



MIDWEST RESEARCH INSTITUTE

425 Volker Boulevard
Kansas City, Missouri 64110
Telephone (816) 753-7600
Telefax (816) 753-8420

December 17, 2001

Ms. Carleen Burgess
ESP, LLC
1071 S. Jefferson Davis Parkway
New Orleans, LA 70125

Subject: MRI Project No. 310303.1.002, "Evaluation of ESP Antimicrobial Lotion"

Dear Ms. Burgess:

Midwest Research Institute (MRI) is pleased to submit the enclosed draft report for MRI Project No. 310303.1.002 titled "Evaluation of ESP Antimicrobial Lotion." If we do not receive a request for changes within 30 days of receipt, we will issue a final report. If you have any questions, or we can be of further assistance, please contact me. Thank you for the opportunity to work with ESP, LLC on this endeavor.

Sincerely,

MIDWEST RESEARCH INSTITUTE

A handwritten signature in black ink, appearing to read "Robert Huebner", with a long horizontal flourish extending to the right.

Robert Huebner, Ph.D.
Project Leader
Section Manager, Biotechnology

A handwritten signature in black ink, appearing to read "Leasa Brown", with a long horizontal flourish extending to the right.

Leasa Brown
Associate Scientist
Biotechnology

Abstract

Chimal Skin Shield is a barrier cream that contains the antibacterial compound triclosan. This study investigated the activity of this compound on five bacterial pathogens *Bacillus anthracis* Sterne strain, *Staphylococcus aureus* strain 12598, *Escherichia coli* strain 25922, *Propionibacterium acne* strain 11827, and *Streptococcus pyrogenes* strain 19615. The antibacterial activity was determined by measuring the growth inhibition of a bacterial lawn surrounding plugs filled with approximately 100 μL of Chimal Skin Shield. The results of these tests show that Chimal Skin Shield was most effective at inhibiting the growth of *S. aureus* and *S. pyrogenes*, effective to a less degree against *E. coli* and had no visible inhibitory effect on the growth of *P. acne* and *B. anthracis*.

Introduction

Barrier creams are a popular means to protect the skin against the insults of everyday activities. Chimal Skin Shield, in addition to being a barrier cream, contains the antibacterial agent triclosan in its formulation and thus has antibacterial activity as well as barrier activity. This study investigated the ability of triclosan to inhibit the growth of five bacterial pathogens *Bacillus anthracis* Sterne strain, *Staphylococcus aureus* strain 12598, *Escherichia coli* strain 25922 and, *Streptococcus pyrogenes* strain 19615. The bacterial strain 11827 of *Propionibacterium acne* served as a negative control in this study.

1. Study Performance

Chimal Skin Shield was tested against six plates of *Bacillus anthracis* Sterne strain. *Propionibacterium acne* strain 11827 served as a negative product control and was done in triplicate. Each *B. anthracis* plate was also tested with a penicillin disk to show *B. anthracis* susceptibility and identity. The antibiotic disk was removed after 24 hr so not to interfere with reading the product effectiveness results. Controls included TSA plates with overlay and each organism, without product, and with product without organism. Sterility controls were also conducted with overlay media, TSA plates, and TSB broth. Controls were incubated overnight at 37°C.

B. anthracis Sterne strain was pulled from a glycerol stock and inoculated into a TSB tube. The culture was passed into TSB after 48 hr. This was incubated at ~ 37°C for 48 hr prior to use in tests. *P. acnes* was removed from glycerol storage and plated on TSA blood and anaerobic blood plates and incubated in anaerobic conditions for 3 days prior to use in tests.

TSA plates were prepared from TSA and agar according to standard directions, and autoclaved for sterility. Plates were made to contain 21 mL of media and stored at 4°C until used. Plates for use with *P. acne* contained 5% FBS sterile filtered and added to cooled media prior to plating.

For preparation of the bacterial lawns, 10 mL of TSA at 45 to 50°C was added to 50 mL polypropylene tubes. To each tube, 100 µL of 48 hr culture was added and mixed by swirling the agar. For the *P. acne* lawn, a loop was used to clear a TSA blood plate of visible growth. The bacteria was resuspended in TSB with 5% FBS. This suspension was used for spiking of overlay media. After the addition of bacterial culture, the overlay media (10 mL volume) was poured onto an agar base. TSA with 5% FBS was used as overlay for *P. acne*. Plates were allowed to solidify by remaining in the Biosafety cabinet for approximately 1 hr.

