Magic Microbiota: Engineered Cutibacterium acnes

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INTRODUCTION

The skin is the largest organ of the human body and home to millions of bacteria, fungi, and viruses. All these microorganisms make up the skin microbiota, which is essential in protecting individuals against invading pathogens.[1] The skin microbiota exists in an equilibrium between commensal and pathogenic bacteria, which can cause disease when disturbed.[1,2] Many bacterial strains are important parts of the skin microbiota including *Cutibacterium acnes* and *Staphylococcus epidermidis*.[3] Though there are benefits to *C. acnes*, such as producing metabolites to fight infection, an abundance of certain strains of this bacteria is associated with acne vulgaris (acne), a common inflammatory disease affecting 85% of individuals aged 12 - 24.[3,4] Acne has various treatments, depending on the individual's age, skin colour, and severity of the infection.[5] Typical treatments include topical retinoids and benzoyl peroxide, but in more severe cases, oral antibiotics, hormonal therapies, or oral isotretinoin may be prescribed.[5] However, there can be drawbacks to some of these agents. Currently, severe acne cases are commonly treated with isotretinoin, a retinoid that increases production of the *LCN2* gene that encodes neutrophil gelatinase-associated lipocalin (NGAL), a protein that induces sebocyte apoptosis to diminish acne symptoms. However, lipocalin can be associated with mood swings and weight change. Oral isotretinoin is also often associated with dryness of skin, lips, and eyes, as well as possible upregulation of liver enzymes.[6] However, many advancements in understanding the skin microbiome have helped scientists understand skin conditions like acne from a novel lens. In an effort to develop new treatments for acne, advancements in using skin microbiota have been explored with promising results.

A CASE STUDY ON C. ACNES

As one of the three main genera that inhabit the skin microbiota, the interplay between *C. acnes* and other microbial communities is essential for maintaining healthy skin.[7] Predominantly found in sebaceous glands, *C. acnes* is critical in regulating skin homeostasis and preventing colonisation by other harmful pathogens.[7] As a gram-positive bacterium, its genes encode for lipase enzymes, like triacylglycerol lipase and lysophospholipase.[8] These enzymes aid in sebum lipid degradation, helping maintain an acidic skin pH.[8] However, *C. acnes* has also long been implicated in the pathophysiology of acne due to its role in increased sebum production, hyperkeratinisation of the pilosebaceous unit, and inflammation.[7] Still, research has shown no quantitative difference in *C. acnes* between subjects with and without acne, leading to hypotheses that some strains may improve skin health, whereas others may become pathogenic.[8] Using sequence comparison of either *recA* or *tly* genes, chosen based on their functions as a phylogenetic marker and for allowing bacteria to coexist with hosts, researchers categorized *C. acnes* strains into phylotypes IA, IB, II, and III.[7,9] A 2010 study by Lomholt and Kilian identified that the phylotype IA was strongly associated with moderate to severe acne due to its preferential proliferation in inflammatory microenvironments.[11] Contrastingly, IB, II, and III, were associated with healthy skin.[11] These findings were later confirmed by other studies.[7] As such, *C. acnes* presents as a popular therapeutic target for acne, given its critical role in skin microbiota flora.

ENGINEERING OF C. ACNES

A study conducted by Knödlseder et al. successfully utilised skin microbiota to treat acne by engineering commensal *C. acnes* to secrete NGAL.[11] They identified secretion signals from endogenous proteins known to be highly secreted in *C. acnes*.[11] The *LCN2* gene was then fused to the secretion signals and expressed from a strong promoter in *C. acnes*.[11] When sebocytes were incubated with NGAL-secreting *C. acnes* cells, the *C. acnes* pBR13 protein significantly decreased sebum levels by around twofold after 48 hours.[11] Lastly, researchers tested whether skin could be engrafted with the sebum-modulating *C. acnes* strain by topically applying 0.5% peptone in control, wild-type *C. acnes*, and the engineered NGAL-expressing *C. acnes* on the back of mice for three consecutive days.[11] At each time point, *Cutibacterium sp.* was observed to be engrafted on mouse skin.[11] The study also assessed the expression of inflammatory cytokines like IL-1β, IL-6, and tumor necrosis factor α for potential inflammation derived from *C. acnes* treatment, of which no significant differences were observed between treated groups.[11]

In order to address the antibiotic resistance crisis in developing engineered *C. acnes*, scientists have worked to remove antibiotic resistance cassettes from the bacterium.[11] Antibiotic resistance cassettes are mobile genetic elements that can move around from one organism to another and provide resistance to certain antibiotics.[13] Scientists typically try to avoid targeting antibiotic resistance cassettes in case they are transferred to other harmful organisms, making them resistant to some antibiotics. To mitigate this risk, scientists used dual selection to select the best organism. This process consists of using negative selection where bacterium without a certain trait are rejected and positive selection where those with a desired trait are accepted. First, they did a gene knock-out of the thymidine kinase (*tdk*) gene in *C. acnes*. The *tdk*-deficient strains of *C. acnes* are unable to metabolise 5'-fluoro-2'-deoxyuridine into a toxic compound that inhibits DNA and RNA production. Without synthesising this harmful compound, *C. acnes* strains retain the ability to produce DNA and RNA, facilitating their growth and enabling scientists to identify the *tdk*-deficient variants. Additionally, *tdk*-deficient *C. acnes* can become resistant to erythromycin, an antibiotic that can treat skin infections,

in the presence of an erythromycin-resistance cassette.[11,12] By employing dual selection, scientists eliminate the need for reliance on antibiotic resistance cassettes to identify modified *C. acnes* bacteria, enhancing the safety of the microorganism.

