ORIGINAL RESEARCH

Safety Evaluation of Topical Products Containing Live Cultures and Ferment of *Cutibacterium Acnes* Subspecies *Defendens* Strain XYCM42 in Individuals Predisposed to Acne Vulgaris

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J Clin Aesthet Dermatol. 2025;18(5):44–53.

BACKGROUND: For individuals with acne-prone skin, identifying a topical regimen that does not lead to progression of their inflammatory issues often poses a challenge. A topical skin probiotic regimen containing a specific strain of *Cutibacterium acnes (C. acnes)* subspecies *defendens*, XYCM42, has been shown to be beneficial in improving skin health and appearance in individuals with generally healthy skin, but the use of the skin probiotic has not been sufficiently assessed in individuals with acne-prone skin. **OBJECTIVE:** The purpose of this study was to evaluate the safety and efficacy of daily application of a topical skin biome care regimen containing a living *C. acnes* subsp. *defendens* derivative strain, XYCM42, its ferment, and adjunct topicals in individuals with acne-prone skin. **METHODS:** This eight-week study was conducted at five locations and included 136 total participants. At baseline, Week 1, Week 4, and Week 8, subjects completed product questionnaires and symptom severity surveys. Of the study subjects, 20 were enrolled for clinical efficacy evaluation at all timepoints. Clinical assessments included blemish lesion counts, Investigator's Global Assessment (IGA) of acne lesion severity, and clinical grading of skin cosmetic and safety parameters. **RESULTS:** As early as Week 1 of regimen application, clinical observations demonstrated statistically significant improvements in acne severity scores, with no subjects reporting increased or worsened acne during the study. By Week 4, subjects showed significant changes in nearly all skin cosmetic parameters assessed, including skin texture, clarity, tone, fine wrinkling, undereye dark circles, dryness, and erythema. Lesion counts were significantly reduced from baseline at all timepoints, with 100 percent of subjects experiencing fewer non-inflammatory lesions and 70 percent and 30 percent with fewer papule and pustule inflammatory lesions, respectively, by the end of the study. No adverse events were reported. **CONCLUSION:** This at-home use study demonstrates that use of the XYCM42-based topical skin biome care regimen is both safe and appropriate for individuals with acne-prone skin. More broadly, the outcomes of this study provide further support toward the beneficial and commensal nature of *C. acnes* subsp. *defendens* in promoting skin health. **KEYWORDS:** *Cutibacterium acnes defendens*, *C. acnes*, microbiome, skin probiotic, topical skin products, acne vulgaris, acne-prone

When applying new topical products to skin, especially when formulated with an active ingredient, it is not uncommon for a subset of the population to have some form of transient skin reaction. A notable example of this phenomenon is found with use of formulations containing different forms of retinoids, which, despite their high potential for treating several dermatological conditions, are also notorious for inducing localized side-effects such as skin irritation, erythema, and peeling.[1] Fortunately, such side effects may subside over time as the skin acclimates. They can also be managed by controlling the frequency of application and through modification of active ingredient concentration. However, those with existing inflammatory skin conditions may be reluctant to use such topicals, either therapeutically or cosmetically, given their predisposition to skin irritation and the potential to aggravate their condition. One such population would be those who are predisposed to acne vulgaris. Individuals who have chronic, poorly controlled, or occasional transient acne may not wish to risk aggravation or reemergence of acne lesions by using new topical products. Therefore, it becomes important for clinical investigations to assess how topical products may affect those with active inflammatory skin issues or with a propensity to such issues.[2]

Previously, we reported clinical studies that showed dermatological benefits following consistent topical application of a regimen containing viable cultures of *C. acnes* subsp. *defendens* strain XYCM42 as well as filtered ferments of the same strain.[3,4] In one study of 121 generally healthy subjects, there were no reports of any inflammatory side-effects during or after the study.[3] A second study looking at peri-procedural use of the regimen in conjunction with microneedle-induced skin remodeling (ie, microneedling) also had no reports of any related inflammatory side effects.[4] As the latter study was conducted exclusively in subjects with acne scars, most of the participants were predisposed to acne vulgaris. Therefore, it was noteworthy that none of the study subjects reported exacerbation of acne lesions, and many of the subjects showed improvements in their inflammatory acne over time (up to nine months after initial application).[4]

In this study, we aimed to corroborate the previously observed safety of the XYCM42 regimen by specifically applying the same regimen in a

FUNDING: This study was funded by Crown Laboratories, Inc.
DISCLOSURES: All authors are employed by Crown Laboratories.
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population of individuals prone to inflammatory and/or non-inflammatory acne. The purpose of this at-home use study was to demonstrate that this regimen of products based around the benefits of a live skin-relevant probiotic (XYCM42) and conditioned postbiotic does not induce or stimulate an acneic response in acne-prone individuals. These findings will allow us to further assess the safety and appropriateness of the XYCM42 regimen for those with a propensity for active acne vulgaris.

METHODS

Study design. This eight-week study included clinical efficacy evaluation and claim substantiation of XYCM42-based topical probiotic regimen,[3,4] including sunscreen, (BIOJUVE, Crown Aesthetics; Dallas, Texas) use on acne-prone skin. The claims substantiation portion of the study was conducted at four locations: Calabasas, California; Chalfont, Pennsylvania; Richardson, Texas; and Milwaukee, Wisconsin. The in-clinic efficacy evaluation study was conducted in Dallas, Texas.

All study participants utilized the specified facial cosmetic regimen throughout the duration of the study and completed online questionnaires at baseline, Week 1, Week 4, and Week 8. Study visits occurred at baseline, Week 4, and Week 8 for claims substantiation study sites and at baseline, Week 1, Week 4, and Week 8 for clinical efficacy assessments in Dallas, Texas. The study success criteria was based on the participants' agreement that the facial cosmetic regimen is safe and effective for use in acne-prone skin. A minimum score of 65 percent favorable agreement was designated as meeting the claim substantiation requirement.

Additional assessments completed at baseline, Week 1, Week 4, and Week 8 included blemish lesion counts, Investigator's Global Assessment (IGA) for acne lesion severity, clinical safety grading, and subject-reported symptom severity surveys. Adverse events were monitored throughout the course of the study and clinical grading of skin cosmetic parameters was also completed as a secondary assessment at each visit for the in-clinic clinical evaluation group.

