Title: The Use of Topical *Nitrosomonas eutropha* for Cosmetic Improvement of Facial Wrinkles

Running Head: Topical *Nitrosomonas eutropha* for Improvement of Wrinkles

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Conflicts of interest: CCL, LBW, and LA are employees of AOBiome at the time of the writing of this manuscript. RKS serves as a scientific advisor for Dermveda and as a consultant for Burt's Bees and Dermala.

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Background: Both topical and oral probiotics are becoming widely used. There is increasing interest in the cosmetic potential in topical probiotics. *Nitrosomonas eutropha* is an ammonia oxidizing bacteria.

Aim: The purpose of this study was to assess whether there is any improvement in facial wrinkles with the use of *Nitrosomonas eutropha*, a topical probiotic.

Methods: In this prospective study, high-resolution photographs were obtained in twenty-nine participants at baseline and after using topical *Nitrosomonas eutropha* for seven days.

Results: There was a significant difference in wrinkle depth and severity in the high concentration probiotic group. There was also a statistically significant improvement in pigmentation of the forehead and glabella in the higher concentration group.

Conclusions: *Nitrosomonas eutropha* may have aesthetic benefits in terms of reducing the appearance of wrinkles. Larger studies with longer treatment and follow up periods are required.

Keywords: photography, wrinkles, pigmentation, probiotics

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Introduction

Probiotic and prebiotic therapies have expanded within dermatology with both oral and topical use.[1] Topical probiotics appear to have a range of effects including anti-inflammatory, anti-microbial activities, and cosmetic.[1-3] While many of the studies have focused on health related outcomes, the cosmetic potential of probiotic and prebiotic therapies is gaining interest.

Although there have been few studies assessing the cosmetic results of probiotics on the skin, several studies have suggested that topical probiotics can promote

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aesthetic effects on the skin. Topical application of *Streptococcus thermophilus* on the forearms of 20 healthy women improved skin hydration.[4] Topical application of *Lactobacillus plantarum* HY7714 was found to have multiple aesthetically beneficial effects including a decrease in facial wrinkle depth after 12 weeks of topical application, improvement in facial skin gloss after one week, and an increase in skin elasticity of 13.2% at 4 weeks and 21.7% at 12 weeks.[5]

Nitrosomonas eutropha is a non-pathogenic bacteria that has been postulated to have multiple health benefits. As an ammonia-oxidizing bacteria (AOB), *Nitrosomonas eutropha* utilizes ammonia from sweat to generate nitrite and nitric oxide. Studies have shown that nitrite and nitric oxide are associated with beneficial physiological effects including vasodilation, anti-inflammatory properties, improved wound healing, and reduced blood pressure.[6-9] In pre-clinical studies, *Nitrosomonas eutropha* led to a reduction in pathogenic bacteria on the skin (e.g. *Propionibacterium acnes*), as well as improved skin healing.[10] A phase 2 clinical trial investigating the effects of *Nitrosomonas eutropha* delivered as a topical spray for the treatment of mild to moderate acne has been completed, but the results have not yet been reported.[11] The purpose of this study is to investigate the effect of aerosolized live *Nitrosomonas eutropha* on wrinkles, radiance, and pigment intensity.

Methods

Twenty-nine participants were recruited to participate in the study at the UC Davis Department of Dermatology. The participants were split into two groups with the first group (ages: 23-41; 4 females and 6 males) receiving a low concentration topical treatment and the second group (ages:19-61; 11 females and 8 males) receiving a high concentration topical treatment. The study was approved by the Institutional Review Board of the University of California, Davis. All participants provided written informed consent prior to participation.

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Participants were included if they had clear skin. Participants were excluded if they were pregnant, started any new oral medication within four weeks, use of systemic antibiotics within one month, use of isotretinoin within six weeks, use of topical antibiotics or retinoids within the past two weeks, autoimmune or metabolic disorders, active skin infections, switched brands of oral contraceptives within two weeks, syncopal episodes, concomitant use of anti-hypertensives for less than one year, and concomitant use of nitrates or syncopal episodes.