LIMITATIONS AND FUTURE DIRECTIONS

Despite the modified *C. acnes* showing promise as a treatment for acne, the development of this modified microorganism is still in its early stages. The in vivo study conducted by Knödlseder et al. has its limitations with respect to clinical translation. Namely, mouse skin displays significant differences from human skin.[11] In addition to being four times thinner than human skin, it is separated from underlying connective tissue and has more hair follicles.[14] Although pig skin is a greater match due to the similarities in structure and healing, it is generally not used in research due to its high price, genetic heterogeneity, and lack of knowledge of its physiology relative to mice.[14] To replace animal testing, human skin models containing immune cells and microbial populations are being developed to conduct ex vivo experiments. However, these models require further development to ensure accuracy.[15]

Engineered *C. acnes* also has potential for further research in the field of engineered microorganisms. While the development of engineered *C. acnes* is recent, this research opens doors to developing other modified microorganisms in the skin microbiome to benefit human health. For example, *S. epidermidis*, a bacteria in the skin microbiome, has been genetically engineered to produce melanoma tumour antigens.[15] These antigens can provoke a sufficient immune response to kill aggressive metastatic and localised melanoma.[15] Furthermore, engineered *C. acnes* opens up the door for further research to be done into the field of skin microbiota in order to better understand the interactions between the organisms and their host. Overall, the skin microbiota is a promising avenue for therapeutic development in treating acne and other diseases.

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1. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. *Nat Rev Microbiol*. 2018;16(3):143–55. Available from: doi.10.1038/nrmicro.2017.157.
2. Singh B, Christina E. Bacterial metabolites: An unexplored quarry. *mVOCs*. 2021:205-34. doi:10.1016/B978-0-12-824523-1.00006-7.
3. Eisenstein M. The skin microbiome. *Nature*. 2020;588(7838):209. Available from: doi.10.1038/d41586-020-03523-7.
4. Flowers L, Grice EA. The skin microbiota: Balancing risk and reward. *Cell Host Microbe*. 2020;28(2):190–200. Available from: doi.10.1016/j.chom.2020.06.017.
5. Eichenfield DZ, Sprague J, Eichenfield LF. Management of Acne Vulgaris: A review. *JAMA*. 2021;326(20):2055–67. Availablte from: doi:10.1001/jama.2021.17633.
6. Vallerand IA, Lewinson RT, Farris MS, Sibley CD, Ramien ML, Bulloch AGM, et al. Efficacy and adverse events of oral isotretinoin for acne: A systematic review. *Br J Dermatol*. 2018;178(1):76–85. Available from: doi:10.1111/bjd.15668.
7. Dréno B, Pécastaings S, Corvec S, Veraldi S, Khammari A, Roques C. *Cutibacterium acnes* (*Propionibacterium acnes*) and acne vulgaris: A brief look at the latest updates. *JEADV*. 2018;32:5-14. Available from: doi:10.1111/jdv.15043.
8. Platsidaki E, Dessinioti C. Recent advances in understanding *Propionibacterium acnes (Cutibacterium acnes)* in acne. *F1000Research*. 2018;7. Available from: doi:10.12688/f1000research.15659.1.
9. McDowell A. Over a decade of recA and tly gene sequence typing of the skin bacterium *Propionibacterium acnes*: What have we learnt? *Microorganisms*. 2017;6(1):1. Available from: doi:10.3390/microorganisms6010001.
10. Lomholt HB, Kilian M. Population genetic analysis of *Propionibacterium acnes* identifies a subpopulation and epidemic clones associated with acne. *PLoS One*. 2010;5(8):e12277. Available from: doi:10.1371/journal.pone.0012277.
11. Knödlseder N, Fábrega MJ, Santos-Moreno J, Manils J, Toloza L, Marín Vilar M, et al. Delivery of a sebum modulator by an engineered skin microbe in mice. *Nat Biotechnol*. 2024:1-6. Available from: doi:10.1038/s41587-023-02072-4.
12. Mayo Clinic. *Erythromycin (Oral Route) Side Effects* [Internet]. Available from: https://www.mayoclinic.org/drugs-supplements/erythromycin-oral-route/side-effects/drg-20075495?p=1.
13. Tran TQ, Park M, Lee JE, Kim SH, Jeong JH, Choy HE. Analysis of antibiotic resistance gene cassettes in a newly identified *Salmonella enterica* serovar Gallinarum strain in Korea. *Mobile DNA*. 2023;14(1):4. Available from: doi:10.1186/s13100-023-00292-8.
14. Zomer HD, Trentin AG. Skin wound healing in humans and mice: Challenges in translational research. J *Dermatol Sci*. 2018;90(1):3–12. Available from: doi:10.1016/j.jdermsci.2017.12.009.
15. Niehues H, Bouwstra JA, El Ghalbzouri A, Brandner JM, Zeeuwen PLJM, van den Bogaard EH. 3D skin models for 3R research: The potential of 3D reconstructed skin models to study skin barrier function. *Exp Dermatol*. 2018;27(5):501–11. Available from: doi:10.1111/exd.13531.