A novel *Dekkera bruxellensis* RNA-FISH probe: design and evaluation

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Abstract

RNA-Fluorescence *In Situ* Hybridisation (FISH) technique is based on the hybridisation of fluorescently-labelled oligonucleotide probes targeting to specific regions of the ribosomal RNA [1]. Its use has exponential increased in the last decades for microbial identification. However, one of the main limitations of this technique is the reduced number of specific RNA-FISH probes available. One of the major issues is the design of probes with the desired level of specificity and high hybridisation efficiency. Specificity of probe binding to the target site depends on the stringency conditions, commonly adjust using formamide(FA). Thus, *in silico* and empirical formamide denaturation curves are commonly used searching for the optimal conditions for ensuring specificity [2]. Therefore, this work was focused on the design of an RNA-FISH probe target to a yeast specie (*Dekkera/Brettanomyces bruxellensis*) and on the evaluation of its suitability *in silico* and experimentally.

Methodology

The probe was designed *in silico* by using DECIPHER-program (http://decipher.cee.wisc.edu/DesignProbes.html). It was analysed with mathFISH-program (http://mathfish.cee.wisc.edu/) and blast nucleotide (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to calculate hybridisation efficiency and specificity/coverage, respectively. Experimental evaluation was done by constructing the fluorescence-signal response/formamide concentration curve for the target (*Dekkera/Brettanomyces bruxellensis*) and a non-target yeast (*Candida krusei*) from the same environment of the target (wine). A previously described FISH procedure was applied [3] and fluorescence intensity (FI) was measured by flow cytometry.

Results and Discussion

Analyses *in silico* of D. bruxellensis probe

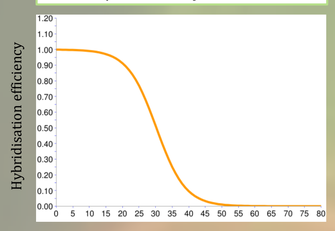
Properties of the probe

% GC content	Hairpin temperature (ºC)	Probe sequence (5´- 3´)
52.90	14.20	CCTTCCTCCTCTCTAGT

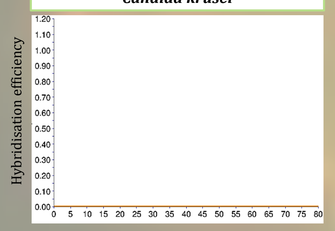
mathFISH-program analyses

Formamide denaturation curve

Target yeast- *Dekkera/Brettanomyces bruxellensis*



Non-target yeast- *Candida krusei*



Blast nucleotide analyses

Taxonomy report

Taxonomy reports sum up the number of hits for a given taxonomic node and place them in a lineage structure

Organism	Blast Name	Score	Number of Hits	Description
Eukaryota	eukaryotes		147	
. Opisthokonta	eukaryotes		137	
. . Bilateria	animals		7	
. . . Euteleostomi	vertebrates		5	
. . . . Osteoglossocephalai	bony fishes		2	
. . . . . Seriola dumerili	bony fishes	34.2	1	Seriola dumerili hits
. . . . . Scleropages formosus	bony fishes	34.2	1	Scleropages formosus hits
. . . . Apteryx australis mantelli	birds	34.2	3	Apteryx australis mantelli hits
. . . Eufriesea mexicana	bees	34.2	1	Eufriesea mexicana hits
. . . Toxocara canis	nematodes	34.2	1	Toxocara canis hits
. . Brettanomyces bruxellensis	ascomycetes	34.2	105	Brettanomyces bruxellensis hits
. . Brettanomyces sp. DCY-2016	ascomycetes	34.2	2	Brettanomyces sp. DCY-2016 hits
. . uncultured Dekkera	ascomycetes	34.2	23	uncultured Dekkera hits
. Neospora caninum Liverpool	apicomplexans	34.2	2	Neospora caninum Liverpool hits
. Arachis duranensis	eudicots	34.2	7	Arachis duranensis hits
. Spirodela polyrhiza	monocots	34.2	1	Spirodela polyrhiza hits

Summary of mathFISH and blast nucleotide analyses

	mathFISH and blast nucleotide analyses		
Microorganism	% Hybridisation efficiency*	Match target organism**	Match ecosystem organisms**
Dekkera/Brettanomyces bruxellensis	99.89	130	0
Candida krusei	1.0901E-08		

*Theoretical hybridisation efficiency analysed by mathFISH (http://mathfish.cee.wisc.edu/) with 0% of formamide. **Number of sequences with 100% of match identity in 500 sequences found in blast nucleotide (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

Excellent performance of the probe was observed with high maximal theoretical *in silico* hybridisation efficiency (99.89 %)
and specificity (130 matches of the target organism in 500 sequences and 0 matches for organisms from the same ecosystem of the target organism).

