Skin microbiome dynamics in patients with polymorphic light eruption in response to UV radiations

Yacine Amar,[1] Sebastian Niedermeier,[1] Rafaela Silva,[1] Susanne Kublik,[2] Michael Schloter,[2] Tilo Biedermann,[1] Martin Köberle[1†] and Bernadette Eberlein[1†]

1 Department of Dermatology and Allergy, School of Medicine, Technical University of Munich, Munich 80802, Germany.
2 Research Unit Comparative Microbiome Analysis, Helmholtz Center Munich, Munich, 85764, Germany.

†These authors contributed equally to this study

Corresponding author: Yacine Amar

Email: yacine.amar@tum.de

Funding sources: This work was supported by the Ulm Dermatology Prize for the year 2023 which was awarded by the University of Ulm (Germany) to Dr. Yacine Amar. This work was also supported by grants from Deutsche Forschungsgemeinschaft (CRC1371 (TP06; project number 395357507), RTG 2668 (TPA2; project number 425874434 and BI 696/14-1 project number 527318848) attributed to Pr. Tilo Biedermann.

Conflicts of interest: The authors declare that they have no competing interests.

Data availability: The datasets supporting the conclusions of this article are included within the article and its additional files. The generated 16S sequencing files have been uploaded to the NCBI database and made available at the following link: https://submit.ncbi.nlm.nih.gov/subs/sra/SUB14820309/files.

Ethics statement: This study was approved by the ethics committee of the Technical University of Munich under the study number 2024-216-S-CB. Individual written informed consent was obtained in advance of photoprovocation testing and of any sample collection.

Patient consent: Written patient consent for publication was obtained.

What is already known about this topic?

- Polymorphic light eruption (PLE) shows a similar pattern to a delayed-type hypersensitivity reaction to a yet unknown allergen.
- Microbial antigens were suggested to exacerbate PLE inflammation.

What does this study add?

- This is the first study investigating the cutaneous microbiome changes in PLE patients upon photoprovocation with UVB and UVA irradiation, offering new insights into disease pathogenesis.
- The growth patterns of different isolated skin commensals and pathogens based on our microbiome data analysis were assessed upon exposure to UVB and UVA irradiation.

What is the translational message?

- PLE skin is characterized by a dysbiotic microbiome, already at baseline, with significantly reduced diversity and noticeable colonization by S. aureus.
- The bactericidal effects of UVR and particularly UVA might transiently scramble the microbiome composition, favoring the development of a dysbiotic microbiome.
- An overgrowth of dysbiotic taxa in PLE lesions upon photoprovocation may be accompanied by the release of microbial ligands able to trigger a strong inflammatory response, given the lack of immune-suppression in PLE patients.

Abstract

Background Polymorphic light eruption (PLE) is the most frequent photodermatosis in Europe with an estimated prevalence of 10 to 20%, particularly in temperate climates. Itching or burning lesions appear only in sun-exposed areas, predominantly on the chest, the arms and forearms within a few hours following exposure. The disease's cause is still unknown, yet studies have suggested that skin microbial elements may play a role in its pathogenesis.

Objectives We investigated in this cohort the skin microbiome of PLE patients upon exposure to ultraviolet radiation (UVR), to assess its role in the onset of PLE lesions.

Methods Forty-one skin swabs have been collected from eleven PLE patients at baseline and following a three-day exposure to UVR and from healthy controls. The collected swabs were analyzed for their microbial composition using a 16S amplicon sequencing approach.

Results The PLE skin showed a dysbalanced microbiome, already at baseline, with significantly reduced microbial diversity and noticeable colonization by bacterial pathogens as *Staphylococcus aureus*. Upon UVR exposure, the PLE microbiome exhibited a further loss of diversity and decline of beneficial skin commensals. In line with this, we observed that UVR exerted strong antimicrobial effects *in vitro* against representative skin residents.

Conclusions Taken together, UVR can lead to profound skin microbiome changes, allowing the proliferation of dysbiotic members that can release a variety of elements able to trigger PLE lesions. This is the first study investigating the cutaneous microbiome changes in PLE patients upon UVR, offering new insights into disease pathogenesis, so far unexplored.

Introduction

Polymorphic light eruption (PLE) is the most common photodermatosis in Europe, with an estimated prevalence of 10 to 20%, particularly in temperate climates. It usually appears in the third decade of life and is up to four times more frequent in women than men. PLE affects all skin types, with the highest prevalence in people with skin type I (Fitzpatrick classification).[1,2] Itching or burning lesions appear only in sun-exposed areas predominantly on the V-area of the chest and on the arms and forearms within a few hours after UVR exposure.[3]

The cause of the disease is still unknown. Yet, it has been suggested that mutations in glutathione S-transferases (GSTs) genes may be responsible for a reduced capacity to neutralize reactive oxygen species produced upon UV exposure in PLE patients.[4] However, we have previously examined the relationship between GSTs polymorphism for GSTM1, GSTT1 and GSTP1 genes and possible role in PLE, but could not find any significant association.[5] The cutaneous immunity has also been hypothesized to play a role in PLE development. Indeed, it was demonstrated that PLE patients exhibit less UV-induced immunosuppression due to low IL-10 cytokine levels. This may facilitate immune responses against cutaneous or microbial neo-antigens released upon UVR, with similar patterns to a delayed-type hypersensitivity (DTH) reaction.[6] In line with this, high levels of the pro-inflammatory cytokine IL-36 were detected in the skin and peripheral blood of PLE patients, indicating not only the activation of local but also systemic immune responses.[7] Also, Langerhans cells were reported to resist UVR in PLE patients unlike controls and remain in the skin to further potentiate the DTH reaction.[8] Furthermore, an impaired immunosuppressive function of the regulatory T cells has been reported in PLE[9], whereas tissue-resident memory $CD8^+$ T cells, were shown to infiltrate PLE lesions.[10]

Moreover, microbial antigens were suggested to exacerbate PLE inflammation by inducing the release of antimicrobial peptides (AMPs).[11] This is supported by the unique profile of differentially expressed AMPs detected in PLE lesions.[12] Importantly, a skin microbiome dysbiosis was observed in several inflammatory disorders[13,14] yet no study has carried out a detailed analysis of the skin microbiome of PLE patients to investigate its role in the pathogenesis of this disease. Here we characterize the skin microbiome changes in PLE patients before and following a photoprovocation, looking for microbiome signatures that may explain the onset and/or exacerbation of PLE lesions. To this aim we selected 11 PLE patients under photoprovocation with healthy matched controls and analyzed their skin microbiome using 16S rRNA gene metabarcoding to assess changes in bacterial community structure.