The study design and protocol adhered to the ethical considerations expected for a clinical study and was approved by the relevant Institutional Review Board (IRB; Allendale IRB, 30 Neck Road Old Lyme, Connecticut, 06371; IRB study number CL-AC-24-01). Informed consent was obtained from all subjects before participating, and each participant signed a photography/digital photo release form.

Participants. Adult male and female subjects with Fitzpatrick skin types I to VI aged between 18 and 40 were eligible to be screened for this study. Participants with facial blemishes or self-perceived acne-prone skin, sensitive skin, or skin prone to breakouts or frequent facial bumps that met the eligibility criteria were enrolled in the study. Though enrollment required subjects to be predisposed to acne, it was not required that subjects had active acne lesions at the time of enrollment to be eligible for the study.

Individuals with known allergies to skincare products, compromised or infected skin, recent facial trauma, skin conditions (ie, psoriasis, pemphigus, active skin cancer) currently under physician's care, as well as systemic disorders or complicating factors that could interfere with study outcomes were excluded to ensure participant safety and study integrity. Participants currently taking medications such as immunosuppressive drugs, antihistamines, anti-inflammatory drugs, anti-androgens, and topical or oral antibiotics were excluded from the study. Participants who used any medication, such as over the counter retinols, prescription retinoids, or physician-prescribed benzoyl peroxide in the three months prior to enrollment were also excluded. Additionally, individuals with upcoming or recent (within the last year) facial cosmetic treatments such as skin tightening procedures, filler or neurotoxin injections, and laser treatments were not eligible to participate in the study. Pregnant women were not included in this study and subjects were required to discontinue use of the product if they became pregnant during the study.

For enrollment in the clinical efficacy evaluation study at the Crown Laboratories Research Clinic site (Dallas), subjects in generally good health with facial acne IGA scores of 1 or greater (as determined by the Study Investigator) were eligible to participate. Enrolled women of child-bearing potential agreed to use a form of birth control during the study.

All participants agreed to discontinue their typical skincare regimen and blemish products beginning three days prior to the start of test product application and to refrain from any aesthetic procedure to the face throughout their participation in the study. Subjects were permitted to continue use of their normal facial make-up so long as brands remained unchanged. Subjects were also instructed to avoid excessive sun exposure.

Regimen application. Subjects received two complete regimen kits (dispensed at baseline and Week 4). All subjects were trained on proper product application as well as morning and evening usage requirements. Morning product use included the prebiotic cleanser, XYCM42 ferment-based serum, hydrating barrier cream, and sunscreen (SPF 50+). Evening product use included the prebiotic cleanser, followed by the live XYCM42 gel and prebiotic activating mist (mixed thoroughly in the palm of the hand for five seconds prior to application on the face). Product ingredients were reported previously.[4] Products were returned upon completion of the study for accountability purposes.

Imaging and porphyrin measurement. Standardized color photographs were taken at baseline, Week 4, and Week 8 at claims substantiation sites. Three facial profile views were taken for each subject: left, right, and frontal. For the in-clinic group (Dallas, Texas), images were acquired using VISIA-CR photo imaging (Canfield Scientific Inc, Parsippany-Troy Hills, New Jersey) at each visit. Subjects were instructed to adopt neutral, non-smiling expressions. Before imaging procedures, study personnel confirmed that the face and neck were free of makeup and jewelry. All subjects wore a black headband and a cloth over their clothing during imaging.

In addition to obtaining images, the VISIA-CR was also used to measure porphyrin intensity using quantitative fluorescence. Porphyrins were excited at 405±10nm, and spectral filters were applied to the camera to separate Protoporphyrin IX (PpIX) (emission >630nm) from Coproporphyrin III (CpIII) (570–630nm) fluorescence signals.[5–12] For quantitative fluorescence measurement, captured fluorescence images were corrected for non-uniform light distribution and tissue absorption using excitation light reflectance images.[13]

Subject-reported outcomes. At Weeks 1, 4, and 8, all subjects completed online claims questionnaires to assess their agreement with statements about the product regimen as a

whole. Subject agreement was assessed using a five-point Likert scale (strongly agree/agree/neutral/disagree/strongly disagree) and a minimum of 65 percent agreement was required for claims to be considered met. Subjects also completed symptom severity surveys based on 5 or 10-point scales (none, mild, moderate, severe, very severe) at baseline and Weeks 1, 4, and 8, and adverse events were monitored and reviewed throughout the course of the study.

Clinical assessments. At baseline and Weeks 1, 4, and 8, the Study Investigator performed clinical grading assessments of skin cosmetic and safety parameters based on a grading scale of 0–9 (0=none; 1–3=mild; 4–6=moderate; 7–9=severe). Skin cosmetic grading parameters included texture, clarity (lack of), even skin tone, discrete pigment, mottled pigment, fine wrinkling, coarse wrinkling, laxity, undereye dark circles, turgor (lack of), undereye puffiness, and overall photodamage. In addition, the Study Investigator noted any new adverse events from the use of test products by grading the following safety parameters: erythema, scaling/peeling, edema, burning/stinging, and itching. The investigator also performed blemish lesion assessments at every site visit. Lesion assessments included inflammatory (open or closed comedones, nodules/cysts) and non-inflammatory (papules, pustules) lesion counts as well as blemish lesion severity based on the five-point IGA scale (0=none; 4=severe).

Data analysis. For comparing outcomes between visits, each subject's baseline clinical grading, blemish lesion count, and IGA scores were subtracted from the respective scores at subsequent visits for all assessment parameters. Each subject's score differentials at Weeks 1, 4, and 8 (compared to baseline) were averaged to calculate mean and 95-percent confidence intervals. Mean values were used to calculate percent change, and paired *t*-tests were used to compare outcomes between visits. Statistical significance was reached if $p<0.05$. Clinical grading score and lesion count differentials were used to determine the percentage of subjects with changes in skin appearance. Claims substantiation analysis was based on the percentage of those with a favorable opinion toward statements in the claims questionnaire.

RESULTS

Subject demographics. A total of 140 subjects were enrolled in the study, including 20 subjects who were enrolled in the in-clinic efficacy evaluation portion. Of the 140 subjects enrolled, 136 completed the study. Three subjects were lost to follow-up, and one was discontinued from the study due to emergency treatment of kidney stones. Subject demographics are summarized in Table 1.

