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The combined application of the anti-*RAS1* and anti-*RIM101* 2′-*O*MethylRNA oligomers enhances *Candida albicans* filamentation control

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Abstract

Although antisense oligomers (ASOs) have been successfully utilized to control gene expression, they have been little exploited to control *Candida albicans* virulence's determinants. Filamentation is an important virulence factor of *C. albicans*, and *RAS1* and *RIM101* genes are involved in its regulation. Thus, the main goal of this work was to project ASOs, based on 2′-*O*Methyl chemical modification, to target *RAS1* and *RIM101* mRNA and to validate its application either alone or in combination, to reduce *Candida* filamentation in different human body fluids.

It was verified that both, anti-*RAS1* 2′*O*Me and anti-*RIM101* 2′*O*Me oligomers, were able to reduce the levels of *RAS1* and *RIM101* genes' expression and to significantly reduce *C. albicans* filamentation. Furthermore, the combined application of anti-*RAS1* 2′*O*Me oligomer and anti-*RIM101* 2′*O*Me oligomer enhances the control of *C. albicans* filamentation in artificial saliva and urine.

Our work confirms that ASOs are useful tools for research and therapeutic development on the control of candidiasis.

Lay Summary

This work aimed to project antisense oligomers to control *Candida albicans* filamentation. The results revealed that the projected oligomers, anti-*RAS1* 2′*O*Me and anti-*RIM101* 2′*O*Me, were able to control *RAS1* and *RIM101* gene expression and to significantly reduce *C. albicans* filamentation.

Key words: *Candidiasis*, virulence, nucleic acid mimics, therapeutic oligomers.

Introduction

Antisense oligomers (ASOs) are short strands of nucleic acids that are complementary to a specific target mRNA that will bind via standard Watson-Crick base pairing.[1] Normally, ASOs are chemically modified[1,2] in order to protect them against the action of nucleases, to improve delivery and biodistribution and to enhance RNA affinity.[1–3] The 2'-OMethylRNA (2'OMe) was one of the early developed chemical modifications, and it is characterized by sugar modification with the introduction of an oxygenated group. This chemical modification does not allow the recruitment of RNase H, however, to overcome this limitation, a central unmodified sequence, called as *gapmer*, was inserted to allow RNase activity.[4,5]

Candidiasis represents a public health problem of major importance. *Candida* species have the ability to cause superficial infections, affecting urinary and oral tracts, or systemic infections, involving major body organs.[6–10] *Candida albicans* remains the most prevalent of all *Candida* species in the world[11–13] and its pathogenicity is attributed to certain virulence factors, being the switch from yeast to filamentous form one of the most important.[14,15] *RAS1* and *RIM101* are known as two important regulator genes of *C. albicans* filamentation.[16,17]

RAS1 gene regulates the mitogen-activated protein (MAP kinase) [51,55] and the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA)[18–20] cascades of genes.[21,22] Furthermore, *RIM101* gene, was described as one transcription factor associated to *C. albicans* yeast-to-hyphae transition as response to pH environment.[21,23] The increase of *C. albicans* infections rate, coupled with an increased resistance to the traditional antifungal therapies, highlights the need of new alternative therapies, with new mechanisms of action and low. In this sense, this work is based on key hypothesis that if a pathogen's genetic sequence is recognized as determinant of virulence (as is the case of *RAS1* and *RIM101*), it will be possible to synthesize nucleic acid mimics that will be bind to the correspondent mRNA and degrade it and, consequently, reduce its phenotype (in this case of *C. albicans* filamentation). Thus, the main goal of this work was to project and synthesize 2'-OMethylRNA ASOs targeting the *RAS1* and *RIM101* mRNA and to perform *in vitro* validation of its single and combined applicability to reduce filamentation on different human body fluids (artificial saliva and urine).

Methods

Design and synthesis of anti-RAS1 and anti-RIM101 oligomers

To design ASOs against *RAS1* and *RIM101*, the target regions of each gene were selected based on a search conducted at the *Candida* Genome Database (http://www.candidagenome.org/cgibin/compute/blast_clade.pt). Different sequences of *RAS1* and *RIM101* were aligned, to ensure the use of conserved regions of each gene. Also, a BLAST was conducted to ensure that the selected sequences do not target any human genome sequence or a similar region in another *C. albicans* gene.