Methods

Subjects

Medical ethical committee approval (study number 2024-216-S-CB) and individual written informed consent were obtained in advance of any sample collection and photoprovocation. Eleven patients (1 male, 10 females; mean age 45.6 years, range: 28 to 76 years) with a history of PLE have been recruited for this study in addition to healthy age and gender matched controls (1 male, 10 females; mean age 42.6 years, range: 27 to 62 years). The skin type of the study participants was classified according to Fitzpatrick grading[15] and various clinical parameters were recorded using questionnaires. Microbiome samplings have been performed according to guidelines from the Human Microbiome Project. [16] Briefly, participants were instructed to not use any antibiotics or corticosteroids before sampling for at least 7 days if applied topically or 4 weeks in case of systemic administration. Inclusion criteria for healthy controls consisted of the absence of any current or prior chronic skin disorders or use of systemic antibiotics in the preceding 6 months. The participants were also asked not to shower or wash the sampling area at least 24h before swabs' collection (See Supplementary methods).

Photoprovocation

The minimal erythema dose (MED) for UVB-rich irradiation was determined prior to provocation of PLE on the lower back: six skin areas were exposed to increasing doses of broadband UVB radiation ranging from 25 mJ/cm^2 to 150 mJ/cm^2 in steps of 25 mJ/cm^2. After 24 h the MED was determined by visual assessment of a sharp erythema. To induce PLE, three test areas (each 8x5 cm) on the lower arm were irradiated on three consecutive days. One test area was irradiated with 1.5 MED, the second with 100 J/cm^2 UVA and the third test area with a combination of 1.5 MED and 100 J/cm^2 UVA. Evaluation criteria for the photoprovocation test were papules/vesicles and itching at the test sites. Development and severity of these criteria were scored according to the following scheme: grade 0 (absent), grade 1 (mild), grade 2 (moderate) and grade 3 (severe). The final evaluation was done 24 h after the last irradiation (See Table 1). Noteworthy, controls did not undergo a photoprovocation.

Specimen collection

For microbiome analysis, skin swabs were collected from eleven patients with PLE history at baseline (D0) before a photoprovocation phase of three days, then one day (D4) and one week (D10) after photoprovocation from areas exposed to a combined UVB and UVA radiations. Additionally, microbiome samples were taken from eleven healthy matched controls at the same skin areas, namely the lower arm. About 20 µl of the collected suspension was diluted and plated on non-selective agar plates for the

determination of bacterial colony forming units following an incubation period of 48 h at 37°C. The microbial DNA was extracted using a benzonase pre-digest approach that we optimized to assess the living skin microbiota[17] and the obtained DNA samples were stored at -80°C until further processing.

16S rRNA gene amplification and downstream processing

A 16S rRNA gene amplicon sequencing approach targeting the V3-V4 regions was used to explore the microbial diversity.[14] Next, the PCR products were indexed using the Nextera XT Index Kit v2 Set B. A composite pool was prepared by combining 4 nM of purified amplicon samples ensuring equal representations of barcoded libraries. Control samples did not generate amplicons and therefore have been added at equal volumes instead. The final pool was sequenced on an Illumina MiSeq platform with a PE300 v3 cartridge generating up to 25 million of 2×300 bp reads. The obtained V3-V4 reads were analysed according to the UPARSE method as implemented in the IMNGS platform.[18] We have previously optimized the workflow for samples handling and analysis using skin mock communities, and our method enabled accurate taxa identification with up to 99% similarity[17] (See Supplementary methods).

Effects of UVR on the growth of selected skin commensals and pathogens

In order to assess the direct effects of UVR on the cutaneous microbiome, we isolated different skin commensals and pathogens based on our microbiome data analysis and evaluated their growth patterns upon exposure to UVR. The inhibitory action of UVA, UVB or their combination was tested against a total of twelve skin commensal and pathogenic strains that were respectively collected from healthy and AD patients from a previous cohort. Three *Staphylococcus aureus* strains (1-3) have been collected from the lesional skin of patients with severe atopic dermatitis and the other 9 strains, namely three *Staphylococcus epidermidis* (1-3), two *Staphylococcus hominis* (1, 2), two *Micrococcus luteus* (1, 2), one *Corynebacterium stearicum* and one *Moraxella osloensis* were isolated from healthy volunteers. Similar to the cohort design of phototesting in PLE patients, the isolated strains were exposed to comparable UVR doses on 3 consecutive days. Bacterial suspensions were exposed either to UVA (100 J/cm²), to UVB (100 mJ/cm²), or their combination (See Supplementary methods).

Results

Photoprovocation

Three patients had a MED of 50 mJ/cm^2, three of 75 mJ/cm^2 and five of 100 mJ/cm^2. In two patients the phototesting resulted in a severe PLE, in three patients a moderate PLE was induced and in two patients a mild PLE. In four patients the PLE-lesions could not be provoked. Two patients did not attend the last

sampling visit on day 10, due to personal reasons and the missing data were disregarded. For the participants developing PLE the combined UVB/UVA test area was always affected (For details see Table 1).