TABLE 1. Subject demographics by gender, age, skin type, and self-reported skin conditions

DEMOGRAPHICS	PERCENT PARTICIPANTS (N=136)
Gender	
Female	51%
Male	49%
Age	
18–24	21%
25–30	34%
31–40	45%
Fitzpatrick Skin Type	
I	9%
II	28%
III	36%
IV	22%
V	4.5%
VI	3.5%
Subject facial skin conditions of concern	
Blemishes	78%
Facial bumps	52%
Skin breakouts	75%

Shown is the percentage of subjects in each group (n=136)

Subject-reported outcomes. At Week 1, Week 4, and Week 8, all subjects completed consumer questionnaires about the product regimen as a whole. Based on Likert scale (strongly agree/agree/neutral/disagree/strongly disagree) responses, subjects held favorable agreement with 20 of the 21 statements about the product regimen at all timepoints (if applicable) (Table 2). At baseline and Week 8, subjects were also asked to rate their perceived symptom severity to assess changes in breakouts, redness, skin bumps, dryness, and itchiness (if any) by the end of the study. Of the 136 subjects who completed the surveys, a total of 94 subjects (69%) reported experiencing breakouts at baseline. At Week 8, a notable number of participants (35%) noted a transition in their acne breakouts from severe or moderate severity to mild severity, yielding a 53.8-percent reduction in breakout severity scores within the eight-week period of applying the regimen (Figure 1A). When asked about skin bumps, a total of 87 subjects (64%) reported experiencing skin bumps at baseline. This number decreased to 60 subjects (44%) at Week 8, representing a 31-percent decrease (Figure 1B). There was also a 50-percent reduction in those with six or more lesions.

TABLE 2. Claims substantiation results (n=136)

CLAIM (% FAVORABLE AGREEMENT)	WEEK 1	WEEK 4	WEEK 8
Spreads evenly onto skin	92%	94%	91%
Hydrates/moisturizes my skin	93%	89%	92%
Leaves my skin soft and smooth	93%	89%	91%
Is something appropriate I can use all over my face	96%	90%	90%
Is good for everyday use	93%	87%	89%
Leaves my skin feeling healthy	93%	90%	90%
Has a nice texture and feel	89%	80%	88%
Is good for my skin type	90%	82%	79%
Enhances skin clarity	90%	79%	82%
My skin is visibly clearer	–	75%	81%
Is good for blemish-prone skin	88%	81%	80%
Within 4–8 weeks, my skin had noticeably improved	–	75%	77%
Visibly improved clarity and imperfections	80%	76%	79%
Does not leave skin feeling greasy	72%	66%	68%
Skin is clear and radiant	81%	73%	72%
It balanced my skin within 4–8 weeks	–	74%	75%
Is good for any blotchy skin areas	90%	82%	82%
Transformed my skin and boosted my overall confidence	–	75%	78%
Provides for all of my blemish-prone skincare needs	79%	71%	65%
Corrects any problem areas my skin might have	74%	73%	71%
My skin is blemish-free	–	48%	53%

All claims were met (>65% agreement) except one

A total of 76 subjects (56%) reported experiencing redness on their face at baseline. Fewer subjects, a total of 51 (38%), reported redness at the final visit. In addition, subjects reported milder redness severity after eight weeks of regimen use compared to baseline, with a 20-percent reduction in those who reported moderate and severe redness (Figure 1C). It should be noted that one subject (2%) reported very severe redness at the end of the study. Following the conclusion of the study, this subject was contacted for validation. Upon thorough review of the subject's images, clinical grading data, and testimonial, the principal Study Investigator concluded that the subject's redness did not meet the defining characteristics of very severe redness and instead represented those of a moderate severity rating.

Dryness and itchiness showed similar trends to the other symptoms, with 21-percent and 18-percent fewer subjects, respectively, who reported experiencing these symptoms at the end of the study (Figure 1D–E). Severity was also improved, with a 77-percent reduction in the number of subjects who reported moderate or severe dryness and a 67-percent reduction in the number of subjects who reported moderate or severe itchiness (Figure 1D–E).

Clinical grading. As a secondary evaluation of subject outcomes, the Study Investigator performed clinical grading assessments of cosmetic skin parameters at each clinic visit for the subset of 20 subjects enrolled in the in-clinic evaluation portion of the study (Dallas, Texas). Clinical grading scores were compared to baseline for each subject and the percentage of subjects with changes in skin appearance over baseline were determined after each visit for all parameters (Figure 2). As early as Week 1, subjects demonstrated statistically significant improvements in skin texture, clarity, evenness of skin tone, fine wrinkling, and undereye dark circle appearance when compared to baseline. At Weeks 4 and 8, clinical grading scores with significantly reduced for all grading parameters except for mottled pigment (Table 3). Most notably, the majority of subjects showed clinically significant improvements in the appearance of skin texture (85%), clarity (90%), evenness of skin tone (85%), fine wrinkling (90%), turgor (85%), and undereye dark circles (80%) at the end of the eight-week period of regimen use (Figure 2).