The *RAS1* sequence 5′ ATCCGCTTTAACCA 3′ and *RIM101* sequence 5′ ATGCTACGTCACC 3′ were selected as targets, taking into account its high specificity to *C. albicans* genome, the gene region (the first 200 nucleotides), the number of nucleotides (between 12 and 20), the melting temperature (Tm) (between 39°C and 42°C), and the content of guanine-cytosine (GC) (around 50–60%).[1,24,25] The ASOs were modified in each end of the sequence using the 2'OMe chemical modification, and the central regions were constituted by DNA nucleotides to ensure the RNase H activity.[5,26]

The anti-*RAS1* 2'OMe and the anti-*RIM101* 2'OMe oligomers were synthesized with standard phosphodiester linkages in the Biomolecular Nanoscale Engineering Center (Department of Physics, Chemistry and Pharmacy University of Southern Denmark, Odense M, Denmark) according to the user own specifications, with custom added 5′- and 3′- modifications and then purified by HPLC. The original aliquots of ASOs were prepared to 100 μM in sterile ultrapure water and stored at −20°C. The stock was prepared as an aliquot of 4 μM and stored at −20°C in sterile ultrapure water.

Cytotoxicity evaluation

The cytotoxicity of the each ASO was determined against the 3T3 cell line (Fibroblast cells, Embryonic tissue, Mouse from CCL3, American Type Culture Collection). For that, the 3T3 cell line was cultured in Dulbecco's Modified Eagle's Medium (D-MEM, Biochrom, Germain) supplied with 10% of fetal bovine serum (FBS, Sigma Aldrich) and 1% of antibiotic containing penicillin and streptomycin (P/S - Biochrom, Germain). After detachment, a suspension of 1×10^5 cells ml^{-1} was added on a 96-well plate and incubated at 37°C and 5% CO_2 until attaining 80% of confluence. Prior to the cytotoxicity assays, the wells were washed twice with phosphate-buffered saline solution (PBS; pH 7, 0.1 M). A concentration 40 nM of each ASO was prepared in D-MEM medium and 50 μL of each ASO were added to each well. Negative control was prepared with 50 μl of DMSO and positive control 50 μl of D-MEM medium was used. The plates were incubated for 24 h at 37°C and 5% CO_2. After incubation, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium solution (MTS (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega); 1% of D-MEM without phenol) was added to each well and incubated during 1 h in the dark. Last, the absorbance was recorded at 490 nm with a microplate reader (Biochrom EZ Reader 800 Plus, Cambridge, England). The cytotoxicity results were expressed as the percentage of viable cells corresponding the optical density 490 (OD_{490}) of cells grown without ASO as 100% cell viability.

Microorganism and growth conditions

The *Candida albicans* SC5314 reference strain from the *Candida* collection of the Biofilm Group of the Centre of Biological Engineering was used during this study. For all experiments, *C. albicans* was subcultured on Sabouraud dextrose agar (SDA; Merck, Darmstadt, Germany) and incubated for 24 h at 37°C. Cells were then inoculated in Sabouraud dextrose broth (SDB; Merck, Darmstadt, Germany) and incubated at 37°C and 120 rpm, overnight. After incubation, the cells' suspensions were centrifuged for 5 min at 6000 × *g* and 4°C and washed twice with PBS. Pellets were suspended in 5 ml RPMI medium (Sigma-Aldrich, St. Louis, MO, USA), and the cellular density was adjusted for each experiment using a Neubauer chamber (Paul Marienfeld, Lauda-Königshofen, Germany) to 1×10^6 cells per milliliter (cells ml^{-1}). All experiments were performed in triplicate and in a minimum of three independent assays.