A loss of microbial diversity and increased microbial loads upon photoprovocation

The aim of this study was to investigate whether an exposure to UVR would trigger microbiome changes associated with the onset of PLE lesions. Skin swabs were collected prior to and following photoprovocation on three consecutive days as depicted in Figure 1a. A total of 41 samples were analysed using 16S rRNA gene ribotyping yielding 2.08×10^6 filtered reads with an average count of 5.09×10^4 high quality reads per sample. We first performed a β-diversity analysis, a distance-based approach for inter-group comparison using PCoA plots. The overall microbiome composition did not exhibit major differences between matched controls and the PLE group at baseline (D0), at day 4 or 10 days following phototesting (Figure 1b). However, the microbial loads on UV-induced lesions showed significant increases at day 4 that dropped to baseline levels one week after the last photoprovocation (day 10) (Figure 2a). Importantly, we noticed a significantly reduced microbial diversity on PLE patients' skin already at baseline and during UV provocation (Figure 2b). Indeed, compared to controls the values of richness as well as Chao1 and ACE were clearly reduced in PLE patients and even decreased further upon three days of photoprovocation (D4). Also the Shannon index that accounts for both richness and taxa relative abundance showed similar patterns, although not reaching significance. We furthermore observed that at day 10 the microbial diversity was restored to baseline levels in PLE patients. Taken together our data indicate that PLE skin is characterized by a loss of microbial diversity and that members of this dysbiotic microbiome significantly expand on UV-induced lesions.

High baseline S. aureus proportions and loss of commensals in PLE skin upon phototesting

Next, we performed a taxonomy analysis to gain insight into bacteria thriving on PLE skin. Analysis at phylum level revealed, *Actinobacteria*, *Firmicutes* and *Proteobacteria* as the topmost abundant phyla, comprising 97.41% of all sequences, across all individuals and both prior and after photoprovocation. In contrast to healthy controls, a lower abundance of the *Firmicutes* phylum and dominance of *Actinobacteria* was observed in PLE skin at baseline. After photoprovocation we detected a slight increase in the *Firmicutes* and *Proteobacteria*, whereas the proportions of *Actinobacteria* and *Cyanobacteria* showed a clear decrease (Figure 3a, Figure S1a). In line with that *Staphylococci*, the major genus present in *Firmicutes*, prevailed on the skin of healthy controls (60.5%) in comparison with PLE patients at baseline (45.25%). Nevertheless, upon photoprovocation this genus showed a noticeable increase at day 4 in PLE

patients (52.86%) that was maintained a week later at day 10 (51.42%). Also, the *Corynebacteria* group representative of *Actinobacteria* phylum was significantly decreased in PLE patients compared to healthy controls (Figure 3b, c, Figure S1b, c). Analysis at species level (≥ 99% similarity) showed decreased proportions of key *Staphylococci* commensals such as *Staphylococcus hominis* and *Staphylococcus epidermidis* on PLE skin (26.69% and 7.92%, respectively) in comparison to healthy matched controls (38.84% and 17.58%, respectively). In contrast, the proportions of *Staphylococcus aureus* were significantly higher (p=0.04) in the PLE group at baseline with 8.71% compared to controls with 0.68%. Remarkably, higher *S. aureus* abundances and increased microbial loads were observed in PLE patients with moderate to severe lesions after UVR in contrast to patients with mild lesions or no response, displaying furthermore distinct microbiomes at baseline (Figure S2a-d). Upon photoprovocation the relative abundances of *S. epidermidis* and *S. caprae* increased in tendency, whereas interestingly *S. aureus* exhibited a drop in three patients out of four compared to baseline. Of note, patients 2, 6 and 7 displaying moderate to severe symptoms upon photoprovocation had high proportions of *S. aureus* on their skin at baseline of 31.18%, 18.44% and 52.51%, respectively. Also, other potentially beneficial commensals including *Cutibacterium acnes*, *Micrococcus luteus*, *Moraxella osloensis* and *Chroococcidiopsis HQ189092* showed a decrease of relative abundance upon photoprovocation that returned to baseline values one week after UVR-exposure (Figure 4a, b, Table S1). In light of these results, the skin microbiome composition of PLE patients at baseline showed clear differences compared to controls, with lower abundances of commensals and high proportions of pathogenic *S. aureus*. Upon UV challenge the relative abundances of both potentially beneficial commensals and *S. aureus* exhibited a transient drop.

UV-radiations exhibit strong antimicrobial effects against skin resident bacteria

Finally, we evaluated the direct effects of UVA and UVB radiations on the growth of representative skin commensal and pathogenic bacteria. Twelve strains were exposed to UVR in a similar setting as for the PLE patients. Then, bacterial growth kinetics were assessed upon incubation for 48h at 37°C. Remarkably the UVA radiation exerted strong bactericidal effects leading to a complete growth inhibition of all tested bacteria compared to untreated controls. On the other hand, UVB displayed a bacteriostatic effect characterised by a transient inhibition of bacterial growth, resulting in a delay of the exponential phase as seen with the different *S. aureus 1-3*, *S. epidermidis 1,2* and *S. hominis 1,2* strains (Figure 5, Figure S3). Of note, a number of strains were insensitive to UVB as seen with *M. luteus 1, 2* and *C. stearicum*. Also, the growth inhibitory effect of UVB seems strain-dependent as *S. epidermidis 3* was unresponsive to UVB light in contrast to *S. epidermidis 1,2*. The combination of UVA and UVB showed similar bactericidal effects as

observed with UVA alone, indicating that the later exerts the strongest antimicrobial effects under the studied conditions.