Study investigators also monitored reactions

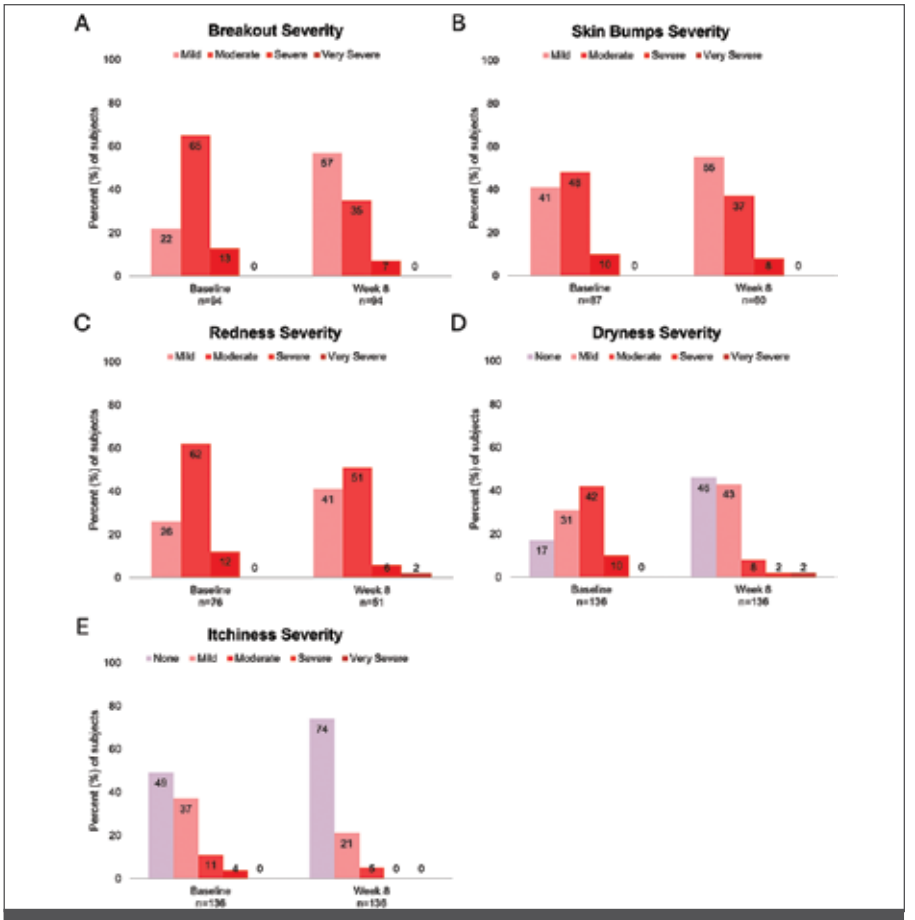

FIGURE 1. Subject-reported symptom severity at baseline and Week 8. Shown is the percentage of total subjects (n) who reported experiencing each symptom by severity category and visit. (A) Breakout severity (B) Skin bumps severity (C) Redness severity (D) Dryness severity (E) Itchiness severity

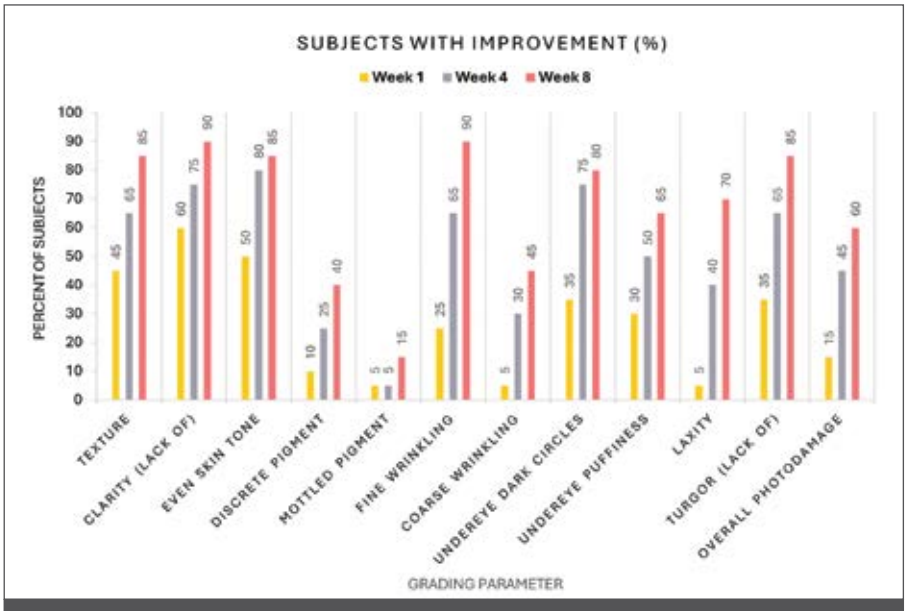

FIGURE 2. Subject improvement in skin cosmetic parameters. Values represent percentage of subjects with improvement in skin cosmetic parameter appearance based on clinical grading at Weeks 1 (yellow), 4 (gray), and 8 (red) compared to baseline (n=20)

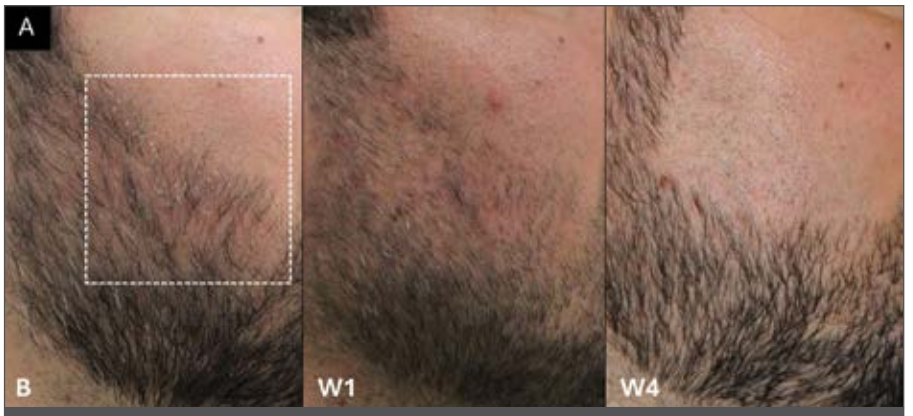

FIGURE 3. Facial profile photos highlight improvements in safety grading parameters including scaling/peeling following regimen use. Right profile of a 40-year-old male with Fitzpatrick skin type III at baseline (B), Week 1 (W1), and Week 4 (W4). Dashed box marks scaling/peeling observed at baseline