Antisense oligomers effect on filamentation

To evaluate the effect of the anti-*RAS1* 2'OMe oligomer and the anti-*RIM101* 2'OMe oligomer on *C. albicans* filamentation, yeast cells were incubated with each ASO during 24 h, in an Erlenmeyer flask. For that, 10 ml of *C. albicans* suspensions at

Table 1. Genes studied and the respective primer sequence, melting temperature (Tm) and amplification product

Specie	Gene name	Systematic name	Sequence (5′-3′)	Primer	Tm (°C)	Amplification (BP)
Candida albicans	*RAS1*	C2_10210C_A (orf19.1760)	5′-TGCAAATCAACAAGGTCAAG-3′	Foward	55	164
			5′-GACCAGAAGAAACACCTCCA-3′	Reverse		
	RIM101	C1_14340C_A (orf19.7247)	5′-TCCATGTCCCATTGAAGC-3′	Foward	57	169
			5′-TGTTGTTGCTTGGCCTCT-3′	Reverse		
	PMA1	C3_00720W_A (orf19.5383)	5′-TTGCTTATGATAATGCTCCATACGA-3′	Foward	57	66
			5′-TACCCCACAATCTTGGCAAGT -3′	Reverse		

1×10^6 cells ml^{-1}, prepared in RPMI medium, were incubated in the presence of 40 nM of each ASO and incubated at 37°C under gentle agitation (120 rpm) during 24 h. The positive control was prepared only with 10 ml of the same yeast cell concentration on RPMI. After 12 and 24 h, aliquots were recovered, and filaments were counted using a Neubauer chamber. The results were presented as percentage (%) of filamentation reduction, through the following formula:

$$\%\ \text{of filamentation inhibition} = [(\%\ \text{of filaments cells on control}) - (\%\ \text{of filaments cells in presence of ASO})]/ (\%\ \text{of filamentscells on control}).$$

Epifluorescence microscopy analysis was used to confirm, qualitatively, the effect of ASOs on *C. albicans* filamentation. *Candida albicans* cells grown in the presence and absence of ASOs were stained with 1% (v/v) of calcofluor (Sigma-Aldrich, St. Louis, MO, EUA) for 15 min in dark conditions, centrifuged for 5 min, and washed twice with ultra-pure water. An Olympus BX51 microscope (Olympus Portugal, Porto, Portugal) coupled with a DP71 digital camera and the filter used was DAPI — 360-370/420 (blue channel), and the images were acquired with the program FluoView FV100 (Olympus).

Antisense oligomers effect on gene expression

To analyze the effect of the anti-*RAS1* 2'OMe oligomer and anti-*RIM101* 2'OMe oligomer on the respective gene expression, quantitative RT-PCR studies were performed. For that, *C. albicans* cell suspensions were prepared, as described previously, and aliquots recovered at 12 and 24 h for RNA extraction. The RNA was extracted using the E.Z.N.A® Total RNA kit I (Omega, Bio-TEK).[14] Then, to avoid potential DNA contamination, samples were treated with DNase I (DNase I, Amplification Grade, Invitrogen), and RNA concentration was determined by optical density measurement with the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). The cDNA was synthetized using the iScript Reverse Transcriptase (Bio-Rad) in accordance with the manufacturer's instructions. qRT-PCR (CFX96, Bio-Rad) was performed on a 96-well microtiter plate using EvaGreen Supermix (Bio-Rad, Berkeley, CA, USA). The expression of the *RAS1* and *RIM101* genes was normalized with the *PMA1 Candida* reference gene. No-reverse transcriptase controls (NRTs) and no template controls (NTCs) were included in each run. Each reaction was performed in triplicate, and mean values of relative expression were determined for each gene. The primers were designed using the Primer 3 web-based software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and are described in Table 1.

Performance in simulated body fluids

Simulated body fluids

To mimic human body fluids, artificial urine (AU) and saliva (AS) were used during this work. AU (pH 4 and 5.8) and AS (pH 7) were prepared with slight modifications to that previously described by Silva et al (2010) and (2013), respectively.[27,28] The composition of AU was (0.65 g/L), $MgCl_2$(0.65 g/L), NaCl (4.6 g/l), Na_2SO_4 (2.3 g/l), Na_3C_3H5O $(CO2)_3$ (0.65 g/l), $Na_2C_2O_4$ (0.02 g/l), KH_2PO_4 (2.8 g/l), KCl (1.6 g/l), NH_4Cl (1.0 g/l), urea (25 g/l), creatinine (1.1 g/l), and glucose (3 g/l); and the composition of AS was peptone (5 g/l), glucose (2 g/l), mucin (1 g/l), NaCl (0.35 g/l), $CaCl_2$ (0.2 g/l), and KCl (0.2 g/l).