Discussion

It has been hypothesized that PLE is triggered by elements resulting from UV-induced damages to skin microbial communities, leading to a DTH reaction and characteristic skin rash of the disease.[11] To verify that we investigated in this study the cutaneous microbiome changes in PLE lesions induced upon combined UVA and UVB irradiations. Interestingly, the UV-induced lesions seem to favor a bacterial overgrowth with significantly higher loads measured one day after photoprovocation that dropped to baseline levels a week later. This observation is in agreement with previous reports of bacterial expansion on inflamed skin lesions from AD[19] and psoriasis.[20] The increased relative abundance of *Staphylococci* on PLE lesions upon UVR exposure is likely associated with the observed gain of microbial load. Noteworthy, the expansion of *Staphylococci* was paralleled by a significant drop of microbial diversity, mirroring AD key microbiome features.[21] The values of α-diversity were clearly decreased in PLE patients compared to controls, prior to photoprovocation and even dropped further upon UVR. This suggests a somewhat dysbiotic microbiome already at baseline that is susceptible to undergo further changes upon UVR exposure.

Remarkably, the microbial diversity was restored to homeostatic levels one week after the last day of photoprovocation, indicating a fast recovery of the microbiome balance in parallel to symptoms' improvement. Similar patterns of microbial diversity restoration in skin lesions post-flares have been reported for AD.[21] Analysis at species level revealed a loss of potentially beneficial commensals particularly *S. hominis*, *S. epidermidis*, *C. acnes*, *M. luteus* and *M. osloensis* on PLE skin at baseline in comparison to healthy controls. Our observations corroborate the results from previous cohorts on various inflammatory skin disorders.[14,21,22] On the other hand *S. aureus* proportions were significantly higher, particularly in patients showing severe lesions in response to UVR, pointing towards a possible role in PLE pathogenesis and severity of the inflammatory response. We also noticed a slight drop of relative abundances of some commensals as well as *S. aureus* upon UVR, suggesting direct antimicrobial effects of UVB/UVA radiations in agreement with previous reports.[23] These findings are supported by the drastic loss of microbial growth that we observed upon exposure of both commensals and pathogenic *S. aureus* to UVA especially and UVB to a certain extent. This might be accompanied by the release of microbial antigens, particularly from *S. aureus* known to express a plethora of virulence factors including toxins, phenol soluble modulins and proteases.[24] The inhibitory action of UVR has often been attributed to the production of reactive oxygen species, causing oxidative damages to cellular macromolecules including DNA.[25] Upon photoprovocation

the less abundant phyla showed a slight increase in *Proteobacteria* represented by *Moraxella osloensis* and a decrease of *Cyanobacteria* dominated by *Chroococcidiopsis HQ189092*. In contrast to our observations, Burns and co-authors reported an increase of *Cyanobacteria* following a one day exposure to UVA and UVB[26], whereas Wilmott *et al* noticed a reduction of the *Proteobacteria* upon sun exposure during holidays.[27] Of note, our findings corroborate those obtained by Dotterud *et al* reporting a similar pattern of decrease of *S. aureus* and increase of *S. epidermidis* counts on AD lesions following a 4 week treatment with UVB.[28]

Our observations support a direct effect of UVR on the cutaneous microbiome leading to a transient dysbiosis that may be linked to PLE lesions. However, UVR may also indirectly alter the microbiome landscape by modulating the immune response.[29] Indeed, it has been shown that UVR can induce the expression of AMPs by keratinocytes which can strongly affect the microbiome composition.[30] On the other hand, the skin microbiome was also reported as a critical modulator of UV-induced cytokine expression, thereby modulating the immune response. In fact, topical disinfection of PLE skin has been shown to reverse cytokine imbalances mediated by the skin microbiome upon UV exposure.[31]

It is still unclear whether cutaneous microbiome alterations precede the development of inflammatory skin disorders including PLE or are rather a consequence of an established disease[32]. Our findings suggest that PLE skin is characterized by a dysbiotic microbiome, already at baseline, with significantly reduced diversity and noticeable colonization by *S. aureus*. Similarly, a microbial dysbiosis with an expansion of this pathogen has been reported to precede the onset of AD in children, suggesting a causative role[33]. Upon exposure to UVR the PLE microbiome composition is transiently scrambled, favoring an overgrowth of dysbiotic members. This may be accompanied by the release of various ligands able to trigger a strong inflammatory response, given the lack of immune-suppression in these patients. Hence, correcting dysbiosis in these patients (eg. using cutaneous probiotics or skin microbiome transfer) might prevent PLE lesions formation and disease exacerbation. Notably, PLE's baseline microbiome showed subtle differences in comparison to controls, likely because this disease follows an acute development, whereas more drastic changes could be expected in chronic inflammatory skin diseases[3]. The main limitation here is the low number of participants and therefore a larger cohort is required to validate these observations.

References

1 Rhodes LE, Bock M, Janssens AS *et al.* Polymorphic light eruption occurs in 18% of Europeans and does not show higher prevalence with increasing latitude: multicenter survey of 6,895 individuals residing from the Mediterranean to Scandinavia. *J Invest Dermatol* 2010; **130**: 626-8.

2 Burfield L, Rutter KJ, Thompson B *et al.* Systematic review of the prevalence and incidence of the photodermatoses with meta-analysis of the prevalence of polymorphic light eruption. *J Eur Acad Dermatol Venereol* 2023; **37**: 511-20.

3 Guarrera M. Polymorphous Light Eruption. *Adv Exp Med Biol* 2017; **996**: 61-70.

4 Millard TP, Fryer AA, McGregor JM. A protective effect of glutathione-S-transferase GSTP1*Val(105) against polymorphic light eruption. *J Invest Dermatol* 2008; **128**: 1901-5.

5 Zirbs M, Pürner C, Buters JT *et al.* GSTM1, GSTT1 and GSTP1 gene polymorphism in polymorphous light eruption. *J Eur Acad Dermatol Venereol* 2013; **27**: 157-62.

6 Lembo S, Raimondo A. Polymorphic Light Eruption: What's New in Pathogenesis and Management. *Front Med (Lausanne)* 2018; **5**: 252.

7 Lembo S, Caiazzo G, Balato N *et al.* Polymorphic light eruption and IL-1 family members: any difference with allergic contact dermatitis? *Photochem Photobiol Sci* 2017; **16**: 1471-9.