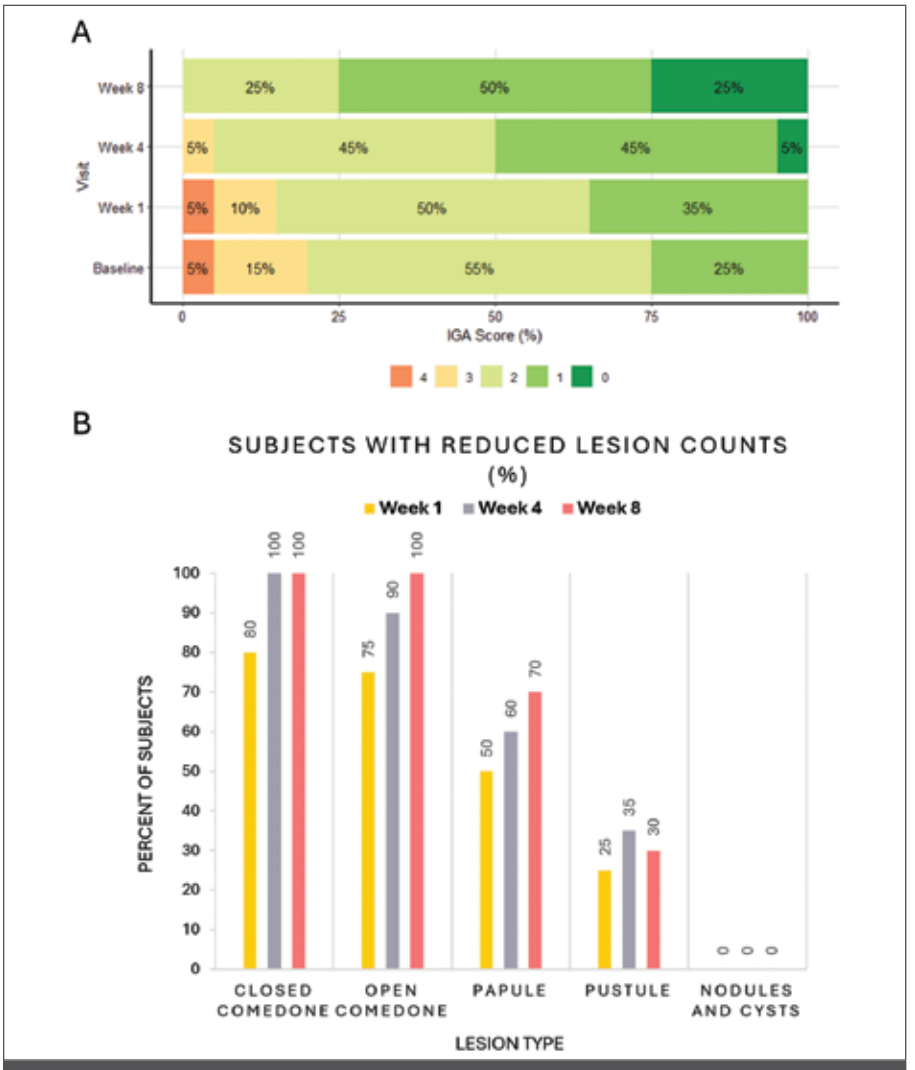

FIGURE 4. Lesion assessment outcomes by visit. (A) Breakdown of IGA scores by visit. Shown is the percentage of subjects in each IGA score category (n=20). (B) Percentage of subjects with reduced non-inflammatory (closed and open comedone) and inflammatory (papule and pustule) lesion counts at Weeks 1 (yellow), 4 (gray), and 8 (red) compared to baseline. Values represent improvement percentage averages across subjects (n=20)

to the test products by performing safety grading assessments of erythema, scaling/peeling, edema, burning/stinging, and itching at each visit. None of the study participants showed worsening of any of these grading parameters with regimen use.

Rather, subjects displayed statistically significant ($p<0.05$) improvements in dryness and erythema grading at all timepoints, including as early as Week 1. Of those who had dryness at Baseline, 88 percent had less dryness at Week 1, while 94 percent had less dryness at Weeks 4 and 8. Of those who had erythema at baseline, 54 percent improved by Week 1 and 77 percent improved by Weeks 4 and 8 (data not shown). Although grading scores improved for subjects who had itching and scaling/peeling at baseline, these changes were not significant due to the small number of individuals who experienced these symptoms at the start of the study. Not only was the product regimen well tolerated in study participants, but subjects also showed visible improvements in skin dryness and erythema throughout the course of the study (Figure 3). In combination with the clinically significant changes in all but one clinical grading parameter, these findings suggest that use of this XYCM42-based regimen can help support skin health and appearance in individuals with acne-prone skin.

Acne lesion assessments. Acne lesion assessments were also performed by the Study Investigator at every Dallas clinic visit (n=20 subjects). Blemish condition severity was assessed using the Investigator's Global Assessment (IGA) scale, and blemish lesion count was determined by the number of lesions (inflammatory and non-inflammatory) present on the face.

Subjects had statistically significant changes in their IGA scores at Weeks 4 and Week 8 when compared to baseline (Table 4). While a total of 15 percent of subjects showed improvement at Week 1, 50 percent of subjects showed IGA score improvement at Week 4, and this number increased to 70 percent of subjects by Week 8 (Figure 4A). It is also worth noting that by the end of the study, the IGA scores of five subjects (25%) were improved (decreased) from baseline by two or more points on the five-point IGA scale.

Blemish lesion counts of both non-inflammatory (open and closed comedones) and inflammatory (papules and pustules)

TABLE 3. Clinical grading parameter improvement over baseline

GRADING PARAMETER (COSMETIC)	SUBJECT IMPROVEMENT OVER BASELINE								
	WEEK 1			WEEK 4			WEEK 8		
	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE
Texture	-0.45	-0.69, -0.21	0.001*	-0.9	-1.33, -0.47	<0.001*	-1.2	-1.65, -0.75	<0.001*
Clarity	-0.65	-0.92, -0.38	<0.001*	-1.55	-2.15, -0.95	<0.001*	-2.1	-2.71, -1.49	<0.001*
Even skin tone	-0.6	-0.95, -0.25	0.002*	-1.35	-1.9, -0.8	<0.001*	-1.85	-2.42, -1.28	<0.001*
Discrete pigment	-0.1	-0.24, -0.04	0.163	-0.3	-0.57, -0.03	0.03*	-0.6	-1.01, -0.19	0.007*
Mottled pigment	-0.05	-0.15, -0.05	0.33	-0.05	-0.15, 0.05	0.33	-0.2	-0.44, 0.04	0.104
Fine wrinkling	-0.25	-0.46, -0.04	0.021*	-0.8	-1.13, -0.47	<0.001*	-1.35	-1.7, -1	<0.001*
Coarse wrinkling	-0.05	-0.15, -0.05	0.33	-0.35	-0.62, -0.08	0.015*	-0.6	-0.95, -0.25	0.002*
Undereye dark circles	-0.35	-0.58, -0.12	0.005*	-0.9	-1.2, -0.6	<0.001*	-1.15	-1.5, -0.8	<0.001*
Undereye puffiness	-0.25	-0.51, -0.01	0.056	-0.4	-0.72, -0.08	0.017*	-0.75	-1.12, -0.38	<0.001*
Laxity	-0.05	-0.15, -0.05	0.33	-0.5	-0.82, -0.18	0.004*	-0.9	-1.27, -0.53	<0.001*
Turgor	-0.35	-0.58, -0.12	0.005*	-0.75	-1.05, -0.45	<0.001*	-1.3	-1.7, -0.9	<0.001*
Overall photodamage	-0.15	-0.32, 0.02	0.083	-0.55	-0.87, -0.23	0.002*	-0.85	-1.26, -0.44	<0.001*

