DNA-FISH Probes for Rapid Detection of Microbial Contaminants in Cosmetic Products

Patrícia Branco [1,2], Nuno Lourenço [1], Beatriz Bernardo[1], Elisabete Maurício [1,3,4]

[1]BIORG—Bioengineering and Sustainability Research Group, Faculdade de Engenharia, Universidade Lusófona, Av. Campo Grande 376, 1749-024 Lisbon, Portugal.

[2]Linking Landscape, Environment, Agriculture and Food (LEAF), Associated Laboratory TERRA, Instituto Superior de Agronomia, University of Lisbon, Tapada da Ajuda, 1349-017 Lisbon, Portugal.

[3]CBIOS—Research Center for Biosciences & Health Technologies, Universidade Lusófona, Campo Grande 376, 1749-024 Lisbon, Portugal

[4]Elisa Câmara, Lda, Dermocosmética, Centro Empresarial de Talaíde, n°7 e 8, 2785-723 Lisbon, Portugal

INTRODUCTION & AIM

Microbial contamination in the cosmetic industry possess significant risks to product safety, potentially causing health issues for consumers and leading to costly recalls (Halla et al., 2018). Common contaminants, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, can be introduced during manufacturing or consumer use, resulting in infections and product spoilage (Jairoun et al., 2020). Conventional detection methods like plate counting are slow, often taking up to a week to yield results, which delays quality control and increases costs. Fluorescence *In Situ* Hybridization (FISH) technique offers a faster, specific, and sensitive alternative for detecting these microorganisms directly in cosmetic products (Ferone et al., 2020).

This study aims to design and validate new DNA-FISH probes, optimized to function without formamide, for the rapid identification of cosmetic microbial contaminants.

METHOD

1 DNA-FISH probes design

ProbeCheck - Check the pre-existence of DNA-FISH probes of the *target* microorganisms: *E. coli*, *P. aeruginosa* and *S.aureus*.

NCBI and nBLAST - Acquire 23S ribosomal RNA sequences from *target* microorganisms in FASTA format.

BioEdit-AlignmentEditor - Align acquired sequences from *target* and *non-target* microorganisms in FASTA format.

Decipher-DesignProbes - Design of DNA-FISH probes specific to the *target* microorganism.

2 Microorganism: strains and growth conditions

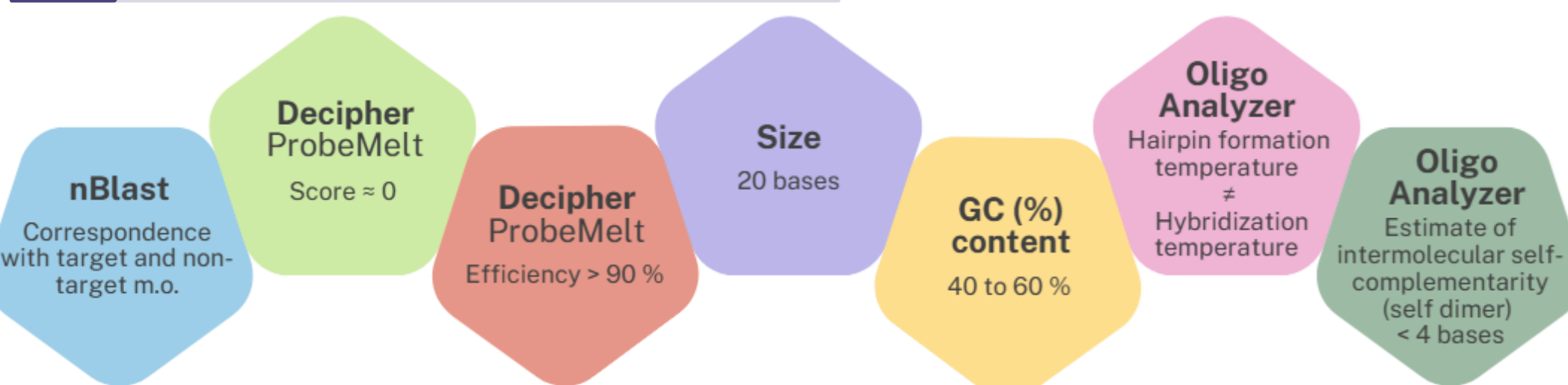*E.coli* ATCC 8739
LB broth
12-24h 37°C without agitation