8 Kölgen W, van Meurs M, Jongsma M *et al.* Differential expression of cytokines in UV-B-exposed skin of patients with polymorphous light eruption: correlation with Langerhans cell migration and immunosuppression. *Arch Dermatol* 2004; **140**: 295-302.

9 Schweintzger N, Gruber-Wackernagel A, Reginato E *et al.* Levels and function of regulatory T cells in patients with polymorphic light eruption: relation to photohardening. *Br J Dermatol* 2015; **173**: 519-26.

10 Patra V, Strobl J, Atzmüller D *et al.* Accumulation of Cytotoxic Skin Resident Memory T Cells and Increased Expression of IL-15 in Lesional Skin of Polymorphic Light Eruption. *Front Med (Lausanne)* 2022; **9**: 908047.

11 Patra V, Wolf P. Microbial elements as the initial triggers in the pathogenesis of polymorphic light eruption? *Exp Dermatol* 2016; **25**: 999-1001.

12 Patra V, Mayer G, Gruber-Wackernagel A *et al.* Unique profile of antimicrobial peptide expression in polymorphic light eruption lesions compared to healthy skin, atopic dermatitis, and psoriasis. *Photodermatol Photoimmunol Photomed* 2018; **34**: 137-44.

13 Fyhrquist N, Muirhead G, Prast-Nielsen S *et al.* Microbe-host interplay in atopic dermatitis and psoriasis. *Nat Commun* 2019; **10**: 4703.

14 Amar Y, Rogner D, Silva RL *et al.* Darier's disease exhibits a unique cutaneous microbial dysbiosis associated with inflammation and body malodour. *Microbiome* 2023; **11**: 162.

15 Gupta V, Sharma VK. Skin typing: Fitzpatrick grading and others. *Clin Dermatol* 2019; **37**: 430-6.

16 Peterson J, Garges S, Giovanni M *et al.* The NIH Human Microbiome Project. *Genome Res* 2009; **19**: 2317-23.

17 Amar Y, Lagkouvardos I, Silva RL *et al.* Pre-digest of unprotected DNA by Benzonase improves the representation of living skin bacteria and efficiently depletes host DNA. *Microbiome* 2021; **9**: 123.

18 Lagkouvardos I, Joseph D, Kapfhammer M *et al.* IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. *Sci Rep* 2016; **6**: 33721.
19 Simpson EL, Schlievert PM, Yoshida T *et al.* Rapid reduction in Staphylococcus aureus in atopic dermatitis subjects following dupilumab treatment. *J Allergy Clin Immunol* 2023; **152**: 1179-95.
20 Quan C, Chen XY, Li X *et al.* Psoriatic lesions are characterized by higher bacterial load and imbalance between Cutibacterium and Corynebacterium. *J Am Acad Dermatol* 2020; **82**: 955-61.
21 Kong HH, Oh J, Deming C *et al.* Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; **22**: 850-9.
22 Seite S, Flores GE, Henley JB *et al.* Microbiome of affected and unaffected skin of patients with atopic dermatitis before and after emollient treatment. *J Drugs Dermatol* 2014; **13**: 1365-72.
23 Sandri A, Tessari A, Giannetti D *et al.* UV-A Radiation: Safe Human Exposure and Antibacterial Activity. *Int J Mol Sci* 2023; **24**.
24 Geoghegan JA, Irvine AD, Foster TJ. Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship. *Trends Microbiol* 2018; **26**: 484-97.
25 Santos AL, Oliveira V, Baptista I *et al.* Wavelength dependence of biological damage induced by UV radiation on bacteria. *Arch Microbiol* 2013; **195**: 63-74.
26 Burns EM, Ahmed H, Isedeh PN *et al.* Ultraviolet radiation, both UVA and UVB, influences the composition of the skin microbiome. *Exp Dermatol* 2019; **28**: 136-41.
27 Willmott T, Campbell PM, Griffiths CEM *et al.* Behaviour and sun exposure in holidaymakers alters skin microbiota composition and diversity. *Front Aging* 2023; **4**: 1217635.
28 Dotterud LK, Wilsgaard T, Vorland LH *et al.* The effect of UVB radiation on skin microbiota in patients with atopic dermatitis and healthy controls. *Int J Circumpolar Health* 2008; **67**: 254-60.
29 Patra V, Byrne SN, Wolf P. The Skin Microbiome: Is It Affected by UV-induced Immune Suppression? *Front Microbiol* 2016; **7**: 1235.
30 Gläser R, Navid F, Schuller W *et al.* UV-B radiation induces the expression of antimicrobial peptides in human keratinocytes in vitro and in vivo. *J Allergy Clin Immunol* 2009; **123**: 1117-23.
31 Zarfl M, Patra V, Bordag N *et al.* Eradication of skin microbiota restores cytokine production and release in polymorphic light eruption. *Exp Dermatol* 2024; **33**: e15034.
32 Yang Y, Qu L, Mijakovic I *et al.* Advances in the human skin microbiota and its roles in cutaneous diseases. *Microb Cell Fact* 2022; **21**: 176.
33 Williams MR, Gallo RL. Evidence that Human Skin Microbiome Dysbiosis Promotes Atopic Dermatitis. *J Invest Dermatol* 2017; **137**: 2460-1.

Figure 1. β-diversity analysis of PLE skin prior to and following a photoprovocation using combined UVB/UVA radiations. a) Workflow of photoprovocation and microbiome samples collection and processing. Created with BioRender.com. **b)** β-diversity analysis using principal coordinate analysis (PcoA) plots of healthy controls, PLE patients at baseline (PLE_D0), PLE patients after one day (PLE_D4) and one week (PLE_D10) following photoprovocation. Each dot represents a swab sample. The Bray Curtis index was used to calculate similarity between samples and PERMANOVA to test the statistical significance between the groups based on the distance matrix.

