32nd IFSCC Congress
London 19-22 September 2022

EFFECTS OF MARINE EXOPOLYSACCHARIDES ON BACTERIAL ADHESION TO HUMAN SKIN CELLS AND ON BIOFILM PRODUCTION, APPLICATIONS FOR COSMETICS

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Introduction:

Some marine bacteria have the capability to produce exopolysaccharides (EPS) to protect themselves, especially against dehydration during prolonged period out of water, but also to attach themselves to natural supports (rock, wood, algae, ...).
These EPS have very variable structures. Some EPS contain acid groups (GlcA, GalA), sulfate or acetate groups, or even amino acids (Ala, Ser). These structures give them interesting biological activities, but also effects on microbial adhesion.
We evaluated the ability of some marine EPS to inhibit the adhesion of bacteria on human skin cells (corneocytes) using an original adhesion test.
To better understand potential interactions between bacteria on the skin surface (corneocytes), we realised additional experiments on C. acnes biofilm formation and glycoprofiling.

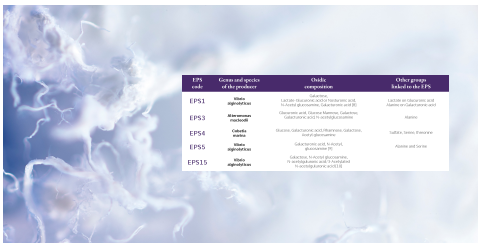

Figure 1: *composition of the different EPS*

Materials & Methods:

Production, Isolation and Purification Exopolysaccharide

Marine bacterial strains were isolated from natural sampling, identified and deposited in the CNCM (Collection Nationale de Cultures de Microorganismes / National Collection for Microorganisms Culture).
Exopolysaccharide were produced by fermentation of marine microorganism in a fermenter containing marine broth medium supplemented with sugar at 25°C during 72h. Then, the supernatant, containing the excreted EPS, was purified by filtration through a 1 µm filter sheet, by ultrafiltration (300 kDa) and by precipitation with isopropanol. The obtained EPS were dried to remove isopropanol and crushed.

Corneocytes adhesion assay

Corneocytes used in this study were sampled (according to standardised procedure of patch pressure) on the day of the study from healthy volunteers, using D-Squams® disks purchased from Monaderm (Monaco).
The labelling of microorganism Staphylococcus epidermidis (ATCC 12228), Staphylococcus aureus (ATCC 6538), Cutibacterium acnes (ATCC 11827). is performed with carboxyfluorescein diacetate succinimidylester (CFDA-SE) purchased from Sigma-Aldrich (St. Louis, MO, USA) according to the supplier's technical note.
The assessment of interactions of microorganism on cell surfaces was achieved according GLYcoDiag's protocol.

Biofilm production assay

According to by BioFilm Control knowledge, the 2 strains of C. acnes of the study present an adhesion at 32 h of incubation with, the The BioFilm Ring Test® (BRT) method at 32 h in order to measure the activity of EPS at the bacterial adhesion time, and at a second time (48 h) to discriminate an inhibition activity from a delayed activity.
The initial bacterial suspension was prepared in M20 medium, concentrated at 10^6 CFU/mL by measure of absorbance (OD 600 nm) and filled in each well with the magnetic beads (TON004) at 10 µL/mL. In parallel, several controls were prepared. Then, microplates were incubated under anaerobic condition at 37°C. After 32 h, the microplate was magnetized for 1 min, scanned and analysed with the BFC Elements 3.0 software. Biofilm Formation Index (BFI) was determined for each well to quantify the biofilm formation. Doxycyclin (DOX), the active ingredient from Doxylis, was used as positive activity control at 64 µg/mL.

Results & Discussion:

Adhesion et biofilm production profile for different EPS

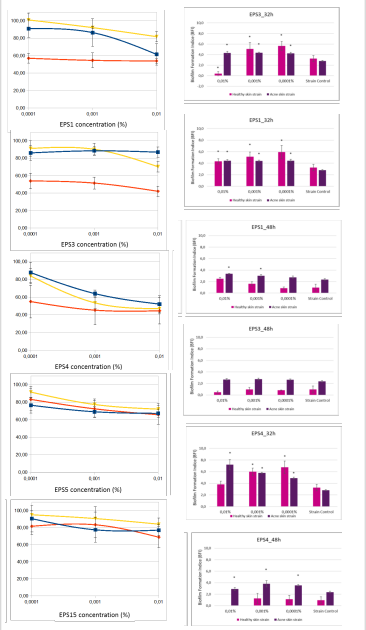

Figure 2: *Adhesion of several strains in percentage against the control condition (without EPS). Blue: C. acnes ; Red: S. aureus ; Yellow: S. epidermidis.*

