SKIN MICROBIOME: PAST, PRESENT, AND FUTURE

THE ECO WELL SKIN CARE SUMMIT – FEBRUARY 25, 2024

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SO MANY TERMS!

Virome

Mycobiome

Archaeome

Holobiont

Prebiotic

Biodiversity

Microbiome

Biogeography

Probiotic

Postbiotic

Microbiota

Dysbiosis

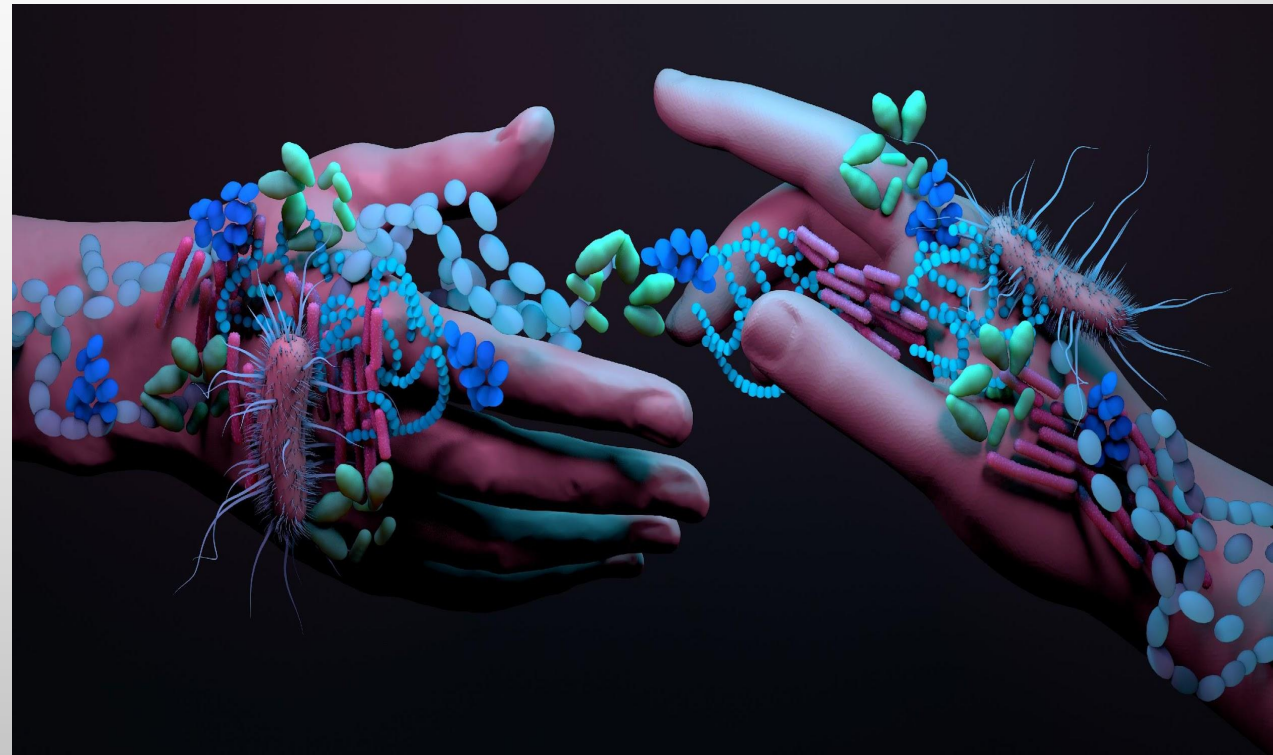
Metagenomics

Synbiotic

16S rRNA Sequencing

WHAT IS THE "MICROBIOME"?

- **MICROBIOME** IS A TERM COINED BY DR. JOSHUA LEDERBERG IN 2001 TO DESCRIBE THE COLLECTION OF GENOMIC MATERIAL PROVIDED BY MICROORGANISMS (**MICROBIOTA**) INHABITING A HIGHER ORGANISM
  - FOCUS ON THE SKIN MICROBIOME
- THESE MICROORGANISMS INCLUDE:
  - BACTERIA
  - FUNGI
  - ARCHAEA
  - VIRUSES
  - PROTISTS (PROTOZOA)



SKIN “BIOGEOGRAPHY”

- **BIOGEOGRAPHY** DESCRIBES THE VARIOUS REGIONS OF THE SKIN THAT HARBOR DIFFERENT MICROBES BECAUSE OF UNIQUE ENVIRONMENTAL CONDITIONS TO THAT AREA
- THESE SKIN ENVIRONMENTS INCLUDE:
  - DRY
  - MOIST
  - SEBACEOUS (OILY)

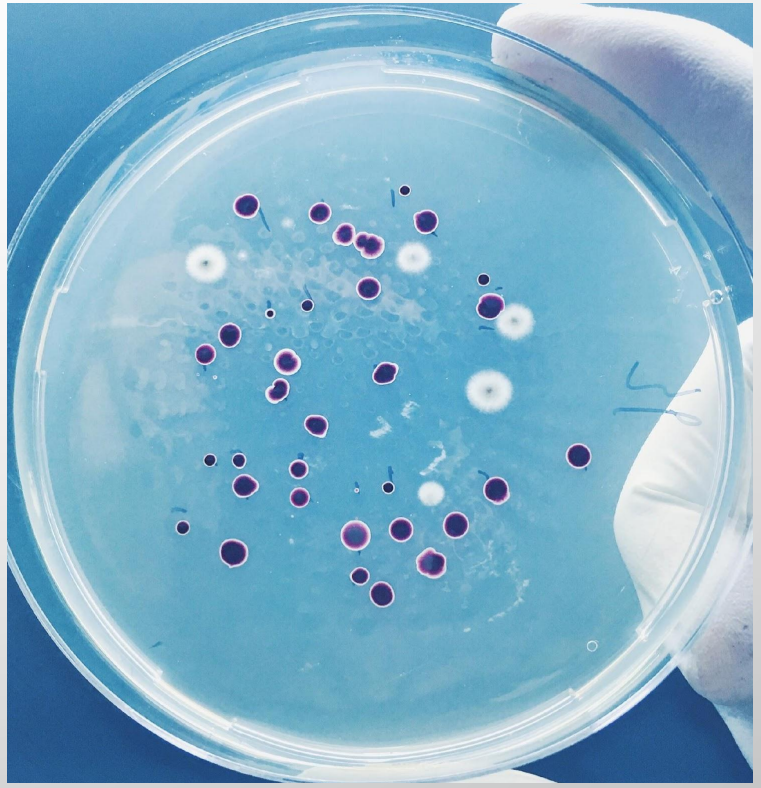
SKIN MICROBIOME:

THE PAST

CULTURE-DEPENDENT CHARACTERIZATION

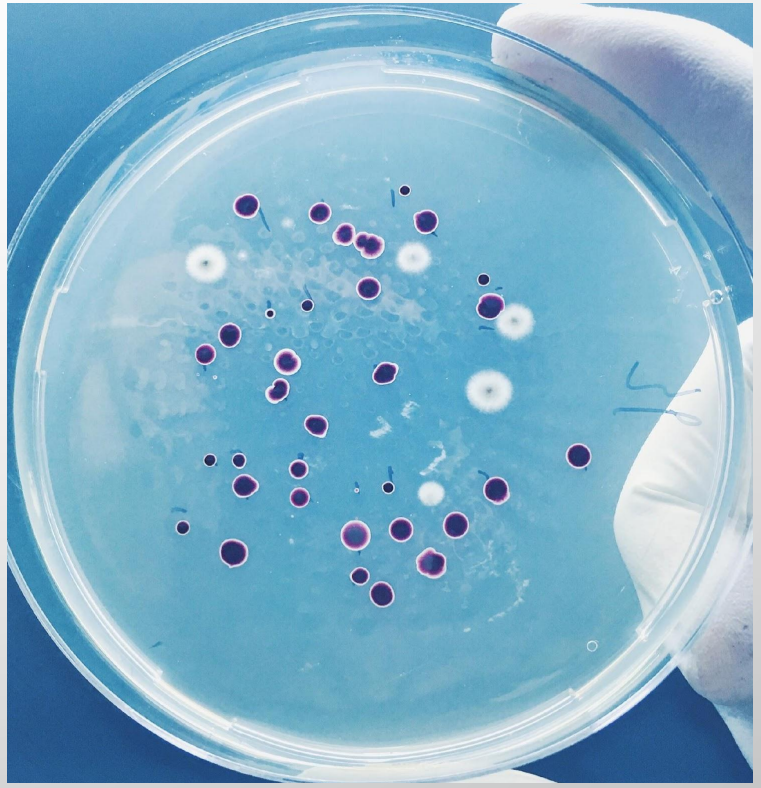
SKIN MICROBIOME STUDIES

- **CULTURE-DEPENDENT** DESCRIBES WHEN THE EXTENT OF OUR UNDERSTANDING OF THE HUMAN MICROBIOME WAS LIMITED TO WHAT WE COULD CULTURE – MEANING WHAT MICROORGANISMS WE COULD SAMPLE AND GROWTH IN THE LAB.
- **THE MAJORITY OF MICROBES CANNOT BE EASILY CULTURED IN THE LAB**



WHAT MICROBES WOULD NOT BE DETECTED BY THIS APPROACH?

- ARCHAEA
- VIRUSES
- MANY PROTISTS
- MANY FUNGI

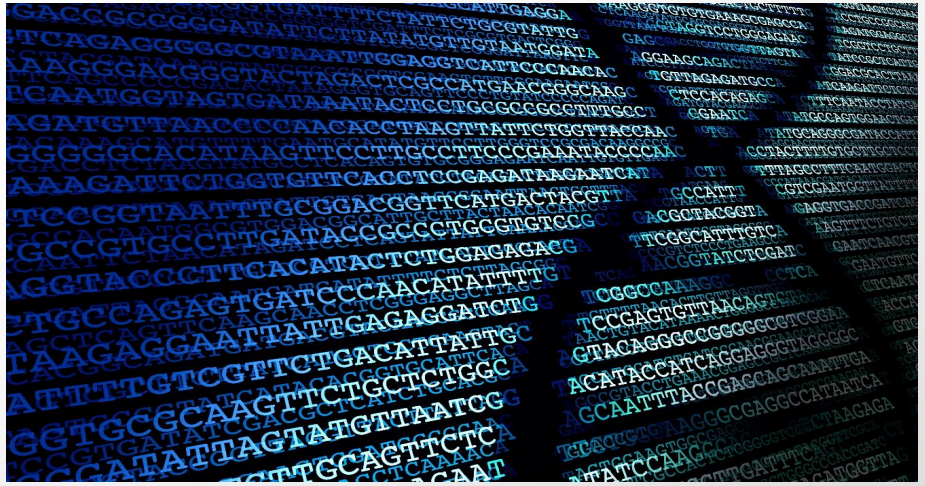


SKIN MICROBIOME:
THE PRESENT

CULTURE-INDEPENDENT CHARACTERIZATION

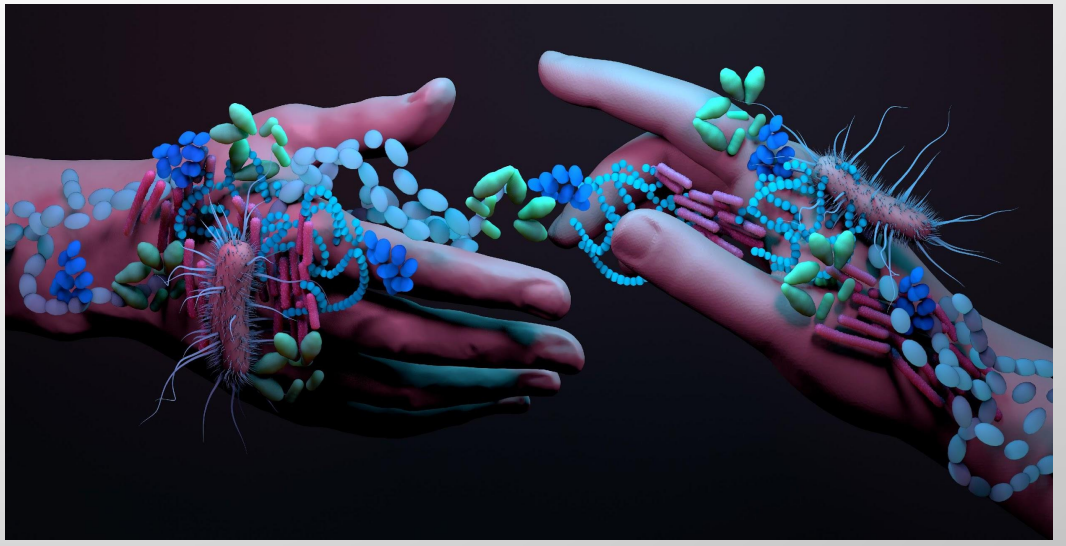
SKIN MICROBIOME STUDIES

- **CULTURE-INDEPENDENT** INVOLVES BYPASSING WHAT IS "CULTURABLE" AND GENETICALLY SEQUENCING MICROBES IN SAMPLES COLLECTED
- FIRST WITH **16S rRNA SEQUENCING**
  - LIMITED TO BACTERIA
- NEXT WITH **ITS2 GENE SEQUENCING**
  - LIMITED TO FUNGI
- NOW WITH **METAGENOMIC SEQUENCING** CAPTURES **ALL MICROORGANISMS**