Each participant completed a seven-day washout period using Mother Dirt Cleanser (Mother Dirt, Cambridge, MA) and returned for their baseline visit without washing their face or showering for 24 hours. BTBP 3D Clarity Pro® Facial Modelling and Analysis System (Brigh-Tex BioPhotonics, San Jose, CA) was utilized to obtain high-resolution facial photographs for all study participants. These images were obtained at baseline. Participants were then supplied with aerosolized live *Nitrosomonas eutropha* in buffer (AOBiome) to apply twice a day. The first ten subjects were provided with *Nitrosomonas eutropha* at a concentration of $1x10^9$ cells/ml (low concentration group) and the remaining nineteen subjects were provided with *Nitrosomonas eutropha* at a concentration $8x10^9$ cells/ml strength (high concentration group). Images were then obtained after a week of using the product.

Facial wrinkles, pigmentation and radiance were quantified through computer algorithm based modelling. Three cameras simultaneously obtained images to allow for triangulation of wrinkle to assess severity, which is a combination of depth and length. Pigmentation and radiance were based on intensity of lightness and reflection, respectively. The photographs were obtained using a chin platform and forehead stop allowing for reproducible position of the face for image and analysis purposes. The algorithm utilized for the image analysis has been previously clinically validated for all of the measures.[12-14]

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This group of participants was originally recruited to assess changes in the facial microbiome and the results of this assessment will be reported elsewhere. Post-hoc power analysis showed that in both groups there was greater than 90% power in detecting a 10% change in wrinkle width at an alpha = 0.05.

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Results

Wrinkles

Wrinkle depth and severity did not change in the low concentration group (Figure 1). However, both wrinkle severity and depth improved in the high concentration mist group on the forehead, glabella and the overall face.

Pigmentation

The low concentration group did not have any change in pigment (Figure 2). The high concentration group had a statistically significant improvement on the forehead and the glabella, but there was not overall change in pigment intensity for the face overall.

Radiance

The low concentration group statistically improved in facial radiance on the glabella (0.81%) and the face overall (0.77%) while the high concentration group statistically improved in radiance overall on the forehead (0.9%), glabella (0.48%), and the face overall (0.96%). However, the changes were subtle and likely not clinically significant. Results are shown in Figure 3.

Overall changes in facial features are depicted in Figure 4.

Discussion

Environmental factors (e.g. extensive sun exposure) and aging lead to skin wrinkle formation, skin radiance reduction, and pigment changes. Topical probiotics seem

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promising in that they have the potential to improve facial skin function and provide aesthetic benefits.

In this study we demonstrated that there was a significant reduction in the wrinkle severity on the glabella with the use of a probiotic mist containing *Nitrosomonas eutropha* (Figure 2) at a higher concentration. The same results were not seen with the lower concentration. In this study, the low concentration group utilized the same topical vehicle as the high concentration group and the results appear to be related to the concentration of the bacteria used. Although there are no previous studies on the use of *Nitrosomonas eutropha* for treating facial wrinkles, our findings are in agreement with Lee et al,[5] which demonstrated that topical application of probiotic *Lactobacillus plantarum* HY7714 reduced facial wrinkle depth. Although we did not identify a specific mechanism of action, ammonia oxidizing bacteria are known to modulate nitric oxide [15] and changes in local nitric oxide appear to be important in cutaneous aging .[16]

The pigment intensity was not changed while there was a subtle improvement in radiance (likely not perceptible). In this study, the recruited participants did not have cutaneous disease and those with overt signs of melasma were excluded. Our results show no evidence that topical *Nitrosomonas eutropha* could be helpful with pigmentation. The change noted in radiance was likely not clinically significant although it was statistically significant. However, since our study was only over the course of a week, it may warrant a follow up study with the higher concentration mist over a longer period of time.

Our study has several limitations. This study was an open-label study which can introduce the possibility of bias. However, the assessments were performed with use of computer based assessments that allows for objective assessments and reduces the possibility of bias. A second limitation is that the topical treatment was used only over the course of a week. This likely mitigates the ability to capture changes in pigmentation and radiance. However, the fact that we noticed a change in

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wrinkles is notable, especially since both the low and high concentration groups utilized the same vehicle. A change purely due to the vehicle is less likely. Therefore, the change in wrinkles is interesting and warrants further study. Finally, our study was conducted as a pilot study and the future studies with an expanded number of subjects will be needed to follow up on the results reported here.