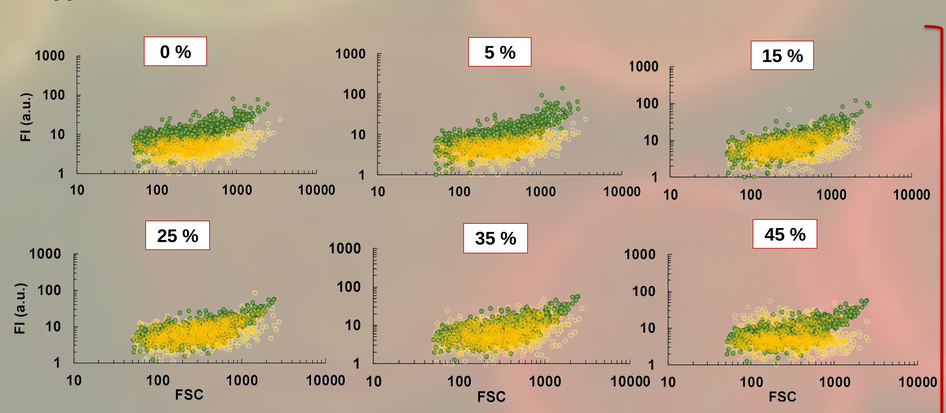
Experimental evaluation of D. bruxellensis-ATTO 647N probe applying FISH procedure

Fluorescence signal response analysed by flow cytometry at different formamide [FA] concentrations

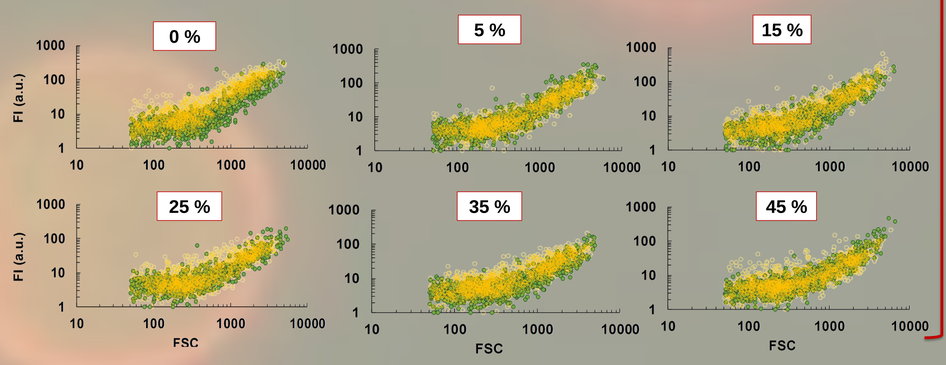
Dot plots (FI/FSC)

Negative Control- ○ EUB338-ATTO 647N probe
Test- ● D. bruxellensis-ATTO 647N probe

Target yeast- *Dekkera/Brettanomyces bruxellensis*

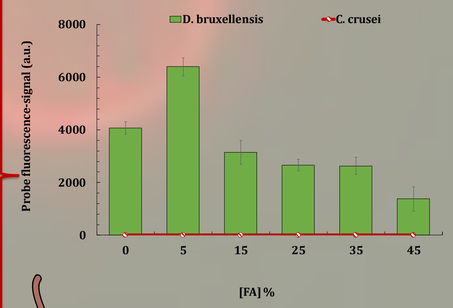


Non-target yeast-*Candida krusei*



Flow cytometry results (Fluorescence Intensity (FI) arbitrary units (a.u.)/Forward SCattering (FSC)) of : **A**- Target cells (*Dekkera/Brettanomyces bruxellensis)* hybridised with D. bruxellensis-ATTO 647N and EUB338-ATTO 647N probes. **B**- Non-target cells (*Candida krusei*) hybridised with D. bruxellensis-ATTO 647N and EUB338-ATTO 647N probes. All assays were performed with increasing concentrations of formamide from 0% to 45%.

Probe fluorescent signals for the target and non-target cells



Probe fluorescence-signal = Σ FI of fluorescent cells* after FISH treatment with D. bruxellensis-ATTO 647N probe

*We considered fluorescent cells those that showed FI values higher than the FI maximal value of FISH treated cells with EUB 338 ATTO 647N probe

The experimental evaluation revealed that the D. bruxellensis-ATTO 647N probe has high specificity with 5% of formamide (showing its maximal fluorescence-signal response for the target and none for the non-target yeast).

References:
[1]- Moter, A. *et al.* (2000) J. Microbiol. Methods **41**:85–112
[2]- Yilmaz *et al.* (2008) Environ. Microbiol. **10**: 2872–2885
[3]- González-Pérez, M. *et al.* (2017) Appl. Phys. A 142:1-11

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