Individual ASOs performance

To study the individual performance of each ASO, *C. albicans* cells were incubated on AS and AU during 24 h. For that, 10 ml of *C. albicans* suspensions at 1×10^6 cells ml^{-1} were prepared in AU (pH 4 and 5.8) and AS (pH 7) and incubated in the presence of 40 nM of each ASO. The positive control was prepared with 10 ml of *C. albicans* cells on simulated body fluids in the absence of ASO. After 12 and 24 h, aliquots were recovered and the percentage of filaments enumerated using a Neubauer chamber, as described earlier.

Combined ASOs performance

To evaluate the ASOs combined performance, *C. albicans* cells were incubated on AS (pH 5.8) and AU (pH 7) in the presence of

Table 2. Oligomers sequences, respective size, melting temperature (Tm) and GC content (% GC)

Gene	Sequences	Length	Tm (°C)	%GC
Anti-*RAS1* 2'OMe	mUmGmG TTA AAC GGmG mAmU	14	42.0	42.9
Anti-*RIM101* 2'OMe	mGmGmU GAC GTA GmCmAmU	13	44.9	53.8

m - 2'O-Methyl RNA; **A** - Adenine; **T** - Thymine; **C** - cytosine; **G** - Guanine; **U** - Uracil.

both ASOs (40 nM of each one), during 24 h. The positive control was prepared as described previously and the filamentation enumeration was evaluated as described before.

Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of a least three independent experiments. Results were compared using Two-way analysis of variance (ANOVA) using GraphPad Prism 6® (GraphPad Software, CA, USA). All tests were performed with a confidence level of 95%.

Results

Anti-RAS1 2'OMe and anti-RIM101 2'OMe sequences

The second generation of nucleic acid mimics, 2'OMe chemical modification, was the base for the design of anti-*RAS1* and anti-*RIM101* oligomers. Table 2 describes the characteristics of each ASO that were designed taking in account, the features described in methods section.

Oligomers cytotoxicity

In order to infer about the cytotoxicity of each ASO, the viability of 3T3 fibroblast cells in the presence of 40 nM of anti-*RAS1* 2'OMe and anti-*RIM101* 2'OMe oligomers was determined using the MTS assay (Fig. 1). The results revealed that both ASOs are non-cytotoxic in concentrations up to 40 nM, since the relative cell viability is higher than 70%.[29] Having in consideration the results of cytotoxicity, the next experiments were conducted using 40 nM of each ASO.

Oligomers effect on gene expression and on *C. albicans* filamentation

The effect of anti-*RAS1* 2'OMe and anti-*RIM101* 2'OMe oligomers in *C. albicans* cells was evaluated in terms of genotype (gene expression inhibition) and phenotype (reduction on the number of filamentous forms) (Fig. 2).

Figure 2A and 2B presents, respectively, the levels of *RAS1* and *RIM101* gene expression reduction. It was possible to verify that both ASOs were able to control the gene expression, although at different levels. The anti-*RAS1* 2'OMe oligomer was capable to reduce the levels of *RAS1* gene expression around 95% after 12 h of incubation (P value < 0.05) and around 24% after 24 h (P value < 0.05) (Fig. 2A). Contrariwise, anti-*RIM101* 2'OMe oligomer was able to maintain its performance throughout the 24 h of incubation with ≈ 73% of *RIM101* gene expression reduction (P value < 0.05).