Mean changes in grading scores with 95% confidence intervals (CI) were calculated across subjects (n=20) for each grading parameter and visit. Outcomes were compared to baseline using paired *t*-tests.
*Denotes statistical significance ($p<0.05$)

TABLE 4. IGA score improvement over baseline by visit

VARIABLE	SUBJECT IMPROVEMENT OVER BASELINE								
	WEEK 1			WEEK 4			WEEK 8		
	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE
IGA score	-0.15	-0.32, 0.02	0.083	-0.5	-0.74, -0.26	<0.001*	-1	-1.4, -0.6	<0.001*

Mean IGA score changes and 95% confidence intervals (CI) were calculated across subjects (n=20) and outcomes were compared to baseline using paired *t*-tests.
*Denotes statistical significance ($p<0.05$)

TABLE 5. Lesion count improvement over baseline by visit and lesion type

LESION TYPE	SUBJECT IMPROVEMENT OVER BASELINE								
	WEEK 1			WEEK 4			WEEK 8		
	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE	CHANGE	95% CI	*P*-VALUE
Closed comedone	-2.85	-4.28, -1.42	0.001*	-5.55	-7.98, -3.12	<0.001*	-7.5	-10.7, -4.3	<0.001*
Open comedone	-3.8	-5.45, -2.15	<0.001*	-5.4	-7.45, -3.35	<0.001*	-6.65	-8.83, -4.47	<0.001*
Papule	-1.0	-1.64, -0.36	0.004*	-1.4	-2.24, -0.56	0.002*	-2.05	-2.98, -1.12	<0.001*
Pustule	-0.5	-0.94, -0.06	0.029*	-0.7	-1.29, -0.11	0.023*	-0.75	-1.34, -0.16	0.015*
Nodules and cysts	0	–	–	0	–	–	0	–	–

Mean changes in grading scores with 95% confidence intervals (CI) were calculated across subjects (n=20) and outcomes were compared to baseline using paired *t*-tests.
*Denotes statistical significance ($p<0.05$)

lesion types significantly decreased after just one week of regimen use and continued to decrease throughout the study when compared to baseline (Table 5). In assessing the percentage of subjects with reduced lesion counts, non-inflammatory lesions had the most robust changes, with 100 percent of subjects experiencing a decrease in the number of closed comedones by Week 4 and open comedones by Week 8 (Figure 4B). This is particularly notable, as all subjects enrolled in the in-clinic portion of the study had fewer non-inflammatory acne lesions after eight weeks of regimen application compared to baseline.

While subjects showed less reduction in inflammatory lesion counts, 50 percent of subjects had fewer papule counts at Week 1, and the percentage of subjects with reduced papule counts further increased to 60 percent and 70 percent at Weeks 4 and 8, respectively. Pustules showed the least percent reduction, but subjects still had statistically significant reductions in pustule counts, with 25 to 35 percent of subjects experiencing reductions in pustule lesion counts across visits (Figure 4B and Table 5).

These quantitative measures of acne lesion severity are supported by representative subject images which demonstrate noticeable reductions in both inflammatory and non-inflammatory lesions across visits (Figures 5–8).

Porphyrin analysis. Finally, the VISIA-CR system was used to analyze porphyrin levels to assess whether supplementation of the *C. acnes defendens* derivative XYCM42 strain alters porphyrin abundance on the face. Bacterial porphyrins are often associated with proinflammatory activities, with high levels often linked to human inflammatory diseases such as acne vulgaris. Various strains of both

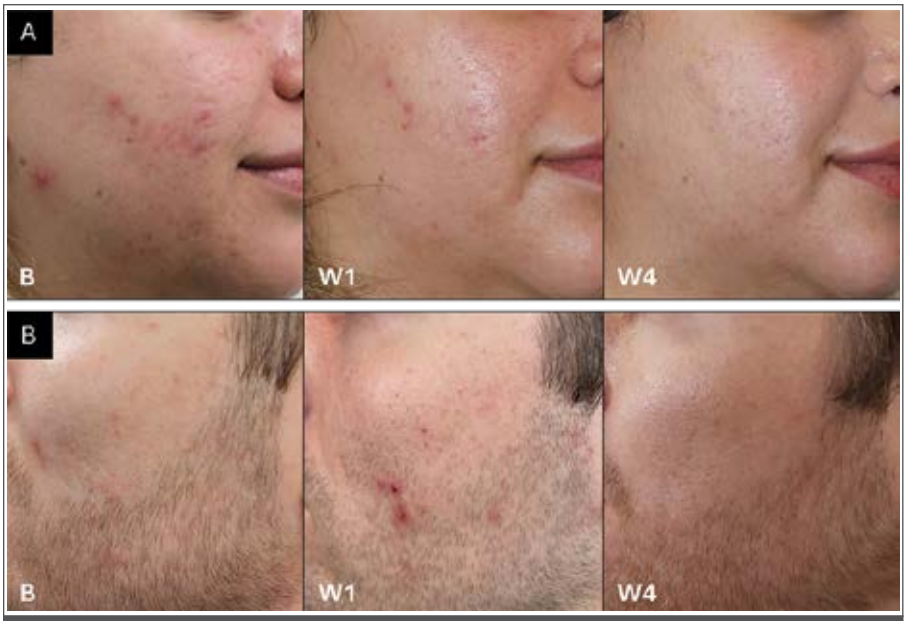

FIGURE 5. Facial profile photos taken at baseline (B), Week 1 (W1), and Week 4 (W4) demonstrate reduced lesion counts and improved appearance of skin texture and clarity as early as one week of regimen use. (A) Right profile of a 25-year-old female subject with Fitzpatrick skin type III. (B) Left profile of a 29-year-old male subject with Fitzpatrick Skin Type II