Conclusion

While our results suggest that aerosolized live *Nitrosomonas eutropha* may provide aesthetic benefits by reducing the appearance of wrinkles. Further research is needed to determine the exact mechanism by which *Nitrosomonas eutropha* provide these aesthetic benefits. Additionally, studies with larger sample size and longer treatment duration and follow up period are needed to confirm the findings presented here. Nevertheless, this study highlights the cosmetic potential of topical probiotics.

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Figure Legend

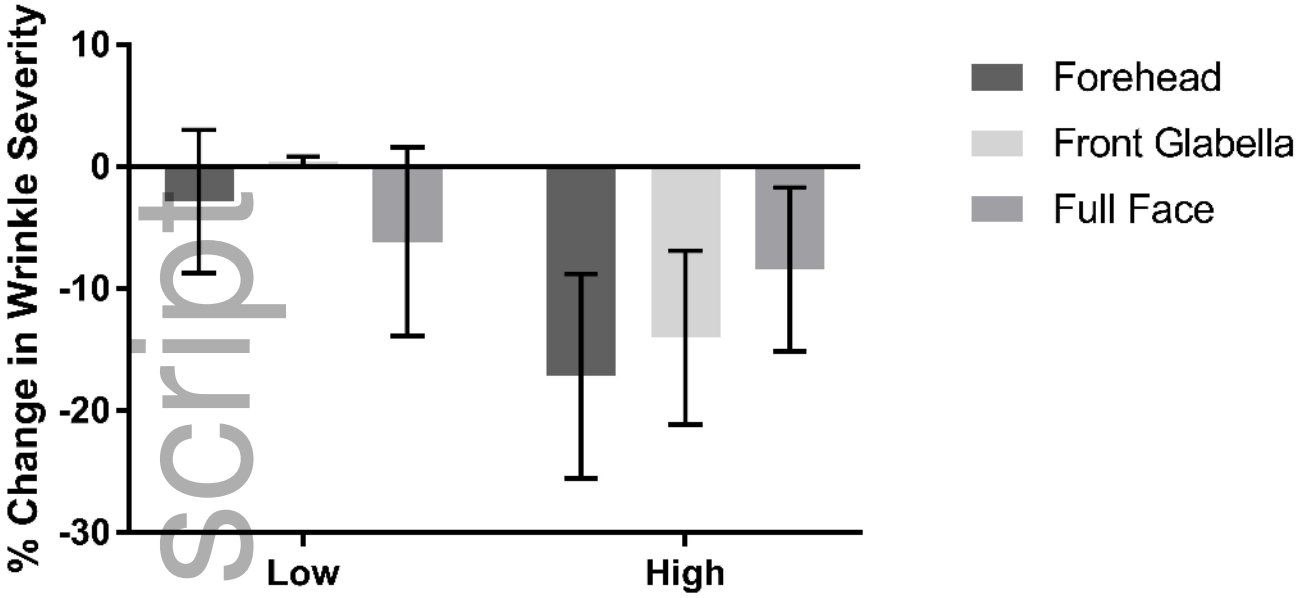
Figure 1. Percentage change in wrinkle severity in the low and high concentration *Nitrosomonas eutropha* groups

Figure 2. Percentage change pigment intensity in the low and high concentration *Nitrosomonas eutropha* groups

Figure 3. Percentage change radiance in the low and high concentration *Nitrosomonas eutropha* groups

Figure 4. Facial photographs before (A) and after (B) the topical application of high concentration *Nitrosomonas Eutropha* for one week