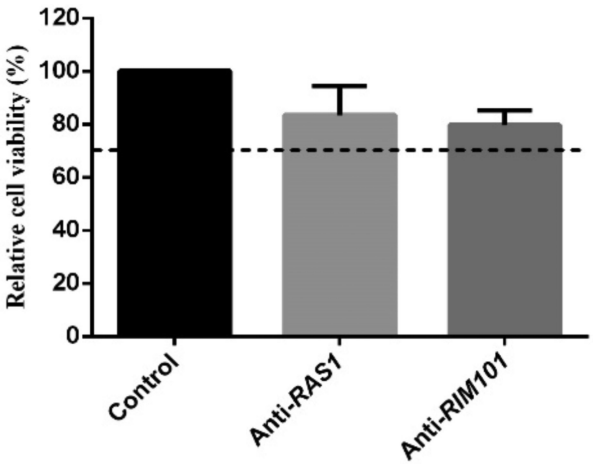

Figure 1. Oligomers cytotoxicity. Relative cell viability (%) determined by the absorbance values (Abs (490 nm)) of formazan product obtained from 3T3 cells treated with 40 nM of anti-*RAS1* 2'*O*Me and anti-*RIM101* 2'*O*Me oligomers. The control is related to the cells incubated in the absence of ASOs. Error bars represented standard deviation.

Figure 2C shows that both ASOs were able to control *C. albicans* filamentation, despite presenting different performances. The anti-*RAS1* 2'OMe oligomer was able to reduce *C. albicans* filamentation around 17% at 12 h and 39% at 24 h (P value < 0.05). In contrast, the anti-*RIM101* 2'OMe oligomer revealed to have a lower ability to control *C. albicans* filamentation compared with anti-*RAS1* 2'OMe oligomer performance, reaching about 23% of inhibition after 24 h (P value < 0.05).

The epifluorescence microscopy images (Fig. 2D) corroborate the quantitative results, showing a decrease on the number of filamentous cells after treatment with each oligomer.

Oligomers performance in simulated human body fluids

To infer about the stability and performance of anti-*RAS1* and anti-*RIM101* 2'OMe on simulated human body fluids, *C. albicans* cells were grown on AU (pH 4 and 5.8) and on AS (pH 7), in the presence of 40 nM of each ASO or their combination.

Individual performance

Figure 3 shows the individual performance of anti-*RAS1* and anti-*RIM101* 2'OMe oligomers on *C. albicans* filamentation. It