To test the effects of the lotion on organism growth, a central plug was removed from the agar plate with the end of a sterile Pasteur pipette. Approximate 100 µL of product was then used to fill the plug via a 5-mL syringe. All plugs were completely filled till the lotion was even with the agar surface. Plates were then incubated upright at approximately 34°C for 48 hr. Plates of *P. acne* were incubated in anaerobic conditions. After 48 hr, the zone of inhibition for each organism was measured in two dimensions to the nearest mm. Because of slow growth on *P. acne* plates, these were checked at 48 hr then allowed to incubate to 72 hr. Results were recorded at both time points. A representative picture of the *B. anthracis* plate results was taken after measurement.

Study Results

A characteristic lawn of bacterial growth was observed for all *B. anthracis* plates at 48 hr. *P. acnes* lawns developed at 72 hr. Zones of inhibition/lysis were measured

around each lotion plug on each plate. Measurements were made by drawing two perpendicular lines through the center of the plug. A ruler was then used to record the diameter of the zone on both the horizontal and vertical lines through the zone. All zones were observed to be nearly symmetrical circles. The data from these measurements is summarized below. The area of the zones of inhibition were calculated by the following formula (horizontal mm/2 x vertical mm/2 x π = zone of inhibition area).

Organism	Zone in mm		Zone area
	Horizontal	Vertical	
<i>B. anthracis</i>	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0
<i>P. acnes</i>	0	0	0
	0	0	0
	0	0	0
Penicillin zone of inhibition			
<i>B. anthracis</i>	27	24	3421.194

Study Conclusions

P. acnes and *B. anthracis* Sterne strain lawns did not show zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs indicating the antibacterial activity in the lotion is not effective against these bacteria.

2. Study Performance

Chimal Skin Shield was tested against six plates of *Staphylococcus aureus* strain 12598. *Propionibacterium acne* strain 11827 served as a negative product control and was done in triplicate. Controls included TSA plates with overlay and each organism, without product and, with product without organism. Sterility controls were also conducted with overlay media, TSA plates, and TSB broth. Controls were incubated overnight at 37°C.

The test organism, *S. aureus*, was pulled from a glycerol stock and inoculated into a TSB tube. The culture was passed into fresh TSB after 48 hr. This was incubated at ~ 37°C for 48 hr prior to use in tests. *P. acnes* was removed from glycerol storage and plated on TSA blood and anaerobic blood plates and incubated in anaerobic conditions for 3 days prior to use in this test.

TSA plates were prepared from TSA and agar according to standard directions, and autoclaved for sterility. Plates were made to contain 21 mL of media and stored at 4°C until used. Plates for use with *P. acne* contained 5% FBS sterile filtered and added to cooled media prior to plating.

For preparation of the bacterial lawns 10 mL of TSA at 45 to 50°C was added to 50 mL polypropylene tubes. To each tube, 100 µL of 48 hr culture was added and mixed by swirling the agar. For the *P. acne* lawn, a loop was used to clear a TSA blood plate of visible growth. The bacteria was resuspended in TSB with 5% FBS. This suspension was used for spiking of overlay media. After the addition of bacterial culture, the overlay media (10 mL volume) was poured onto an agar base. TSA with 5% FBS was used as overlay for *P. acne*. Plates were allowed to solidify by remaining in the Biosafety cabinet for approximately 1 hr.

To test the effects of the lotion on organism growth, a central plug was removed from the agar plate with the end of a sterile Pasteur pipette. Approximate 100 µL of product was then used to fill the plug via a 5 mL syringe. All plugs were completely filled till the lotion was even with the agar surface. Plates were then incubated upright at approximately 34°C for 48 hr. Plates of *P. acne* were incubated in anaerobic conditions. After 48 hr, the zone of inhibition for each organism was measured in two dimensions to the nearest mm. Because of slow growth on *P. acne* plates, these were checked at 48 hr then allowed to incubate to 72 hr. Results were recorded at both time points. A representative picture of the *S. aureus* TSA plate result was taken after measurement.