Figure 2. PLE skin microbiome is characterized by increased microbial loads and a loss of α-diversity upon UVR. a) Total microbial counts in controls and PLE groups prior to (PLE_D0) and following photoprovocation (PLE_D4, PLE_D10). Samples were diluted and plated on non-selective agar plates for 48 h at 37°C. **b)** α-diversity values expressed as effective richness (number of ASVs), Chao1, ACE and Shannon index. Each dot represents a swab sample. The statistical significance was calculated using Kruskal Wallis and Wilcoxon-Mann-Whitney tests respectively for multiple group and pairwise comparisons. Multiple test corrections were performed using the Benjamini and Hochberg procedure. The asterisks indicate statistically significant differences and correspond to *p≤0.05, **p≤0.01.

Figure 3. Microbiome analysis of PLE and matched controls at phylum and genera levels.
Bar chart of taxonomy binning at **a)** phylum and **b)** genus levels. The microbiome composition was assessed by summing up ASVs relative abundances sharing the same taxonomic assignment at phylum and genus levels. The Bayesian classifier from RDP database was used for ASVs classification. **c)** Relative abundances plots of dominant genera *Staphylococcus*, *Cutibacterium*, *Micrococcus*, *Kocuria*, *Moraxella* and *Corynebacterium*. Each dot represents a swab sample. Multiple test corrections were performed and the statistical significance calculated using Kruskal Wallis and Wilcoxon-Mann-Whitney tests respectively for multiple group and pairwise comparisons. The asterisks indicate statistically significant differences and correspond to *p≤0.05, **p≤0.01.

Figure 4. Microbiome analysis of PLE and matched controls at species level. a) Bar chart of taxonomy binning displayed at species level. The composition was assessed by summing up ASVs relative abundances that share the same taxonomic assignment at species level. **b)** Relative abundances plots of dominant taxa *S. hominis, C. acnes, S. aureus*, *S. epidermidis, K. tytonicola, M. luteus, K. rhizophila, M. osloensis,* and *S. caprae*. Each dot represents a swab sample. Multiple test corrections were performed with the Benjamini and Hochberg procedure. The statistical significance was calculated using Kruskal Wallis and Wilcoxon-

Mann-Whitney tests respectively for multiple group and pairwise comparisons. The asterisks indicate statistically significant differences and correspond to *p≤0.05, **p≤0.01.

Figure 5. Effects of UVR on the growth of selected skin commensals and pathogens. Cutaneous pathogens *S. aureus 1*, *2* and skin commensals *S. epidermidis 1, 2*, *S. hominis 1, 2, M. luteus 1* and *C. stearicum* have been exposed to UVA at 100 J/cm², to UVB at a dose of 100 mJ/cm² or their combination for three consecutive days. Upon exposure to UVR bacterial inoculums were prepared at a concentration of 10^5 CFU/ml in tryptic soy broth. The suspensions were incubated in 96 well plates at 37°C for 48h and the growth kinetics assessed at a wavelength of 620 nm. Untreated controls were prepared for each strain. Each dot represents the mean ±SD of 5 replicates. Statistical differences between irradiated vs control plates were analysed using a one-way ANOVA test followed by Tukey's multiple comparisons test. The following symbols *, º, # were used to respectively compare significance of UVA, UVB and UVA+B treatment groups to untreated control within the incubation period from 6 hours to 48 hours. They indicate statistically significant differences and correspond to p≤0.05 (*, º), p≤0.01 (##), p≤0.0001 (****, ####).

Table 01: History and clinical characteristics of PLE patients included in the study.

Patient	Age	Sex	Skin type	Duration/season of PLE	Morphology	Predilection sites	MED-UVB (mJ/cm²)	Month and site of photoprovocation	Wavebands of photoprovocation and morphology	Severity of PLE under photoprovocation
1	41	f	II	Since 24 years in spring and summer	Erythema, vesicules, blisters	Extensor sides of the arms, legs, torso, face	100	February, lower arm	UVB: Papules UVB+UVA: Papules UVA: No specific eruption	Positive, mild
2	28	f	II	Since 5 years in spring	Erythema, papules, blisters	Décolleté, extensor sides of the arms, torso, face	50	August, lower arm	UVB: No specific eruption UVB+UVA: Papules UVA: No specific eruption	Positive, moderate
3	41	f	II	Since 11 years in spring and summer	Erythema, papules, blisters, plaques, crusts, scaling	Décolleté, extensor sides of the arms, torso, face	75	January, upper arm	UVB: No specific eruption UVB+UVA: No specific eruption UVA: No specific eruption	Not provocable
4	37	f	II	Since 8 years in summer	Erythema, papules, vesicules, blisters, plaques	Décolleté, extensor sides of the arms, back of the hands, legs, torso	100	January, upper arm	UVB: No specific eruption UVB+UVA: Papules UVA: Papules	Positive, severe
5	76	f	II	Since 1 year in spring	Erythema, papules,	Décolleté, extensor sides	75	February, lower arm	UVB: No specific eruption	Not provocable

					blisters	of the arms, face			UVB+UVA: No specific eruption UVA: No specific eruption	
6	28	m	III	Since 4 years in summer	Erythema, papules, vesicules blisters, crusts, scaling	Décolleté, extensor sides of the arms, back of the hands, legs	75	April, lower arm	UVB: Papules UVB+UVA: Papules UVA: Papules	Positive, severe
7	34	f	II	Since 15 years in spring and summer	Erythema, papules	Extensor sides of the arms, legs	50	June, thigh	UVB: Papules UVB+UVA: Papules UVA: No specific eruptions	Positive, moderate
8	49	f	II	Since half a year in spring	Erythema, papules	Extensor sides of the arms, back of the hands	50	November, lower arm	UVB: No specific eruption UVB+UVA: No specific eruption UVA: No specific eruption	Not provocable
9	70	f	II	Since two years in spring	Erythema, vesicules, crusts, scaling	Décolleté, extensor sides of the arms, back of the hands	100	February, lower arm	UVB: No specific eruption UVB+UVA: No specific eruption UVA: No specific eruption	Not provocable
10	45	f	II	Since 9 years in spring and autumn	Erythema, vesicules	Décolleté, extensor sides of the arms, back of	100	March, lower arm	UVB: No specific eruption UVB+UVA: Papules	Positive, mild

						the hands, face			UVA: No specific eruption	
11	53	f	II	Since 38 years in spring and autumn	Erythema, Papules, crusts	Décolleté, extensor sides of the arms, legs	100	April, lower arm	UVB: Papules UVB+UVA: Papules UVA: Papules	Positive, moderate