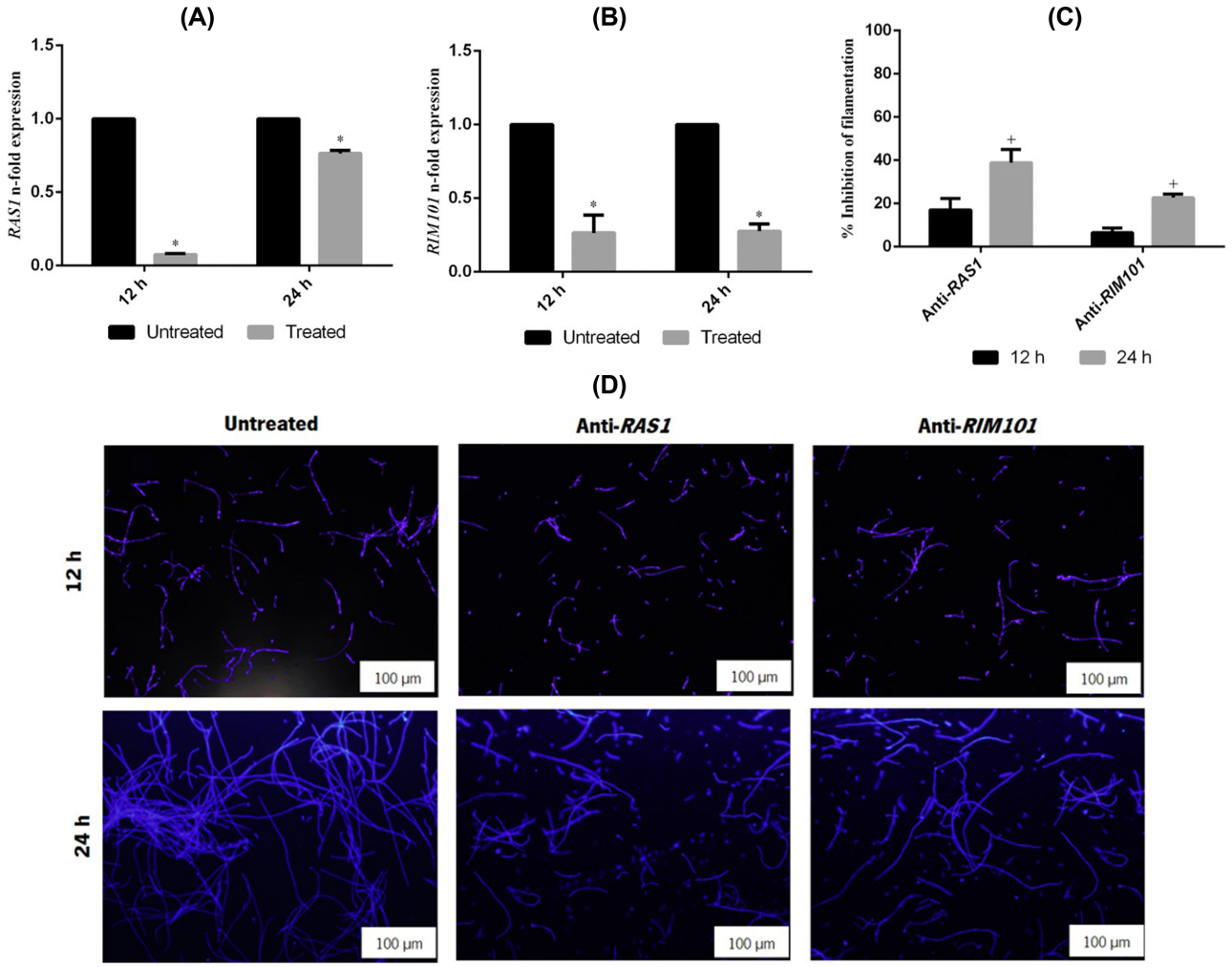

Figure 2. Oligomers effect on gene expression and on *Candida albicans* filamentation. **(A)** Anti-*RAS1* 2′*O*Me oligomer effect on *RAS1* gene expression; **(B)** anti-*RIMI101* 2′*O*Me oligomer effect *on RIM101* gene expression, **(C)** anti-*RAS1* 2′*O*Me and anti-*RIMI101* 2′*O*Me oligomers effect on *C. albicans* filamentation after 12 h and 24 h of incubation and **(D)** Epifluorescence microscopy images of *Candida* cells stained with Calcofluor after treatment with 40 nM of each oligomer at 12 and 24 h. Untreated results represent the positive control (cells grown in RPMI in absence of ASOs). Error bars represent standard deviation. *Significantly differences between cells grown in presence of 40 nM of ASOs and positive controls (*P* value < 0.05). $^{+}$Significant differences between 12 and 24 h for the same condition (*P* value < 0.05).

is important to emphasize that both ASOs maintain their activity in simulated human body fluids. To note, the highest effects were observed on AU (pH 4), with values of reduction around 52% at 12 h and 72% at 24 h for anti-*RAS1* 2'OMe oligomer and 63% at 12 and 24 h for anti-*RIM101* 2'OMe oligomer (*P* value < 0.05). In the case of anti-*RAS1* 2'OMe oligomer, the reduction was effectively lower, not reaching values higher than 23% on AU at pH 5.8 and 14% on AS pH 7, at 24 h. In AS (pH 7), cells grown with anti-*RIM101* 2'OMe oligomer presented an increase on the reduction of filamentation from 12 h for 24 h (26–44%). In contrast, in AU (pH 5.8), the levels of reduction decreased from 24 to 16% over the 24 h.

Combined performance

Figure 4 shows the combined effect of anti-*RAS1* and anti-*RIM101* 2'OMe oligomers on *C. albicans* filamentation in simulated human body fluids. Interestingly, it can be noticed that the combined effect of the two ASOs enhances the individual potential of each one. Importantly, in AU the levels of reduction of *C. albicans* filamentation increased from 28% (at 12 h - Fig. 4A) to 65% (at 24 h - Fig. 4B), corresponding to a duplication of the ASOs individual effect. To note, on AS the combined effect also boosted the individual effect of each ASO, resulting in a reduction of around 50% after 24 h (Fig. 4B) ($P < 0.05$).

