supplementing plasma or tissues with known amounts of SR-E before diluting with methanol. The lowest detectable amount of SR-E in tissues was 100 ng.

Mice vaginas were collected and fixed in 10% buffered formalin. Vaginal sections were examined microscopically by a board certified pathologist (Utah Veterinary Diagnostic Laboratories, Logan, UT).

Statistical Analysis

Statistical analysis was done using Minitab (Minitab Inc., State College, PA). For the vaginal colony counts, an analysis of variance was done on the $\log_{10}$ colony counts for each day of vaginal culture and each drug formulation from days 5 to 7. The student's two-sample *t*-test was then used to determine significant differences between data that had a *P*-value less than 0.1.

For the tissue drug levels, the two-sample *t*-test was used to compare the areas under any peaks that eluted close to the elution time of SR-E. The areas of SR-E-treated groups were compared to the carrier-treated controls.

## Results

Positive/negative cultures taken immediately before challenge were all negative indicating no prechallenge *C. albicans* vaginal colonization. *C. albicans* challenge

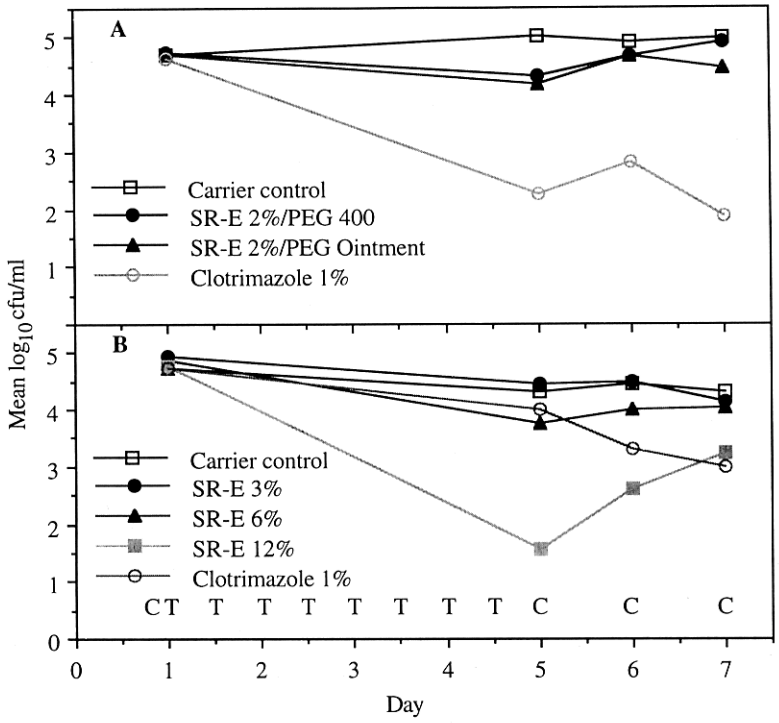
Table 1. Mean cfu/ml and standard deviations for each day the mice were cultured in study I.

Mice were treated with SR-E 2% formulated in PEG-based carriers.

| Treatment group (n=5) | *C. albicans* counts ($log_{10}$ cfu/ml) | | | |
|---|---|---|---|---|
| | Day 1 | Day 5 | Day 6 | Day 7 |
| Carrier control (PEG 400) | 4.69 ± 0.25 | 5.01 ± 0.53 | 4.91 ± 0.45 | 4.98 ± 0.41 |
| SR-E 2% in PEG 400 | 4.73 ± 0.13 | 4.32 ± 0.49[a] | 4.65 ± 0.44 | 4.90 ± 0.35 |
| SR-E 2% in PEG ointment | 4.69 ± 0.10 | 4.18 ± 0.44[b] | 4.68 ± 0.33 | 4.46 ± 0.32[a] |
| Clotrimazole 1% | 4.64 ± 0.20 | 2.27 ± 1.56[c] | 2.83 ± 0.94[c] | 1.89 ± 1.26[c] |

[a] $P \leq 0.06$; compared with carrier control, [b] $P \leq 0.03$; compared with carrier control, [c] $P \leq 0.01$; compared with carrier control.

Fig. 3. Mean $log_{10}$ cfu/ml for animals treated with SR-E in PEG-base carriers (A) and a Aquaphor-based carrier (B).