MAJOR OUTCOMES

- **HUMAN MICROBIOME STUDIES** HAVE REVEALED THAT ALMOST EVERY TISSUE AND ORGAN OF THE HUMAN BODY HAS ITS OWN UNIQUE MICROBIOME, INCLUDING, BUT NOT LIMITED TO:
  - GUT
  - LUNGS
  - SKIN
  - BRAIN
  - BLOOD
  - CANCER

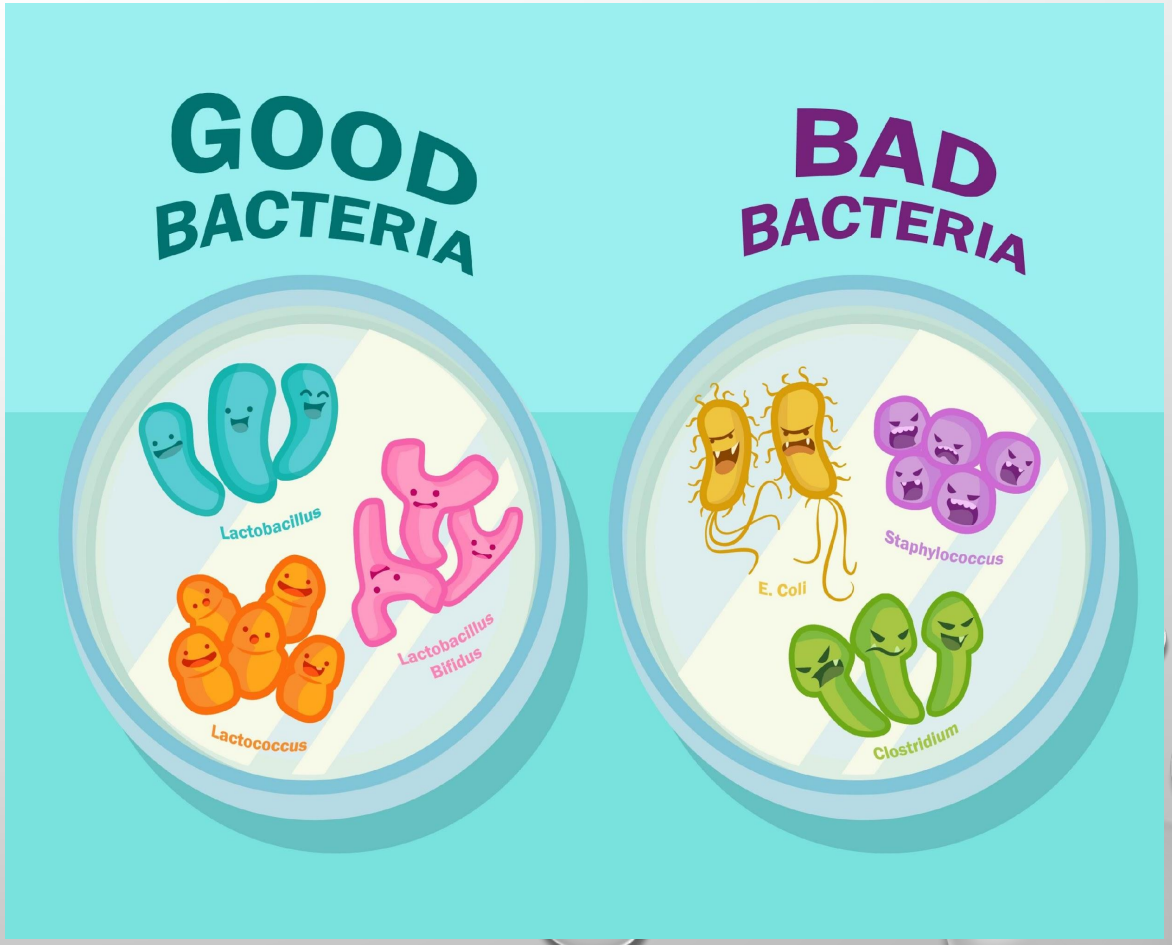


THE BACTERIA

BACTERIA REPRESENT THE MOST NUMEROUS GROUP OF MICROBES FOUND ON THE SKIN – ROUGHLY 1,000 SPECIES

THEY ARE “PROKARYOTES” – MEANING THAT THEY DO NOT HAVE TRUE NUCLEI AND ARE DISTINCTLY DIFFERENT FROM THE CELLS THAT MAKE UP US

MAJOR BACTERIAL PLAYERS



MAJOR BACTERIAL PLAYERS

Phylum Level

ACTINOBACTERIA (36-51%)

- Gram-positive
- *Cutibacteria (Propionibacteria)*
- *Corynebacteria*
- *Micrococcus*

FIRMICUTES (24-34%)

- Gram-positive – "tough skin"
- *Staphylococcus*
- *Streptococcus*

PROTEOBACTERIA (11-16%)

- Gram-negative
- *Acinetobacter*
- *Pseudomonas*
- *Escherichia*

BACTEROIDETES (6-9%)