Discussion

In the last decades, ASOs have been successfully applied for the treatment of many genetic human diseases.[2,25,30] However, the antisense technology has been poorly explored to the control of yeast's virulence determinants.[31,32]

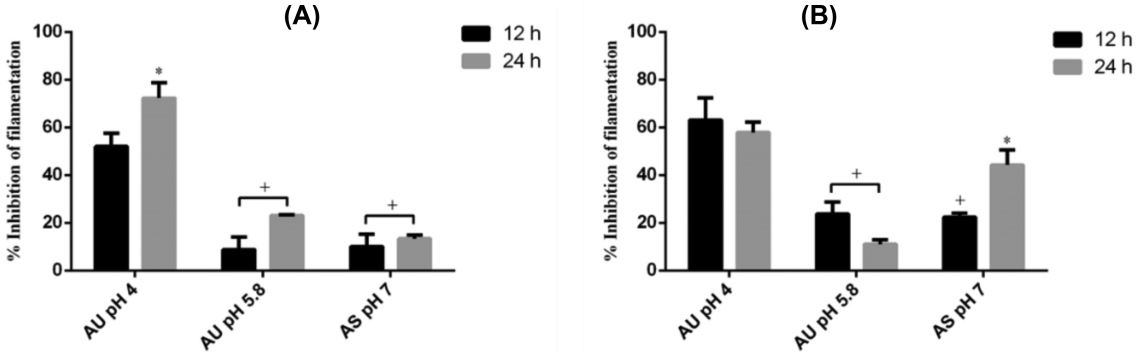

Figure 3. Individual performance of oligomers in stimulated human body fluids. Effect of **(A)** 40 nM of anti-*RAS1* 2′*O*Me oligomer and **(B)** 40 nM of anti-*RIM101* 2′*O*Me oligomer on *Candida albicans* filamentation when grown on AU (pH 4 and 5.8) and AS (pH 7) at 12 and 24 h. Error bars represent standard deviation. *Significant differences between 12 h and 24 h of incubation for the same condition ($P < 0.05$). $^{+}$Significant differences between AU (pH 4) and other tested conditions (P value < 0.05).

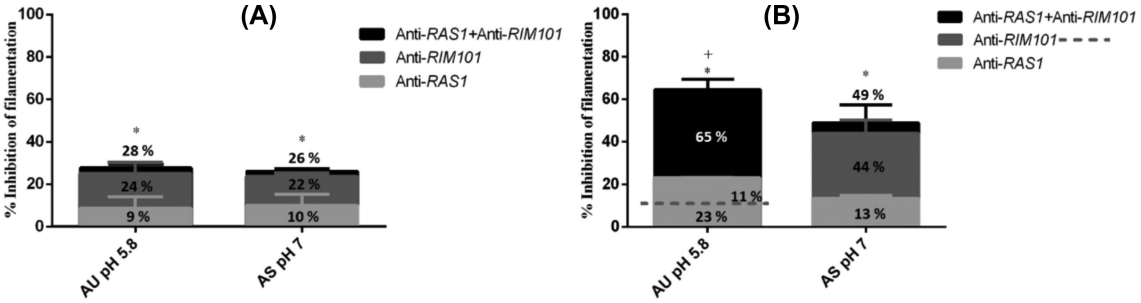

Figure 4. Combined performance oligomers in human simulated body fluids. Effect of anti-*RAS1* 2′*O*Me and anti-*RIM101* 2′*O*Me oligomers on *Candida albicans* filamentation when grown on AU (pH 4 and 5.8) and AS (pH 7) at 12 h **(A)** and 24 h **(B)** of incubation. Error bars represent standard deviation. *Significant differences between single anti-*RAS1* 2′*O*Me oligomer and combined effect for the same time of incubation ($P < 0.05$). $^{+}$Significant differences between single anti-*RIM101* 2′*O*Me oligomer and combined effect for the same time of incubation.