Study Results

A characteristic lawn of bacterial growth was observed for all *S. aureus* plates at 48 hr. *P. acnes* lawns developed at 72 hr. Zones of inhibition/lysis were measured around each lotion plug on each plate. Measurements were made by drawing two perpendicular lines through the center of the plug. A ruler was then used to record the

diameter of the zone on both the horizontal and vertical lines through the zone. All zones were observed to be nearly symmetrical circles. The data from these measurements is summarized below. The area of the zones of inhibition were calculated by the following formula (horizontal mm/2 x vertical mm/2 x π = zone of inhibition area).

Organism	Zone in mm		Zone area
	Horizontal	Vertical	
<i>S. aureus</i>	26	26	3318.307
	27	27	3578.47
	26	27	3369.554
	25	26	3117.245
	25	26	3117.245
	25	24	3019.071
<i>P. acnes</i>	0	0	0
	0	0	0
	0	0	0

Study Conclusions

The *S. aureus* plates showed zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs. This indicates that the antibacterial activity of the Chimal lotion is effective against this *S. aureus* strain. The *P. acnes* plates did not show zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs indicating the antibacterial activity in the lotion is not effective against these bacteria.

3. Study Performance

Chimal Skin Shield was tested against six plates of *Escherichia coli* strain 25922. *Propionibacterium acne* strain 11827 served as a negative product control and was done in triplicate. Controls included TSA plates with overlay and each organism without product and with product without organism. Sterility controls were also conducted with overlay media, TSA plates, and TSB broth. Controls were incubated overnight at 37°C.

The test organism, *E. coli*, was pulled from a glycerol stock and inoculated into a TSB tube. The culture was passed into fresh TSB after 48 hr. This was incubated at ~ 37°C for 48 hr prior to use in tests. *P. acnes* was removed from glycerol storage and plated on TSA blood and Anaerobic blood plates and incubated in anaerobic conditions for 3 days prior to use in this test.

TSA plates were prepared from TSA and agar according to standard directions, and autoclaved for sterility. Plates were made to contain 21 mL of media and stored at 4°C until used. Plates for use with *P. acne* contained 5% FBS sterile filtered and added to cooled media prior to plating.

For preparation of the bacterial lawns 10 mL of TSA at 45 to 50°C was added to 50 mL polypropylene tubes. To each tube, 100 µL of 48 hr culture was added and mixed by swirling the agar. For the *P. acne* lawn, a loop was used to clear a TSA blood plate of visible growth. The bacteria was resuspended in TSB with 5% FBS. This suspension was used for spiking of overlay media. After the addition of bacterial culture, the overlay media (10 mL volume) was poured onto an agar base. TSA with 5% FBS was used as overlay for *P. acne*. Plates were allowed to solidify by remaining in the Biosafety cabinet for approximately 1 hr.

To test the effects of the lotion on organism growth, a central plug was removed from the agar plate with the end of a sterile Pasteur pipette. Approximate 100 µL of product was then used to fill the plug via a 5 mL syringe. All plugs were completely filled till the lotion was even with the agar surface. Plates were then incubated upright at approximately 34°C for 48 hr. Plates of *P. acne* were incubated in anaerobic conditions. After 48 hr, the zone of inhibition for each organism was measured in two dimensions to the nearest mm. Because of slow growth on *P. acne* plates, these were checked at 48 hr then allowed to incubate to 72 hr. Results were recorded at both time points. A representative picture of the *E. coli* TSA plate result was taken after measurement.

Study Results

A characteristic lawn of bacterial growth was observed for all *E. coli* plates at 48 hr. *P. acnes* lawns developed at 72 hr. Zones of inhibition/lysis were measured around each lotion plug on each plate. Measurements were made by drawing two perpendicular lines through the center of the plug. A ruler was then used to record the diameter of the zone on both the horizontal and vertical lines through the zone. All zones were observed to be nearly symmetrical circles. The data from these measurements is summarized below.

The area of the zones of inhibition were calculated by the following formula (horizontal mm/2 x vertical mm/2 x π = zone of inhibition area).