FIGURE 6. Left facial profile images taken at baseline (B), Week 4 (W4), and Week 8 (W8) of regimen use show inflammatory lesion improvement in a 33-year-old male subject with Fitzpatrick skin type II

Propionibacterium and *Cutibacterium* produce different levels of porphyrins and therefore may be more or less associated with inflammatory diseases depending on the strain and its degree of porphyrin production. The measurement of porphyrin expression can be used to guide the development of suitable products for acne and/or acne-prone skin by assessing their ability to modulate the skin microbiome and its production of metabolic materials such as porphyrins.[5–12]

The species of *C. acnes* has been shown to be the main producer of a particular porphyrin, Coproporphyrin III (CpIII), which has a distinct fluorescence emission signal from other porphyrins.[13] Therefore, CpIII porphyrin fluorescence can be used as a measure of *C. acnes* metabolic activity. However, it has been observed that strains of *C. acnes* associated with skin health produce low amounts of porphyrins (if any), while strains associated with acneic skin tend to secrete significantly more.[14]

Quantitative porphyrin fluorescence analysis was performed for all subjects who were observed for in-clinic evaluation at the Dallas clinic site. The results of VISIA-CR porphyrin analysis revealed a rather steady porphyrin count across visits with consistent measurements of 3,000 to 4,000 at all timepoints (Figure 8B), suggesting that the addition of living cultures of strain XYCM42 does not produce clinically significant amounts of porphyrins nor elicit their potentially inflammatory effects.

DISCUSSION

Acne-prone skin is generally considered to be susceptible to factors which can induce either existing skin disease or generate new acneic lesions. Many previous studies have used acne-prone skin to assess topical products. Additionally, it has been shown that skin with a history of acne, even if observationally healthy, continues to express microcomedos and infundibular hyperkeratosis, as measured by reflectance confocal microscopy.[15] This sets this testing and product evaluation model as ideally suited and likely sensitive to any perturbations of the skin to induce observable acneic lesions. The potential for a regimen to worsen or differentially influence the state of acne or acne-associated skin conditions in acne-prone individuals makes this patient base both appropriate and important for this clinical study.

Results from this in-clinic efficacy evaluation study demonstrated that the skin biome care regimen had clinically significant benefit in nearly all measured parameters at all timepoints. This was consistent for all ages, genders, and Fitzpatrick skin types. Among the skin cosmetic clinical grading parameters, only mottled pigment (all timepoints) and laxity, turgor, undereye puffiness, and overall photodamage (Week 1 only) did not reach significance, although each directionally improved (Figure 2). Quantitative measurement of skin porphyrins showed a general reduction in porphyrin counts for the majority of the subjects at Weeks 1 and 4; however, these changes did not reach significance at any timepoint (Figure 8B). Porphyrins are often associated with pro-inflammatory skin conditions. While not significant, these trending reductions may indicate a slight shift of the bacterial composition of the skin. Variations in porphyrin levels may also reflect a change in bacterial levels. Since health-associated strains of *C. acnes defendens* do not secrete significant levels of inflammatory porphyrins,[14,16] increased abundance of an isolated *C. acnes defendens* strain via regimen application may reduce the inflammatory potential of the skin microbiome as a whole. This might explain, in part, the significant reductions in erythema and skin dryness observed at all timepoints. All other safety endpoints were either directionally

improved or never observed during the study period.

In subject-reported questionnaires in which participants rated their experience using the skin biome care regimen, all subjects rated favorable agreement in 20 of the 21 questions (Table 2). Only "my skin is blemish free" did not reach study significance, although 48 percent of subjects by Week 4 and 53 percent of subjects by Week 8 agreed to being "blemish free". It should be reiterated that this regimen is not an over the counter (OTC) or prescription acne treatment. The purpose of this study was to assess if the XYCM42-based regimen is non-acnegenic and safe for individuals with a predisposition to acne. The product regimen was not intended to treat acne or improve skin clarity, yet it is worth noting that a majority of the subjects agreed to having "clear skin" by Week 8. This result is supported by the 54-percent reduction in subject-rated breakout severity scores. Additionally, subjects reported less redness at the end of the eight-week study. More research is needed to characterize how XYCM42 influences skin health and appearance, as observed in this and other studies, following its engraftment in the skin.

All subjects were photographed for study documentation, providing the Study Investigators with a general representation of each subject's skin condition. As was expected, the quality of subject photography varied across research sites. Specifically, lighting was not always consistent between visits, images lacked sufficient resolution in some cases, and most notably, it is difficult to capture blemish lesion severity when imaging full facial profiles. For these reasons, subject images did not always reflect the visible changes seen by eye or the quantitative data reported herein. However, it should be noted that subject photographs were not used for any of the clinical grading assessments.

Previously, this skin biome care regimen based on a living isolated strain of *C. acnes defendens*, XYCM42, has demonstrated benefit in aged and photodamaged skin[3] and after aesthetic microneedling for treatment of acne scars.[4] While there is growing evidence that *C. acnes* is not the only factor relating to acne, including findings that the relative quantity of *C. acnes* are similar between acneic and non-acneic skin, at least in adult skin,[17,18] and that some phylotypes of *C. acnes* are more likely than others to induce acneic or problematic skin than others,[19] there is still a continued effort to develop new technologies that target and eliminate all *C. acnes* bacteria.[20,21] However, targeting all *C. acnes* strains instead of selectively targeting phylotypes linked to acne increases the potential for further disruption of the overall homeostasis of the skin microbiome, thus exacerbating microbiome dysbiosis.[22] Discussions on online forums and social blogs continue to point to *C. acnes*, formerly *Propionibacterium acnes*, as the causation dogma of facial acne and breakout responses, often without much discussion of the considerable evidence to the contrary. Similarly, online communications often fail to present the importance and benefits that non-acneic and commensal *C. acnes* bacteria can have on the skin. By demonstrating that daily application of the *C. acnes defendens* strain XYCM42-based skin biome care regimen does not cause or exacerbate acne, this study further dispels the minimalistic position that as a general bacterial species, *C. acnes* is the major determinate and cause of acne breakouts.