- Gram-negative
- *Prevotella*
- *Porphyromonas*
- *Chryseobacterium*

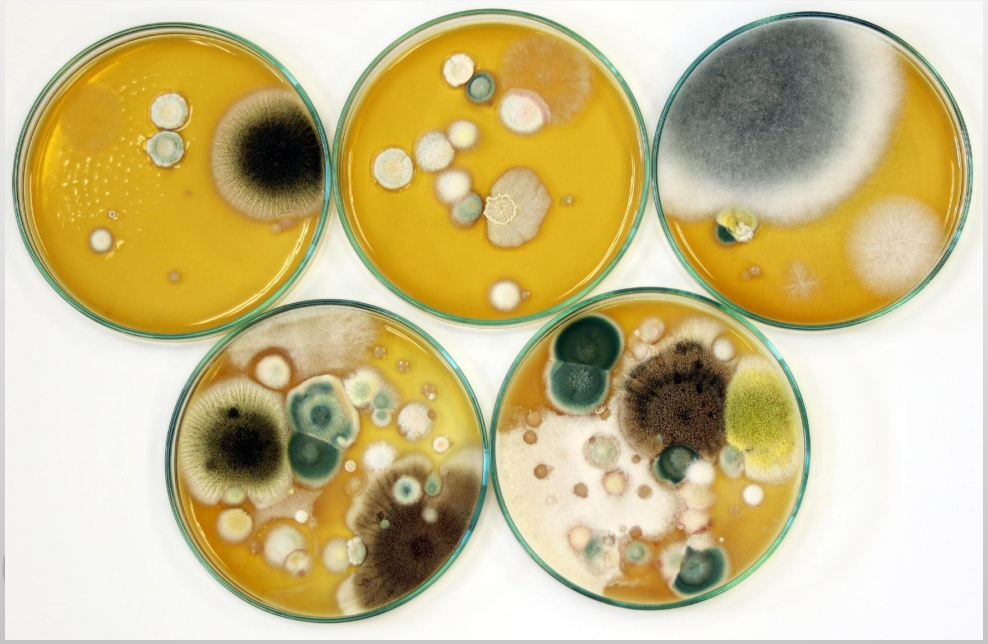
MAJOR BACTERIAL PLAYERS

- ***STAPHYLOCOCCUS EPIDERMIDIS*: ONE OF THE TOP TWO MOST ABUNDANT COMMENSAL BACTERIA ON THE SKIN – PARTICIPATES IN THE REINFORCEMENT OF THE SKIN BARRIER BY EXPRESSING SPHINGOMYELINASE THAT CONTRIBUTES TO CERAMIDE SYNTHESIS**
- ***CUTIBACTERIUM ACNES*: SECOND OF THE MOST ABUNDANT COMMENSAL BACTERIA ON THE SKIN – PRODUCES LIPASES THAT BREAK UP LIPIDS INTO FATTY ACIDS THAT CONTRIBUTES TO THE "ACID MANTLE" OF THE SKIN**
  - ALSO THE MICROBIAL CULPRIT ASSOCIATED WITH ACNE VULGARIS
- **OTHERS INCLUDE:** *MICROCOCCUS LUTEUS, STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA*

THE FUNGI: "MYCOBIOME"

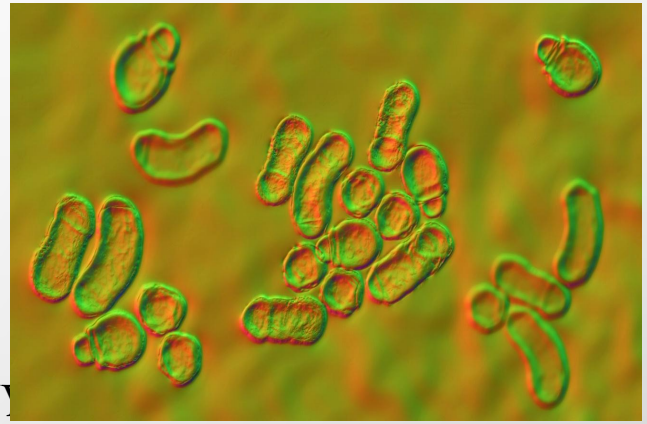
LARGE FOCUS ON THE FUNGI OF THE SCALP

THEY ARE "EUKARYOTES" – MEANING THAT THEIR CELLS ARE SIMILAR TO OUR CELLS



MAJOR FUNGAL PLAYERS

- ***MALASSEZIA* SPECIES**: HIGHLY LIPOPHILIC GENUS OF FUNGI THAT HAVE BEEN ASSOCIATED WITH DANDRUFF AND SEBORRHEIC DERMATITIS
  - BY FAR, THE MOST ABUNDANT FUNGAL GROUP ON THE SKIN
  - *M. RESTRICTA, M. GLOBOSA, M. FURFUR, M. SYMPODIALIS*
  - POSSIBLE LINK TO ATOPIC DERMATITIS, DISTINCT FROM *S. AUREUS*



- **OTHERS INCLUDE**:
  - DERMATOPHYTES: *TRICHOPHYTON*, *MICROSPORUM*, AND *EPIDERMOPHY*
  - *CANDIDA ALBICANS*
  - *RHODOTORULA* SPECIES – "EMERGING SKIN PATHOGEN"

- **WHILE *MALASSEZIA* SPECIES DOMINATE NEARLY EVERY REGION OF THE SKIN BIOGEOGRAPHY, THE MAIN EXCEPTION IS ON THE FEET WHERE THERE IS A FAIR AMOUNT OF FUNGAL DIVERSITY

THE ARCHAEA: "ARCHAEOME"

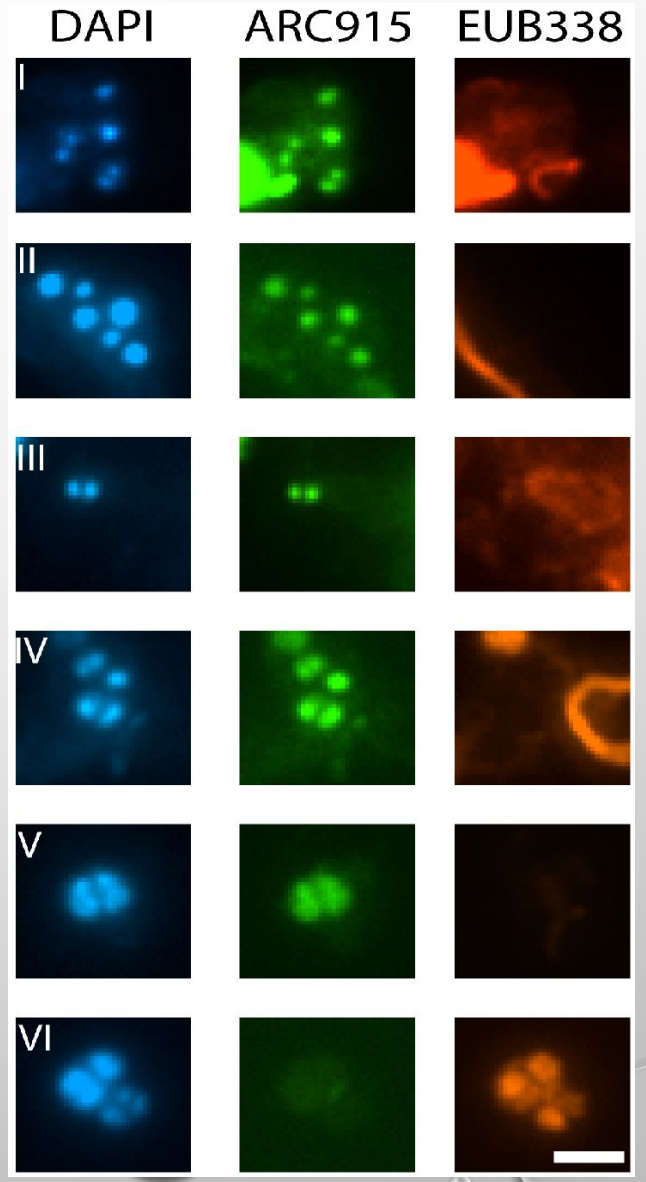
ARCHAEA REPRESENT AN UNDERSTUDIED AND SOMEWHAT ELUSIVE GROUP OF MICROBES FOUND ON THE SKIN