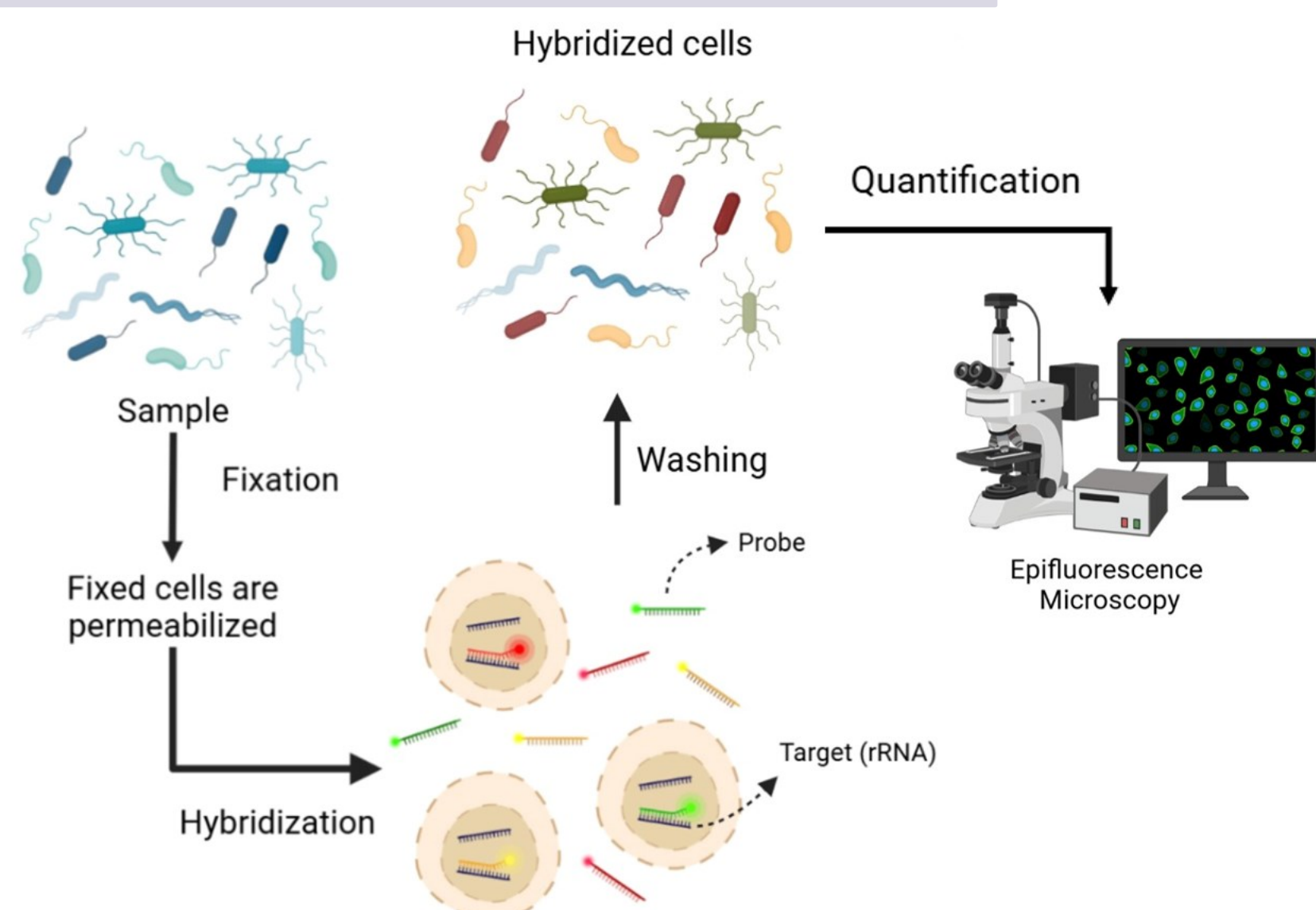*S. aureus* ATCC 6538
BHI broth
12-24h 37°C without agitation

P. aeruginosa ATCC 9027
LB broth
12-24h 37°C without agitation

3 *In silico* evaluation of probes

nBlast — Correspondence with target and non-target m.o.