FIGURE 7. Facial profile photos at baseline (B) and Week 8 (W8) show reduced lesion counts pat the end of the study when compared to baseline. (A) Right profile of a 19-year-old female subject with Fitzpatrick skin type IV. (B) Right profile of a 30-year-old female subject with Fitzpatrick skin type II

Together, this study and the two prior studies have demonstrated benefits from the skin biome care regimen across several skin types, including aged/photodamaged, acne-prone, and with a compromised skin barrier following microneedling.[3,4] No negative skin changes were demonstrated with the use of this regimen in any of these studies. In fact, each study demonstrated an overall positive outcome. Some of the possible bases for these skin benefits have been discussed previously,[3,4] but recent studies have proposed additional potential contributors. One such component is extracellular vesicles that are produced by the live *C. acnes* bacteria. These vesicles are potentially found within the topical bacterial ferment product that is incorporated as part of the skin biome care regimen. Extracellular vesicles, like eukaryote cellular exosomes, may play a role in host cellular signaling and skin microbiome activity such as reducing inflammatory signals and/or modifying cellular production of sebum.[23] Additional work in ascertaining the components released by XYCM42 bacteria and their positive

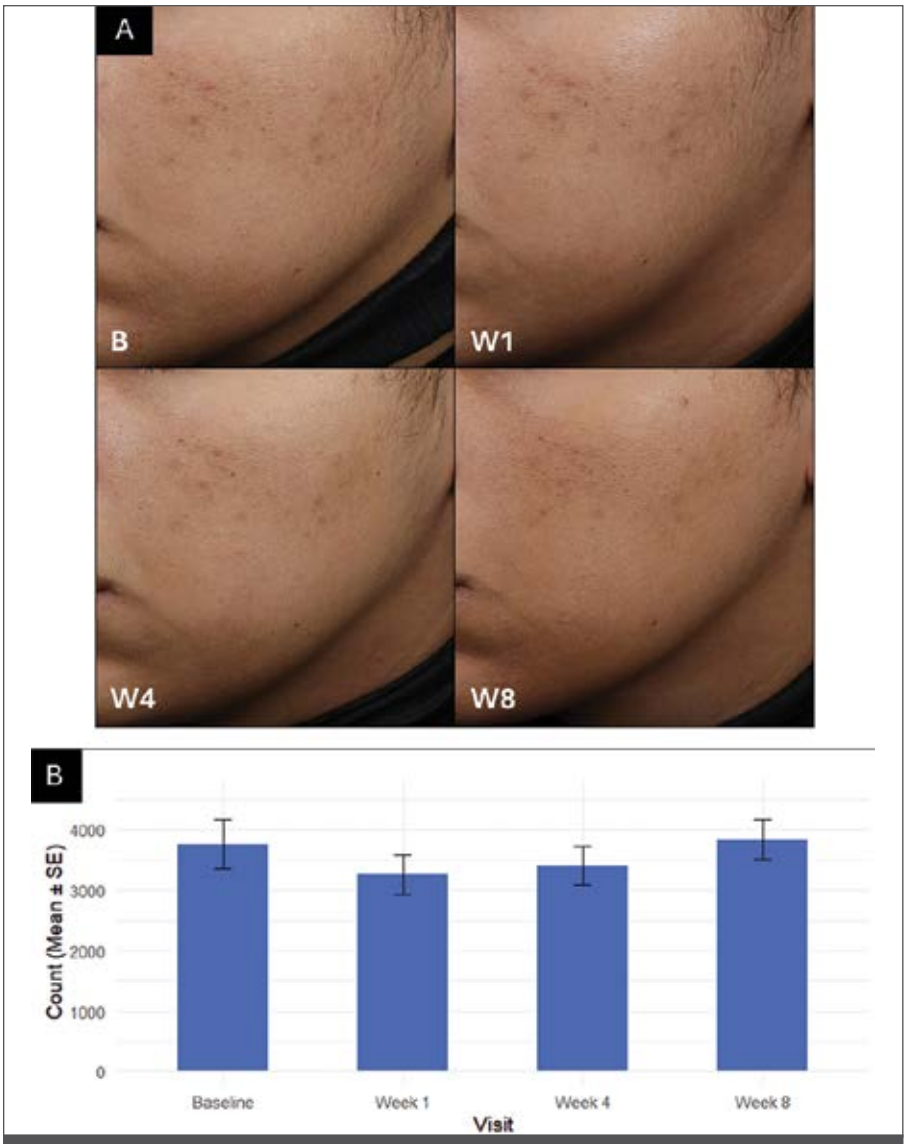

FIGURE 8. Representative VISIA-CR images and mean porphyrin counts at baseline (B), Week 1 (W1), Week 4 (W4), and Week 8 (W8). (A) Left profile of a 19-year-old female subject with Fitzpatrick skin type IV shows reduced appearance of non-inflammatory lesions with regimen use. (B) Mean porphyrin counts by visit. Shown is mean ± standard error (SE) for all Dallas clinic subjects (n=20)

contributions to the skin environment is required to better understand how this XYCM42-based regimen helps promote skin health.

CONCLUSION

The results of this study provide confirmation that the *C. acnes defendens* strain XYCM42-based skin biome care regimen is suitable for individuals with acne-prone skin. Daily application of the previously described and tested live *C. acnes* XYCM42 bacteria along with its conditioned postbiotic ferment derived from the active bacterial culture did not increase the propensity of any indicator of acne or skin reactivity in this subject population. Instead, regimen use yielded a reduction in both the incidence and severity of conditions associated with acne-prone skin, including blemish lesions, redness and erythema, and dryness. Additionally, the study participants' self-reported skin assessments supported an overall favorable outcome at each timepoint.

Overall, the findings presented herein demonstrate that use of the XYCM42-based topical probiotic regimen is both safe and appropriate for individuals with acne-prone skin. The regimen did not exacerbate acne or cause an acnegenic response. While the authors believe the evidence presented in this study is compelling, the results should not be considered conclusive given the size of the study. A larger cohort could be considered for additional studies to allow for greater resolution and opportunity for rare occurrences of possible adverse events over a broader population. Further studies are also required to understand how the XYCM42 bacteria and its postbiotic ferment influence skin health at the cellular level.

ACKNOWLEDGEMENTS

We would like to acknowledge Blueberry Marketing & Sensory Research (Chalfont, Pennsylvania) for their support with subject recruitment, test product management, online questionnaire administration, and data evaluation. We would like to thank Lei Wang for data and statistical analyses. We would also like to acknowledge Abigail Watterson, PhD, for editorial support and assistance with manuscript preparation and John Harbell, PhD, for manuscript review and edits.

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