THEY ARE "PROKARYOTES" – MEANING THAT THEY DO NOT HAVE TRUE NUCLEI AND ARE DISTINCTLY DIFFERENT FROM THE CELLS THAT MAKE UP US

THEY ARE ENVIRONMENTAL EXTREMOPHILES

MAJOR ARCHAEL PLAYERS

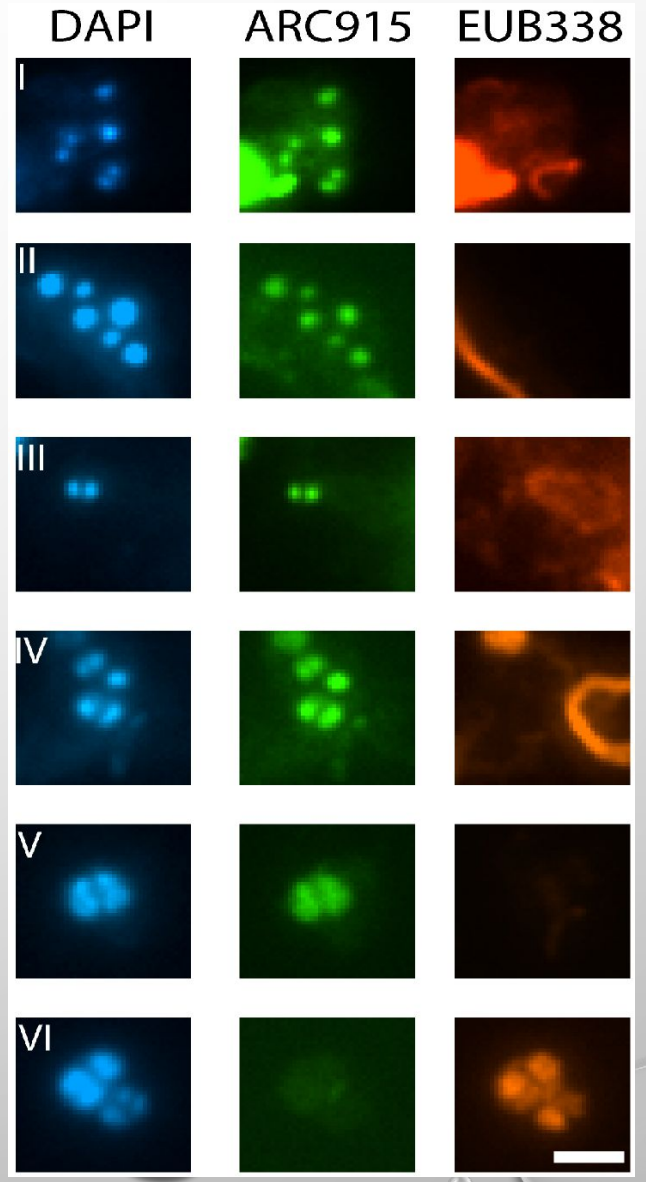
- **ARCHAEA:** (FORMERLY ARCHAEBACTERIA) WHEN DETECTED ON SKIN TEND TO BE IN DRY, LOWER LIPID REGIONS
- ***THAUMARCHAEOTA*** AND ***EURYARCHAEOTA***
- **AMMONIA OXIDIZERS** – THE GROUP THAT WERE DETECTED ON HUMAN SKIN SUGGESTING THEY MIGHT FACILITATE AMMONIA REMOVAL FROM SWEAT THAT CAN CONTRIBUTE TO SKIN DISORDERS
  - LOWER SKIN pH – KEEP PATHOGENS OUT AND REDUCE ODOR ASSOCIATED WITH SWEAT



*borrowed from Probst et al., (2013) *PLoS One*

MAJOR ARCHAEL PLAYERS

- **ARCHAEA:** (FORMERLY ARCHAEBACTERIA) WHEN DETECTED ON SKIN TEND TO BE IN DRY, LOWER LIPID REGIONS
- **AGE COMPONENT** – PREVALENT ON HUMAN SKIN AT AGES: 12 AND YOUNGER, 60 AND OLDER
- **GENDER COMPONENT** – MORE PREVALENT IN WOMEN THAN IN MEN
- **VITAMIN B12 CONNECTION?** MANY MEMBERS OF *THAUMARCHAEOTA* PRODUCE VITAMIN B12



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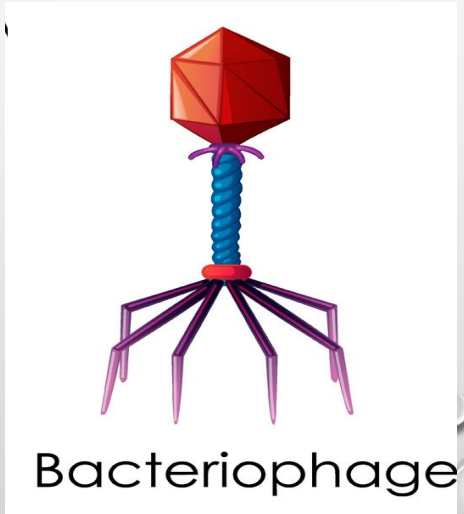
THE VIRUSES: "VIROME"

"ACELLULAR" MICROBES NOT UNIVERSALLY CONSIDERED TO BE LIVING MICROBES



MAJOR VIRAL PLAYERS

- **BACTERIOPHAGES:** ARE VIRUSES THAT ARE EXCLUSIVELY TARGETED TO SPECIFIC BACTERIAL SPECIES AND STRAINS – MAJORITY THAT ARE NORMALLY FOUND ON THE SKIN TARGET *C. ACNES* AND STAPHYLOCOCCI
- **EUKARYOTIC DNA VIRUSES:** ARE VIRUSES THAT DO INFECT HUMAN TISSUE WITH PREVALENCE TOWARD HAVING DNA-BASED GENOMES. SOMETIMES REFERRED TO AS "DNA TUMOR VIRUSES"
  - HERPESVIRUSES, PAPILLOMAVIRUSES, POLYOMAVIRUSES…
- ****NOTE:** MIGHT BE AN UNDER-REPRESENTATION OF **RNA VIRUSES** PRESENT ON THE SKIN…ARTIFACT OF SEQUENCING BIAS

MICROBIOME MODULATION

CURRENTLY, SO-CALLED “MICROBIOME MODULATORY” EFFORTS ARE EXEMPLIFIED BY THREE (3) DIFFERENT STRATEGIES:

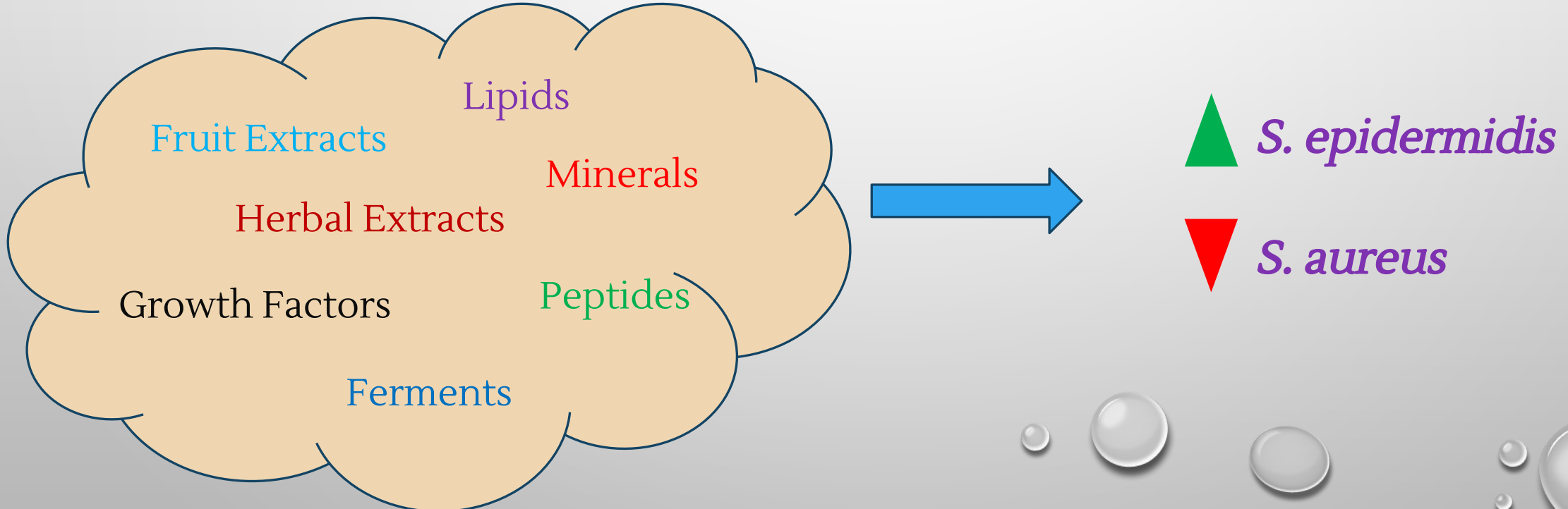
#1: PREBIOTICS

#2: PROBIOTICS

#3: POSTBIOTICS

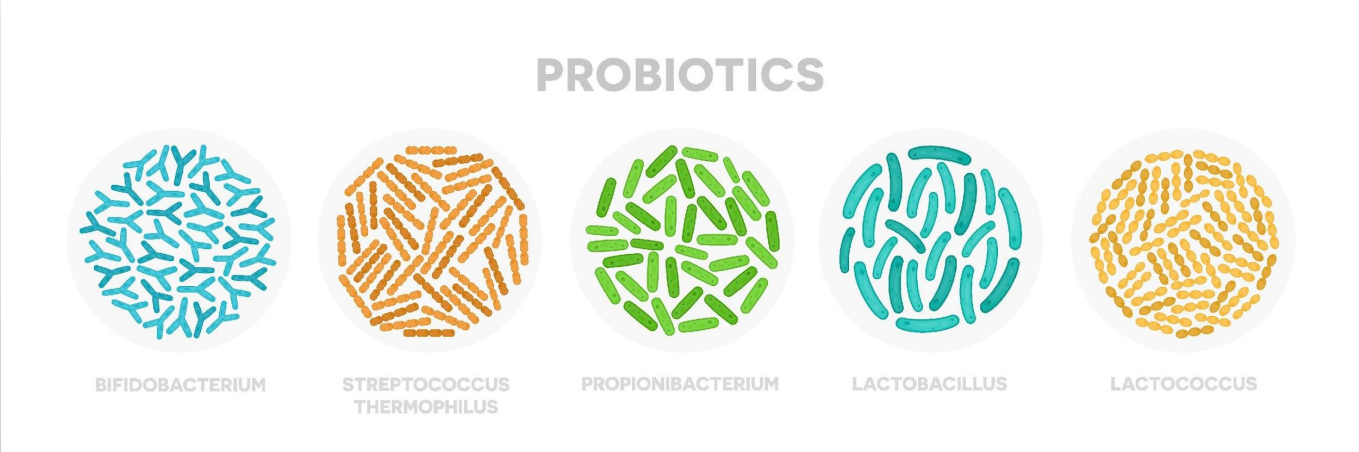
PRE-BIOTICS

- **PREBIOTICS:** ARE BIO-ACTIVE INGREDIENTS APPLIED TO THE SKIN THAT ARE INTENDED TO AUGMENT OR DIMINISH GROWTH OF PARTICULAR MICROBIAL INHABITANTS OF THE SKIN – BY FAR, THE BROADEST CATEGORY



PRO-BIOTICS

- **PROBIOTICS:** ARE LITERALLY LIVE MICROORGANISMS BEING APPLIED TO THE SKIN THAT HAVE POTENTIALLY AMELIORATIVE PROPERTIES IF THEY ESTABLISH A FOOTHOLD – BEST EXEMPLIFIED BY MODERN DAY YOGURTS BEING INFUSED WITH AS MANY BACTERIA AS POSSIBLE FOR GUT HEALTH



PRO-BIOTICS

- **PROBIOTICS:** FOR SKINCARE, ONE OF THE MOST POPULAR LIVE MICROORGANISMS FOR TOPICAL APPLICATION IS *LACTOBACILLUS*

- *Lactobacillus*
- *Lactococcus*
- *Bifidobacterium*

- Anti-inflammatory
- Improved moisturization
- Diminished *C. acnes*
- Reduced *S. aureus* biofilms
- Stimulated collagen production
- Anti-melanogenesis

POST-BIOTICS

- **POSTBIOTICS:** THIS APPROACH ABANDONS COAXING THE GROWTH OF A MICROORGANISM IN ONE DIRECTION OR ANOTHER. RATHER, THE BENEFICIAL MOLECULES FOUND WITHIN THE MICROBE ARE LIBERATED FOR TOPICAL APPLICATION INDEPENDENT OF THE ORGANISM
  - EXTRACTIONS AND FERMENTATIONS
  - MICROBIAL LYSATES

Saccharomyces cerevisiae ferment of sugarcane

Lipotechoic acid from *Lactobacillus*

Ferment lysate of *Lactobacillus*

Lactic acid purified from lactic acid bacteria

Cellular lysate of *Lactobacillus*

PRECISION MODULATION

- THE MAJORITY OF THE **PRE-, PRO-, AND POST-BIOTICS** ON THE MARKET OR IN DEVELOPMENT ARE BROADLY PRO-GROWTH OR ANTIMICROBIAL TO CERTAIN GROUPS OF MICROORGANISMS
- WHAT ABOUT A MORE TARGETED, PRECISE APPROACH?
  - SO-CALLED **"BACTERIOPHAGE THERAPY"** OFFERS SPECIES, SOMETIMES STRAIN, LEVELS OF PRECISE TARGETING