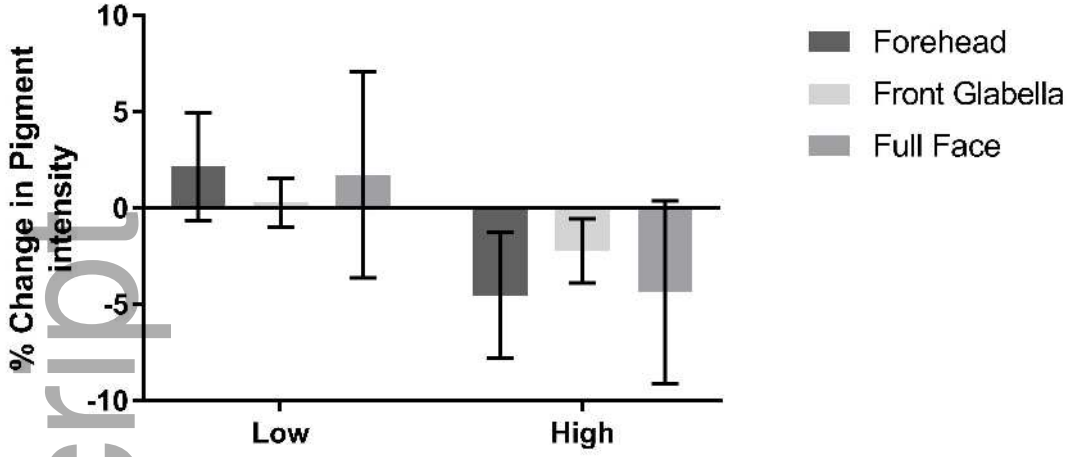
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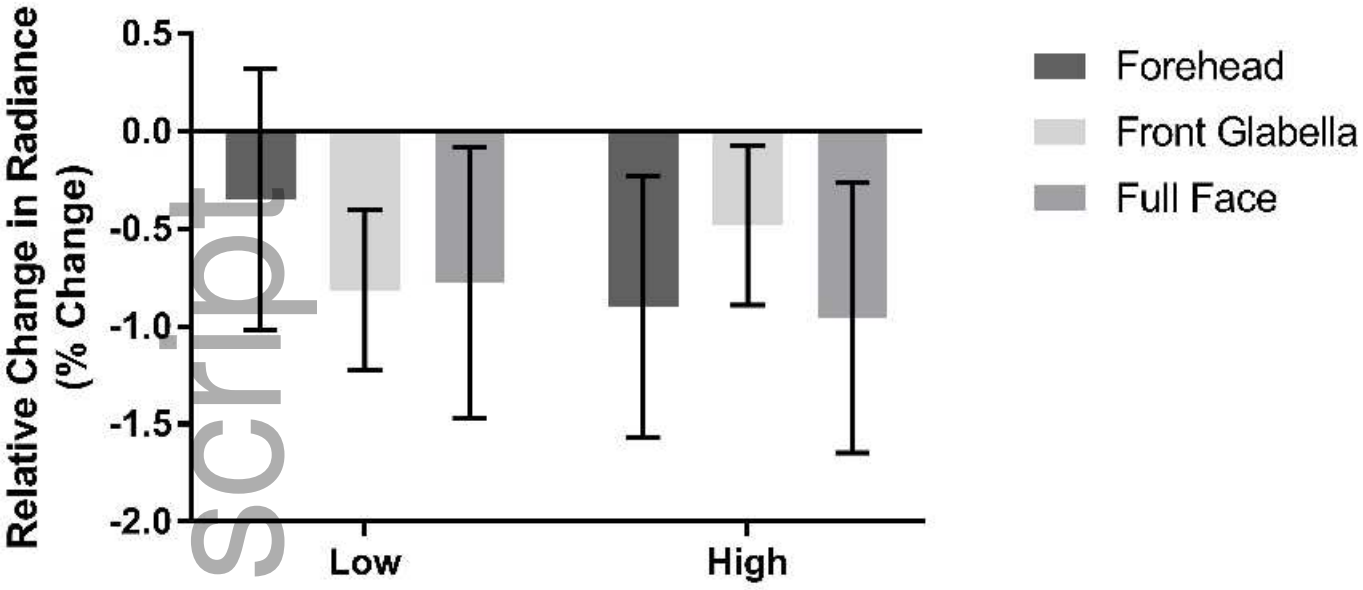
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