Figure 3: *Biofilm Formation Index, for 3 different EPS, 2 strains of C. acnes and 2 times of kinetics (32 and 48h).*

Thanks to complete glycoprofiling of each EPS that gave complementary information about EPS composition and interactions (fig 1), we imagined a competition in lectin/GBP recognition on the skin surface and/or on the bacteria surface. This competition could explain inhibition of bacteria adhesion on any support and modification of their compartment.

Adhesion profiles showed very different results from one EPS to another (fig 2). For example, some of them (EPS1 and EPS3) inhibited S. aureus adhesion in the same manner but EPS1 only decreased C. acnes adhesion (EPS3 remained without any effect on C. acnes), while EPS3 inhibited S. epidermidis adhesion (EPS1 presented no effect on S. epidermidis). Another one (EPS5) inhibited the adhesion of all the strains in a dose dependant manner.
Secondly, we examined the effect of the presence of EPS on biofilm production of two strains of C. acnes (one of healthy skin and one sampled on acneic lesions) and three EPS only (those presenting the main three different profiles of adhesion). On the three selected EPS, only EPS4 exhibited a trend to decrease biofilm production (fig 3). After complementary experiments with different batches of each type of C. acnes, we realised statistical analyses that proved the significant inhibition of biofilm production due to the presence of EPS4 (fig 4-5), in both strains (healthy and pathological). Although the activity of EPS4 is partial and concentration independent on the inhibition of biofilm formation, its activity is significantly higher on strains from acne than on strains from healthy volunteers.

Taking together all the results, we could associate structures of the EPS, their effects on bacteria adhesion and biofilm production. It is interesting to note that EPS presenting the quite same adhesion profiles (EPS5 and 15 for all strains or EPS1 and 3 for Staphylococcus genius) are so different in structure. If we focused on Staphylococcus aureus, EPS 5 presented no effect while EPS1 and EPS4 inhibited adhesion on corneocytes.
The structure of EPS5 appeared simpler than both others. If avoiding simple sugars, presence of glucuronic acid or lactate-glucuronic acid may be an explanation of those differences. It might be confirmed with control experience. In another hand, one EPS (1 or 3) can decrease adhesion of Staphylococcus aureus without any effect on Staphylococcus epidermidis. That suggests different systems of adhesion for bacteria even if they belong to the same biological genius.

Combining adhesion and biofilm data, we saw that on 3 EPS decreasing Cutibacterium acnes adhesion, only EPS4 decreased production of biofilm as well, but biofilm of pathogenic strains only; while none EPS affected bacteria growth.

Focus on EPS4

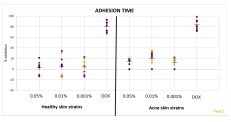

Figure 4: *BFI, Biofilm Formation Inhibition expressed in %, for EPS4, 3*2 strains of C. acnes and 2 times of kinetics.*

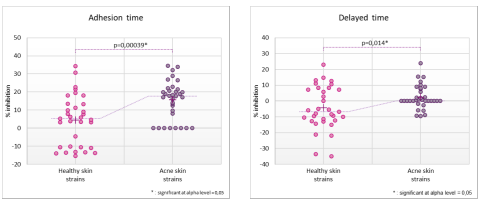

Figure 5: *statistical analysis and the repartition of all points for all the experiments.*

Conclusions:

Results of our study show that marine EPS are not only physical fil-formers. They also play an important role in glycobiological interaction processes. They are recognised by some specific receptors for carbohydrates. Thus, they can interfere with human corneocytes and lock competitively bacterial survey (adhesion on skin surface and biofilm production). That are really interesting for cosmetic applications, and especially for skin that presents unbalanced microbiota (dysbiosis), in order to maintain healthy conditions or to prevent excessive pathogenic strains invasion. Influencing growth is not the only solution, when it is possible to modulate adhesion in a selective manner or to modify the behaviour of cells.

Acknowledgements:

Authors want to thank warmly Polymaris Biotechnology (Brest, France) for implication, access and production of EPS from marine bacteria, GLYcoDiag for their friendly exchanges, for the realisation of glycoprofiles and adhesion tests, and BioFilm Control for quality and reactivity of their study.

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