SKIN MICROBIOME:
THE FUTURE

WHAT WE DO NOT KNOW OR HAVE NOT YET EXPLORED

CAREFUL WITH THE TERMINOLOGY

- **WORRYING TREND OF MISUSE OF MICROBIOME RELATED TERMS AND EXPRESSION**
  - EXAMPLE: CONFLATION OR MISUSE OF "**PREBIOTIC**", "**PROBIOTIC**", AND "**POSTBIOTIC**"
- **NEBULOUS "FEEL GOOD" MARKETING PHRASES**
  - "**MICROBIOME FRIENDLY**" – PERFECTLY FINE IF THERE IS AN ESTABLISHED AND ENFORCED CONSENSUS DEFINITION

NEW FRONTIERS (I)

- **ARCHAEA:** GIVEN THAT THESE ARE GENERALLY CONSIDERED ENVIRONMENTAL EXTREMOPHILES, IT IS EXTRAORDINARY THAT THEY MAKE UP SOME OF THE SKIN RESIDENTS
  - "HUMAN ASSOCIATED ARCHAEA: A NEGLECTED MICROBIOME WORTH INVESTIGATING" – A. GUERRA, *WORLD JOURNAL OF MICROBIOLOGY* (2024)

ARCHAEBIOTICS

- IN THE CASE OF ***THAUMARCHEAOTA***, COULD THESE ORGANISMS BE GROWN UP IN BIOREACTORS AND TOPICALLY APPLIED AS A PROBIOTIC TO IMPROVE AMMONIA OXIDATION OR TO IMPROVE THE LEVELS OF VITAMIN B12?

NEW FRONTIERS (II)

- **VIRUSES:** ALREADY THERE IS MOVEMENT TO UNDERSTAND AND APPLY BACTERIOPHAGES TO BALANCE SKIN BACTERIAL GROWTH, BUT WHAT ABOUT THE EXISTING **EUKARYOTIC DNA VIRUSES**?
  - PAPILLOMAVIRUSES
  - POLYOMAVIRUSES
  - HERPESVIRUSES
  - CIRCOVIRUSES

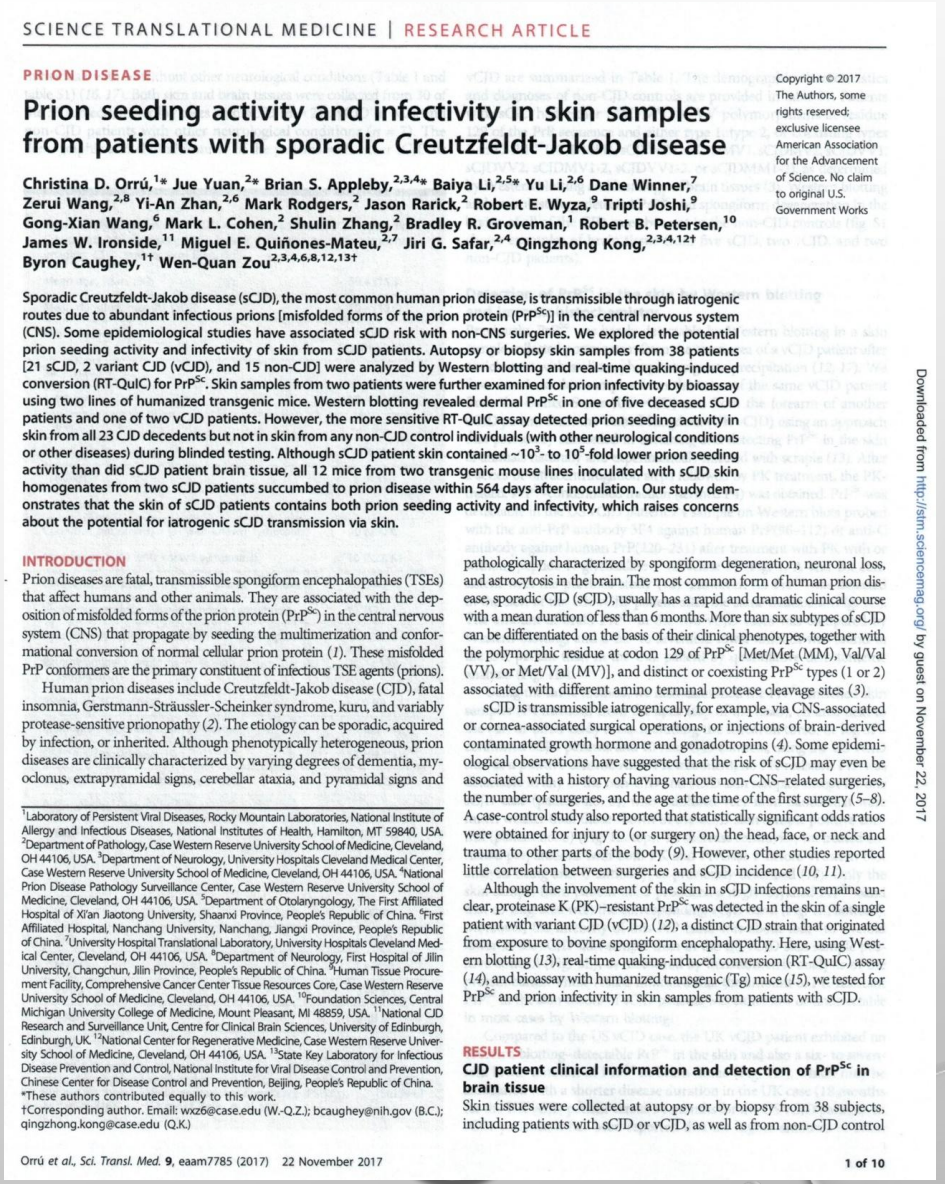
NEW FRONTIERS (III)

- **FUNGI:** AS SOME SKIN CONDITIONS HAVE BEEN LINKED TO PERTURBATIONS IN THE BALANCE OF THE VARIOUS *MALASSEZIA* SPECIES PRESENT ON THE SKIN AND SCALP. CAN HOMEOSTATIC LEVELS BE RESTORED?
  - SEBORRHEIC DERMATITIS
  - DANDRUFF
  - FUNGAL ACNE

- THERE ARE ALSO OTHER FUNGAL SPECIES THAT ARE DESCRIBED AS EMERGING SKIN PATHOGENS LIKE *RHODOTORULA* THAT SHOULD DEFINITELY BE EXPLORED FOR AMELIORATION VIA TOPICAL INTERVENTIONS