Candida albicans remains the most prevalent of all *Candida* species in the world[11–13] and one of the most problematic virulence factors is its ability to switch from yeast to filamentous forms.[33–35] Importantly the *RAS1* and *RIM101* genes are identified as two of the most important regulators of *C. albicans* filamentation.[22,36–38]

Through this work, the performance of ASOs designed to hybridize specifically to *RIM101* and *RAS1* targets (Table 2), was validated regarding its ability to control gene expression and to reduce *C. albicans* filamentation in human body fluids when applied in an individual and combined way.

The *in vitro* ASOs efficacy was evaluated at 40 nM since none of them showed cytotoxicity in concentrations up to 40 nM (Fig. 1). The results demonstrate the capacity of the 2′OMe oligomers to control *RAS1* (Fig. 2A) and *RIM101* (Fig. 2B) gene expression, at different levels. The anti-*RAS1* 2′OMe oligomer was able to reduce by 95% the levels of *RAS1* expression after 12 h, despite having its performance compromised after 24 h (Fig. 2A). In contrast, anti-*RIM101* 2′OMe oligomer maintained its performance over the time with about 73% of reduction on gene expression levels (Fig. 2B). The differences observed in terms of ASOs performance can be related with the different levels of expression of each gene. Of note, the levels of gene expression are a quite different from 12 to 24 h and the expression levels of *RIM101* are superior to those of *RAS1* at 24 h (Supplementary Figure 1). Moreover, the *in vitro* results also demonstrate the capacity of both 2′OMe oligomers to reduce *C. albicans* filamentation of about 20–40% (Fig. 2C). The anti-*RAS1* 2′OMe oligomer was able to reduce *C. albicans* filamentation around 17% at 12 h and 39% at 24 h of incubation. In contrast, the anti-*RIM101* 2′OMe oligomer revealed to have a lower ability for controlling *C. albicans* filamentation compared with anti-*RAS1* 2′OMe oligomer performance, reaching only about 23% of inhibition after 24 h. Despite of knowing the importance of *RIM101* and *RAS1* on regulation of *C. albicans* filamentation, the relevant role of other genes in this phenomenon is also assumed[21,39] thus, it would not be expected to obtain a total reduction on *C. albicans* filamentation.

Bearing in mind future medical applications of ASOs, its performance was evaluated in simulated body fluids. Supplementary Figure 2 shows the *C. albicans* ability to grow as filaments in AU pH 4 (around 10%) and pH 5.8 (around 40%) and in AS pH 7 (around 20%). Interestingly, it can be noticed that both ASOs, anti-*RAS1* and anti-*RIM101*, maintained its ability to control *C. albicans* filamentation on artificial saliva and urine at distinct pH (Fig. 3). To note, the highest effects were observed on

AU (pH 4), achieving values of reduction of 70% (by anti-*RAS1* 2'OMe oligomer) and 60% (by anti-*RIM101* 2'OMe oligomer), and 40% on AS (pH 7) (by anti-*RIM101* 2'OMe oligomer). In contrast, in the human body fluid with higher levels of *C. albicans* filamentation, AU (pH 5.8), the levels of reduction were considerably lower for each oligomer (by 10–20%). Taking in account the promising results of each ASO, their combined effect was analyzed on human body fluids (Fig. 4) and when applied simultaneously, they enhanced twice (by 65%) the reduction of the number of *C. albicans* cells' filamentation on AU. Therefore, this work revealed important results considering future medical applications of ASOs cocktails to control candidiasis.

Conclusion

Our data demonstrates that anti-*RAS1* and anti-*RIM101* 2'OMe oligomers are able to control *RAS1* and *RIM101* gene expression and to prevent *C. albicans* cells filamentation, a major virulence factor, maintaining its performance in human body fluids. It was revealed that the combined effect of anti-*RAS1* and anti-*RIM101* 2'OMe oligomers enhances the *C. albicans'* filamentation control in human body fluids.

Thus, this work reinforces the promising applicability of AST for controlling *Candida* virulence.

Supplementary material

Supplementary data are available at *MMYCOL* online.

Acknowledgments

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Declaration of interest

None.

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