Organism	Zone in mm		Zone Area
	Horizontal	Vertical	
<i>E. coli</i>	11	12	615.7522
	12	13	730.6166
	12	10	660.5199
	11	11	593.9574
	10	12	530.9292
	10	11	510.7052
<i>P. acnes</i>	0	0	0
	0	0	0
	0	0	0

Study Conclusions

The *E. coli* plates showed zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs. This indicates that the antibacterial activity of the Chimal lotion is effective against this *E. coli* strain. The *P. acnes* plates did not show zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs indicating the antibacterial activity in the lotion is not effective against these bacteria.

4. Study Performance

Chimal Skin Shield was tested against six plates of *Staphylococcus pyrogenes* strain 19615. *Propionibacterium acne* strain 11827 served as a negative product control and was done in triplicate. Controls included TSA plates with overlay and each organism without product and with product without organism. Sterility controls were also conducted with overlay media, TSA plates, and TSB broth. Controls were incubated overnight at 37°C.

The test organism, *S. pyrogenes*, was pulled from a glycerol stock and inoculated into a TSB tube. The culture was passed into fresh TSB after 48 hr. This was incubated at ~ 37°C for 48 hr prior to use in tests. *P. acnes* was removed from glycerol storage and plated on TSA blood and Anaerobic blood plates and incubated in anaerobic conditions for three days prior to use in this test.

TSA plates were prepared from TSA and agar according to standard directions, and autoclaved for sterility. Plates were made to contain 21 mL of media and stored at 4°C until used. Plates for use with *P. acne* contained 5% FBS sterile filtered and added to cooled media prior to plating.

For preparation of the bacterial lawns 10 mL of TSA at 45 to 50°C was added to 50 mL polypropylene tubes. To each tube, 100 µL of 48 hr culture was added and mixed by swirling the agar. For the *P. acne* lawn, a loop was used to clear a TSA blood plate of visible growth. The bacteria was resuspended in TSB with 5% FBS. This suspension was used for spiking of overlay media. After the addition of bacterial culture, the overlay media (10 mL volume) was poured onto an agar base. TSA with 5% FBS was used as overlay for *P. acne*. Plates were allowed to solidify by remaining in the Biosafety cabinet for approximately one hr.

To test the effects of the lotion on organism growth, a central plug was removed from the agar plate with the end of a sterile Pasteur pipette. Approximate 100 µL of product was then used to fill the plug via a 5 mL syringe. All plugs were completely filled till the lotion was even with the agar surface. Plates were then incubated upright at approximately 34°C for 48 hr. Plates of *P. acne* were incubated in anaerobic conditions. After 48 hr, the zone of inhibition for each organism was measured in two dimensions to the nearest mm. Because of slow growth on *P. acne* plates, these were checked at 48 hr then allowed to incubate to 72 hr. Results were recorded at both time points. A representative picture of the *S. pyrogenes* TSA plate result was taken after measurement.

Study Results

A characteristic lawn of bacterial growth was observed for all *S. pyrogenes* plates at 48 hr. *P. acnes* lawns developed at 72 hr. Zones of inhibition/lysis were measured around each lotion plug on each plate. Measurements were made by drawing two perpendicular lines through the center of the plug. A ruler was then used to record the diameter of the zone on both the horizontal and vertical lines through the zone. All zones were observed to be nearly symmetrical circles. The data from these measurements is summarized below. The area of the zones of inhibition were calculated

by the following formula (horizontal mm/2 x vertical mm/2 x π = zone of inhibition area).

Organism	Zone in mm		Zone area
	Horizontal	Vertical	
<i>S. pyrogenes</i>	27	28	3631.681
	27	27	3578.47
	27	26	3525.652
	27	28	3631.681
	28	28	3848.451
	27	28	3631.681
<i>P. acnes</i>	0	0	0
	0	0	0
	0	0	0

Study Conclusions

The *S. pyrogenes* plates showed zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs. This indicates that the antibacterial activity of the Chimal lotion is effective against this *S. pyrogenes* strain. The *P. acnes* plates did not show zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs indicating the antibacterial activity in the lotion is not effective against these bacteria.

Project Conclusions

The *E. coli*, *S. aureus*, and *S. pyrogenes* organisms showed zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs. This indicates that the antibacterial activity of the Chimal lotion is effective against these bacteria. The *P. acnes* and *B. anthracis* Sterne strain lawns did not show zones of inhibition in the bacterial lawn surrounding Chimal lotion plugs indicating the antibacterial activity in the lotion is not effective against these bacteria.