Decipher ProbeMelt — Score = 0

Decipher ProbeMelt — Efficiency > 90 %

Size — 20 bases

GC (%) content — 40 to 60 %

Oligo Analyzer — Hairpin formation temperature ≠ Hybridization temperature

Oligo Analyzer — Estimate of intermolecular self-complementarity (self dimer) < 4 bases

4 RNA-FISH procedure and quantification

RESULTS & DISCUSSION

Experimental evaluation of DNA-FISH probes specificity and performance

E.coli ATCC 8739

Table 1: Results obtained by fluorescence microscopy for the RNA-FISH assays performed with different [FA] in the presence of *E.coli ATCC 8739*. Signal intensities were classified into four categories: −, no signal; +/−, low; +, medium; +/+, high (nt, non tested).

E. coli ATCC 8739

[FA] %	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)
0	-	+	+/+	-	-
5	-	+	+	nt	nt
15	-	+	+/-	nt	nt
25	-	+	+	nt	nt
35	-	+	+	nt	nt
45	-	+	+/+	nt	nt

Assay (probe used); [FA]=0%					
Blank (none)	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)

S. aureus ATCC 6538

[FA] %	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)
0	+	+	-	+/+	-
5	+	+	nt	+	nt
15	+	+	nt	+	nt
25	+	+	nt	+	nt
35	+	+	nt	+/-	nt
45	+	+	nt	-	nt

Assay (probe used); [FA]=0%					
Blank (none)	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)

S. aureus ATCC 6538

Table 2: Results obtained by fluorescence microscopy for the RNA-FISH assays performed with different [FA] in the presence of *S. aureus ATCC 6538*. Signal intensities were classified into four categories: −, no signal; +/−, low; +, medium; +/+, high (nt, non-tested).

P. aeruginosa ATCC 9027

Table 3: Results obtained by fluorescence microscopy for the RNA-FISH assays performed with different [FA] in the presence of *P. aeruginosa ATCC 9027*. Signal intensities were classified into four categories: −, no signal; +/−, low; +, medium; +/+, high (nt, non-tested).

P. aeruginosa ATCC 9027

[FA] %	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)
0	-	+	-	-	+/+
5	-	+	nt	nt	+
15	-	+	nt	nt	+
25	-	+	nt	nt	+/-
35	-	+	nt	nt	+/-
45	-	+	nt	nt	-

Assay (probe used); [FA]=0%					
Blank (none)	Negative Control (EUK516-ATTO550)	Positive Control (EUB338-ATTO550)	Test (EC352-ATTO550)	Test (SA606-ATTO550)	Test (PA734-ATTO550)

The designed probes demonstrated **high specificity and efficiency for detecting the target microorganisms in both *in silico* and experimental conditions**. Experimental validation confirmed that the probes could reliably identify *E. coli*, *P. aeruginosa*, and *S. aureus* in controlled cultures, producing distinct fluorescent signals without the need for formamide.

CONCLUSION

This study successfully developed formamide-free DNA-FISH probes for the rapid identification of key microbial contaminants in cosmetic products. The proposed method addresses the limitations of traditional microbiological testing by providing quicker, safer, and more reliable results, thereby improving quality control and product safety in the cosmetic industry.

FUTURE WORK / REFERENCES

For future work, it would be essencial to further test the probes efficiency in common cosmetic formulations that are currently available in the market to guarantee that these are ready to be be put to use in practical setting.

References