Indicated are the sequences for the treatments (T) and cultures (C).

inocula were verified by serial plating on BiGGY agar to be $5.0 \times 10^5$ and $6.8 \times 10^5$ blastospores for studies I and II, respectively. This gave a well-established infection in all challenged animals (Tables 1 and 2) with a range of 4.64 to 4.93 $log_{10}$ cfu/ml on day 1 prior to treatment.

In study I, SR-E formulated in both PEG formulations reduced the number of yeasts in the vaginas on day 5 ($P \leq 0.06$ and $P \leq 0.03$ for SR-E/PEG 400 and SR-E/PEG ointment, respectively) and SR-E/PEG ointment reduced the colonization on day 7 ($P \leq 0.06$) (Table 1 and Fig. 3A). However, no significant differences from the controls were detected on day 6. No visible inflammatory effects were seen as a result of SR-E treatment. The drug remained active in these formulations for the

Table 2. Mean cfu/ml and standard deviations for each day the mice were cultured in study II.

Mice were treated with SR-E formulated in Aquaphor.

| Treatment group (n=5) | *C. albicans* counts ($log_{10}$ cfu/ml) | | | |
|---|---|---|---|---|
| | Day 1 | Day 5 | Day 6 | Day 7 |
| Carrier control | 4.72 ± 0.38 | 4.32 ± 0.96 | 4.45 ± 0.52 | 4.33 ± 0.57 |
| SR-E 3% | 4.93 ± 0.16 | 4.46 ± 0.05 | 4.50 ± 0.45 | 4.15 ± 0.36 |
| SR-E 6% | 4.86 ± 0.13 | 2.75 ± 1.09 | 4.01 ± 0.65 | 4.03 ± 0.65 |
| SR-E 12% | 4.76 ± 0.20 | 1.58 ± 1.52[af] | 2.61 ± 1.53[cd] | 3.24 ± 0.68[be] |
| Clotrimazole 1% | 4.74 ± 0.12 | 4.02 ± 0.32 | 3.29 ± 0.48[a] | 3.01 ± 0.58[a] |

[a] $P \leq 0.01$; compared with carrier control, [b] $P \leq 0.03$; compared with carrier control, [c] $P \leq 0.06$; compared with carrier control, [d] $P \leq 0.03$; compared with clotrimazole 1%, [e] $P \leq 0.08$; compared with SR-E 12% on day 5, [f] $P \leq 0.03$; compared with clotrimazole 1% on day 5.

duration of the study. Clotrimazole reduced the vaginal yeast numbers for all three culture days when compared to the controls ($P \leq 0.01$). The transient reduction in yeast numbers prompted a second study using higher concentrations of SR-E with a different carrier.

In study II, all drugs remained active in their respective Aquaphor formulations throughout the study. A slight decrease ($\leq 3$ mm) in zones of inhibition on SDA plates were seen with most compound formulations on day 5 including clotrimazole. This was most likely due to less drug applied to the sensitivity disks because of the temperature differences of the compounds the time of application.

After 4 days of treatment, mean culture counts for SR-E showed dose-dependent efficacy (Table 2 and Fig. 3B). SR-E 3% had no effect while the 6 and 12% doses reduced the number of yeasts. The 12% dose showed a significant reduction on days 5 ($P \leq 0.01$), 6 ($P \leq 0.06$), and 7 ($P \leq 0.03$) when compared with the drug carrier controls. In addition, on day 5, SR-E 12% was more effective than clotrimazole 1% ($P \leq 0.03$). On days 6 and 7, mean colony counts increased for both SR-E 6 and 12%. This increase was only significant for SR-E 12% and did not occur until day 7 ($P \leq 0.08$) when compared to day 5.

Clotrimazole 1% reduced the number of yeasts on all three culture days, but did not significantly reduce the number of yeasts in the vaginas until days 6 ($P \leq 0.01$) and 7 ($P \leq 0.01$) when compared to the drug carrier controls.

No SR-E above 100 ng (8 $\mu$g/g of tissue) could be detected in the plasma or tissues of animals treated with SR-E 12%, nor in random tissue samples from animals treated with SR-E 3% and 6%. HPLC peaks with retention times similar to that of SR-E were observed in kidney. However, these were also observed in kidney extracts of carrier only treated animals.