1

2

Figure 1

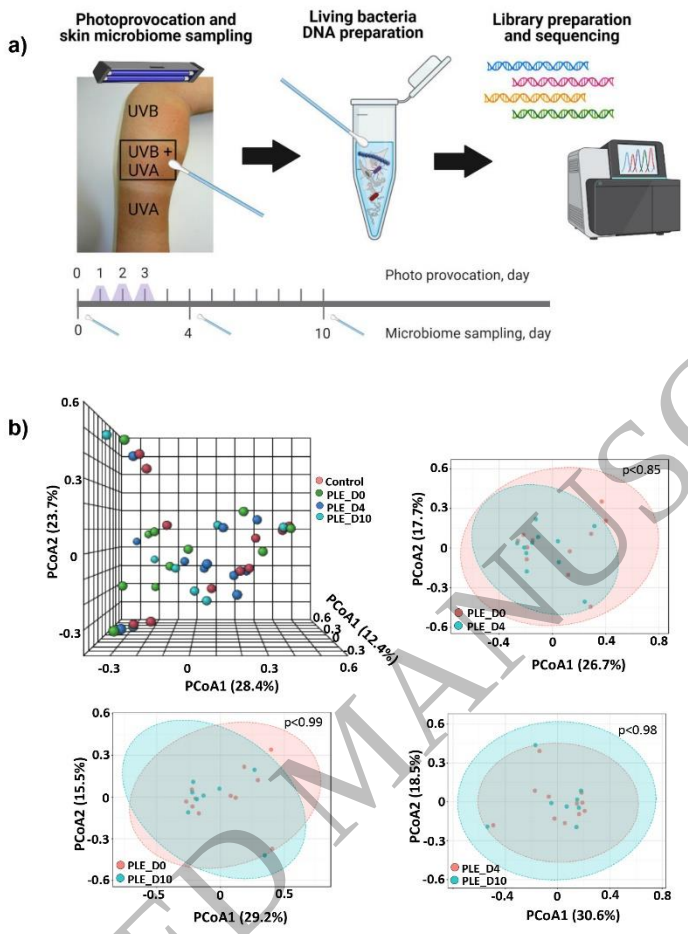

Figure 1
111x160 mm (x DPI)

Figure 2

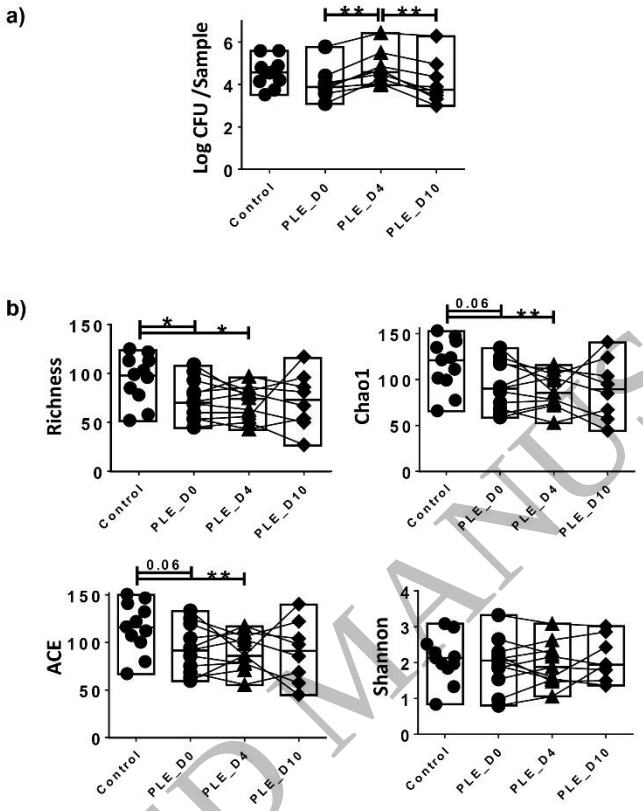

Figure 2
111x160 mm (x DPI)