NEW FRONTIERS (IV)

- **PRIONS**: ARE INHERITED AS WELL AS INFECTIOUS PATHOGENIC PROTEINS THAT CAUSE SLOW-INCUBATING NEUROLOGICAL DISEASES
  - "MAD COWS DISEASE", CREUTZFELDT-JAKOB'S DISEASE
- RECENT EVIDENCE THAT SOME PRIONS BECOME "SEEDED" IN THE SKIN – POSSIBLY LEADING TO ANOTHER MECHANISM OF TRANSMISSION



SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

PRION DISEASE

Prion seeding activity and infectivity in skin samples from patients with sporadic Creutzfeldt-Jakob disease

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Sporadic Creutzfeldt-Jakob disease (sCJD), the most common human prion disease, is transmissible through iatrogenic routes due to abundant infectious prions [misfolded forms of the prion protein (PrP^{Sc})] in the central nervous system (CNS). Some epidemiological studies have associated sCJD risk with non-CNS surgeries. We explored the potential prion seeding activity and infectivity of skin from sCJD patients. Autopsy or biopsy skin samples from 38 patients [21 sCJD, 2 variant CJD (vCJD), and 15 non-CJD] were analyzed by Western blotting and real-time quaking-induced conversion (RT-QuIC) for PrP^{Sc}. Skin samples from two patients were further examined for prion infectivity by bioassay using two lines of humanized transgenic mice. Western blotting revealed dermal PrP^{Sc} in one of five deceased sCJD patients and one of two vCJD patients. However, the more sensitive RT-QuIC assay detected prion seeding activity in skin from all 23 CJD decedents but not in skin from any non-CJD control individuals (with other neurological conditions or other diseases) during blinded testing. Although sCJD patient skin contained ~10^3- to 10^5-fold lower prion seeding activity than did sCJD patient brain tissue, all 12 mice from two transgenic mouse lines inoculated with sCJD skin homogenates from two sCJD patients succumbed to prion disease within 564 days after inoculation. Our study demonstrates that the skin of sCJD patients contains both prion seeding activity and infectivity, which raises concerns about the potential for iatrogenic sCJD transmission via skin.

INTRODUCTION

Prion diseases are fatal, transmissible spongiform encephalopathies (TSEs) that affect humans and other animals. They are associated with the deposition of misfolded forms of the prion protein (PrP^{Sc}) in the central nervous system (CNS) that propagate by seeding the multimerization and conformational conversion of normal cellular prion protein (*1*). These misfolded PrP conformers are the primary constituent of infectious TSE agents (prions).

Human prion diseases include Creutzfeldt-Jakob disease (CJD), fatal insomnia, Gerstmann-Sträussler-Scheinker syndrome, kuru, and variably protease-sensitive prionopathy (*2*). The etiology can be sporadic, acquired by infection, or inherited. Although phenotypically heterogeneous, prion diseases are clinically characterized by varying degrees of dementia, myoclonus, extrapyramidal signs, cerebellar ataxia, and pyramidal signs and pathologically characterized by spongiform degeneration, neuronal loss, and astrocytosis in the brain. The most common form of human prion disease, sporadic CJD (sCJD), usually has a rapid and dramatic clinical course with a mean duration of less than 6 months. More than six subtypes of sCJD can be differentiated on the basis of their clinical phenotypes, together with the polymorphic residue at codon 129 of PrP^{Sc} [Met/Met (MM), Val/Val (VV), or Met/Val (MV)], and distinct or coexisting PrP^{Sc} types (1 or 2) associated with different amino terminal protease cleavage sites (*3*).

sCJD is transmissible iatrogenically, for example, via CNS-associated or cornea-associated surgical operations, or injections of brain-derived contaminated growth hormone and gonadotropins (*4*). Some epidemiological observations have suggested that the risk of sCJD may even be associated with a history of having various non-CNS–related surgeries, the number of surgeries, and the age at the time of the first surgery (*5*–*8*). A case-control study also reported that statistically significant odds ratios were obtained for injury to (or surgery on) the head, face, or neck and trauma to other parts of the body (*9*). However, other studies reported little correlation between surgeries and sCJD incidence (*10*, *11*).

Although the involvement of the skin in sCJD infections remains unclear, proteinase K (PK)–resistant PrP^{Sc} was detected in the skin of a single patient with variant CJD (vCJD) (*12*), a distinct CJD strain that originated from exposure to bovine spongiform encephalopathy. Here, using Western blotting (*13*), real-time quaking-induced conversion (RT-QuIC) assay (*14*), and bioassay with humanized transgenic (Tg) mice (*15*), we tested for PrP^{Sc} and prion infectivity in skin samples from patients with sCJD.

RESULTS

CJD patient clinical information and detection of PrP^{Sc} in brain tissue

Skin tissues were collected at autopsy or by biopsy from 38 subjects, including patients with sCJD or vCJD, as well as from non-CJD control

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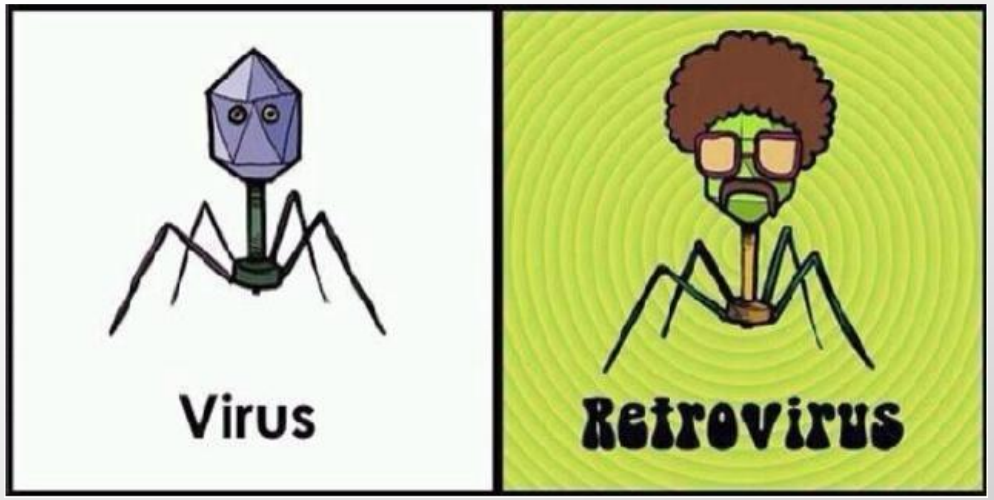
QUESTIONS???

THANK YOU VERY MUCH FOR YOUR TIME AND ATTENTION



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