Histological examination of vaginas found no inflammatory effects of SR-E treatment.

## Discussion

Experimentally induced vaginal candidiasis in this model is hormonal dependent[15], making treatment with estradiol valerate essential. All animals had well-established infections on day 1 with very little variability. The carrier-treated controls maintained an excellent level of infection throughout the studies (day 7). This was an indication that all animals received a proper dose of estrogen and an appropriate challenge inoculum.

Drug dosages and clinical dosing regimens vary greatly when comparing commercially available vaginal preparations. These differences make direct comparisons between compounds difficult. One way to compare various formulations is to subject them to the same dosing regimen and allow the concentration of drug to be the variable. In previous work with the rodent vaginal candidiasis model, clotrimazole 1%, which is generally recommended to be given clinically once a day for 7 days, was instead given b.i.d. for 4 days and gave reproducible reductions of yeast numbers[16].

Study I showed a short term reduction of yeasts in the vagina with SR-E 2% in two different PEG-based carriers when compared with controls. This prompted the more

extensive study with higher concentrations of SR-E in an Aquaphor-based carrier that gave prolonged efficacy at SR-E 12%. It was found that SR-E 3% in an Aquaphor-based carrier had no effect in this model. Since SR-E 2% in a PEG-based carrier showed efficacy, the Aquaphor-based carrier may be responsible for the decreased efficacy at the higher concentration. This may be related to availability of the drug and absorption properties of PEG.

SR-E at the doses tested did not completely eliminate yeasts from the vagina and the mean colony counts for SR-E 6% and 12% increased on days 6 and 7. This was unexpected because SR-E exhibits fungicidal action *in vitro* against a broad range of pathogenic fungi including *C. albicans*[1]. However, in other studies using similar animal models, other antifungals only reduced yeast populations[12] or reported percentages of vaginas cured[13]. Infection rebounds after short-duration treatment have also been reported[18]. Clotrimazole 1% was a consistent positive control in the model used in these studies. It rarely totally eliminates the infection.

SR-E was active for the duration of the studies. The carrier formulations were acidified to pH 6 because SR-E is irreversibly inactivated at basic pH[19,20]. However, the *in vitro* evaluation of SR-E[1] and its production in minimal media[21] were both done at pH 7 and SR-E activity was not affected even at this higher pH.

In conclusion, SR-E 12% provided an effective treatment of vaginal candidiasis in this model. The results suggest that SR-E 12% may be more effective than clotrimazole 1% at reducing the number of yeast in vaginas. Additional research on the formulation may lead to improved efficacy.

Acknowledgments

This research was supported by the Willard L. Eccles Charitable Foundation and the Utah Agriculture Experiment Station (project 607). This is journal paper No. 5060 of the Utah Agriculture Experiment Station.