Figure 3

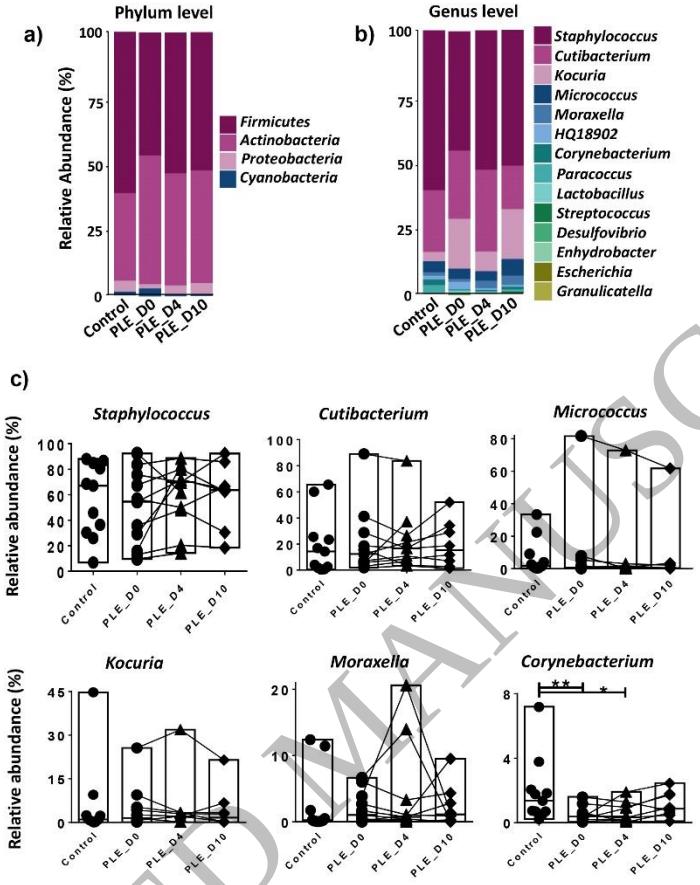

Figure 3
111x160 mm (x DPI)

Figure 4

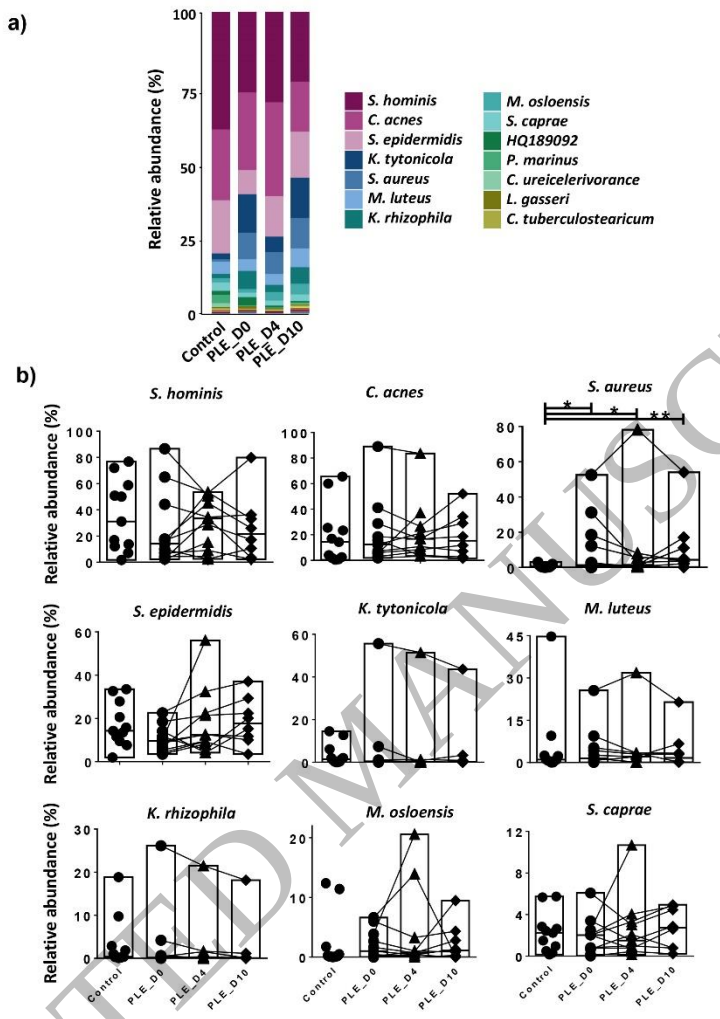

Figure 4
111x160 mm (x DPI)

Figure 5

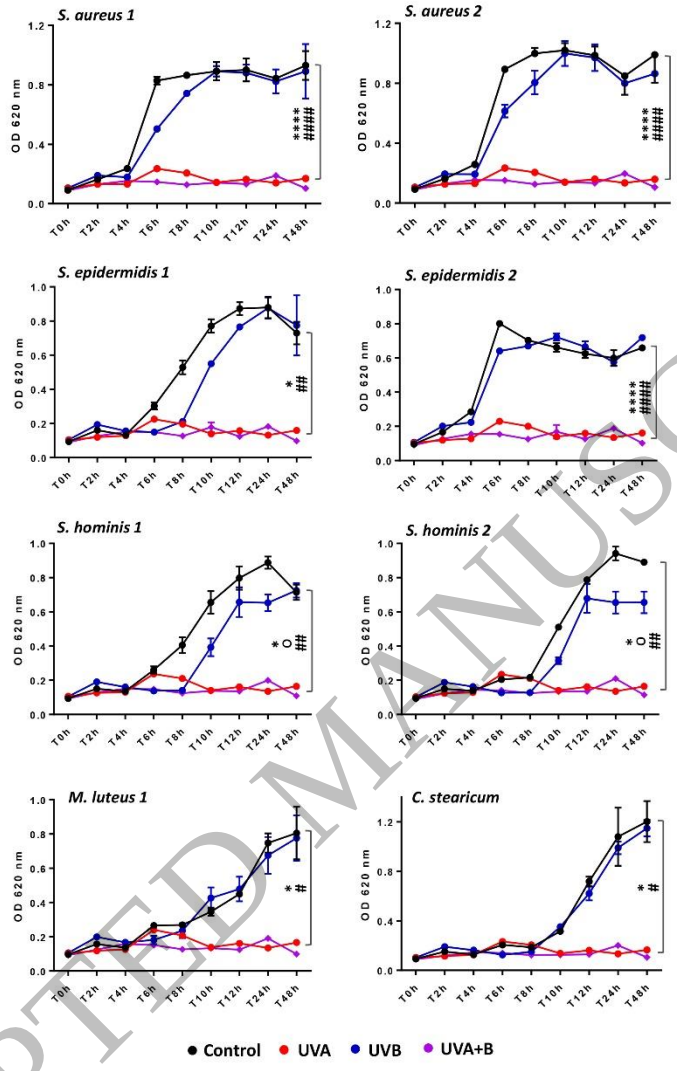

Figure 5
111x160 mm (x DPI)