## References

1) SORENSEN, K. N.; K.-H. KIM & J. Y. TAKEMOTO: *In vitro* antifungal and fungicidal activities and erythrocyte toxicities of cyclic lipodepsinonapeptides produced by *Pseudomonas syringae* pv. *syringae*. Antimicrob. Agents Chemother. 40: 2710～2713, 1996
2) BIDWAI, A. P. & J. Y. TAKEMOTO: Bacterial phytotoxin, syringomycin, induces a protein kinase-mediated phosphorylation of red beet plasma membrane polypeptides. Proc. Natl. Acad. Sci. USA 84: 6755～6759, 1987
3) BIDWAI, A. P.; L. ZHANG, R. C. BACHMANN & J. Y. TAKEMOTO: Mechanism of action of *Pseudomonas syringae* phytotoxin, syringomycin: stimulation of red beet plasma membrane ATPase activity. Plant Physiol. 83: 39～43, 1987
4) SUZUKI, Y. S.; Y. WANG & J. Y. TAKEMOTO: Syringomycin-stimulated phosphorylation of the plasma membrane $H^+$-ATPase from red beet storage tissue. Plant Physiol. 99: 1314～1320, 1992
5) ZHANG, L. & J. Y. TAKEMOTO: Effects of *Pseudomonas syringae* phytotoxin, syringomycin, on plasma membrane functions of *Rhodotorula pilimanae*. Phytopathology 77: 297～303, 1987
6) ZHANG, L. & J. Y. TAKEMOTO: Syringomycin stimulation of potassium efflux by yeast cells. Biochim. Biophys. Acta 987: 171～175, 1989
7) FEIGIN, A. M.; J. Y. TAKEMOTO, R. WANGSPA, J. H. TEETER & J. G. BRAND: Properties of voltage-gated ion channels formed by syringomycin E in planar lipid bilayers. J. Membr. Biol. 149: 41～47, 1996
8) HUTCHISON, M. L.; M. A. TESTER & D. C. GROSS: Role of biosurfactant and ion channel-forming activities of syringomycin in transmembrane ion flux: a model for the mechanism of action in the plant-pathogen interaction. Mol. Plant Microb. Interact. 8: 610～620, 1995
9) CLIFTEN, P.; Y. WANG, D. MOCHIZUKI, T. MIYAKAWA, R. WANGSPA, J. HUGHES & J. Y. TAKEMOTO: *SYR2*, a gene necessary for syringomycin growth inhibition of *Saccharomyces cerevisiae*. Microbiology 142: 477～484, 1996
10) TAGUCHI, N.; Y. TAKANO, C. JULMANOP; Y. WANG, S. STOCK, J. TAKEMOTO & T. MIYAKAWA: Identification and analysis of the *Saccharomyces cerevisiae SYR1* gene reveals that ergosterol is involved in the action of syringomycin. Microbiology 140: 353～359, 1994
11) FIDEL, P. L., Jr.; M. E. LYNCH & J. D. SOBEL: *Candida*-specific cell-mediated immunity is demostratable in mice with experimental vaginal candidiasis. Infect. Immun. 61: 1990～1995, 1993
12) PALACIN, C.; A. SACRISTAN & J. A. ORTIZ: *In vivo* activity of sertaconazole in experimental candidiasis in the mouse. Drugs Exp. Clin. Res. 16: 469～473, 1990
13) PETRANYI, G.; J. G. MEINGASSNER & H. MIETH: Activity of terbinafine in experimental infections of laboratory animals. Antimicrob. Agents Chemother. 31: 1558～1561, 1987
14) RYLEY, J. F. & S. MCGREGOR: Quantification of vaginal *Candida albicans* infection in rodents. J. Med. Vet. Mycol. 24: 455～460, 1986
15) SOBEL, J. D.; G. MULLER & J. F. MCCORMICK: Experimental chronic vaginal candidosis in rats. J. Med. Vet. Mycol. 23: 199～206, 1985
16) ALLEN, S. D.; K. N. SORENSEN & L. MEYERSON: Efficacy of topical SP-1101 in a rat *Candida albicans* model of vaginitis. *In* the 92nd General Meeting of the American Society for Microbiology. p. 514, Abstract F-97, American Society for Microbiology, Washington, DC, New Orleans, LA, 1992
17) MAYHEW, J. W.; C. FIORE, T. MURRAY & M. BARZA: An internally-standardized assay for amphotericin B in tissues and plasma. J. Chromatogr. 274: 271～279, 1983
18) VALENTIN, A.; C. BERNARD, M. MALLIE, M. HUERRE & J.-M. BASTIDE: Control of *Candida albicans* vaginitis in

mice by short-duration butoconazole treatment *in situ*. Mycoses 36: 379~384, 1993

19) SEGRE, A.; R. C. BACHMANN, A. BALLIO, F. BOSSA, I. GRGURINA, N. S. IACOBELLIS, G. MARINO, P. PUCCI, M. SIMMACO & J. Y. TAKEMOTO: The structure of syringomycins A1, E and G. FEBS Lett. 255: 27~31, 1989

20) SINDEN, S. L.; J. E. DEVAY & P. A. BACKMAN: Properties of syringomycin, a wide spectrum antibiotic and phytotoxin produced by *Pseudomonas syringae*, and its role in the bacterial canker disease of peach trees. Physiol. Plant Pathol. 1: 199~213, 1971

21) MO, Y.-Y. & D. C. GROSS: Plant signal molecules activate the *syrB* gene, which is required for syringomycin production by *Pseudomonas syringae* pv. *syringae*. J. Bacteriol. 173: 5784~5792, 1991