Dimethylaminododecyl methacrylate Inhibits *Candida albicans* and oropharyngeal candidiasis in a pH-dependent manner

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Abstract

The prevalence of stomatitis, especially that caused by *Candida albicans*, has highlighted the need for new antifungal agents. We previously found that a type of quaternary ammonium salts, dimethylaminododecyl methacrylate (DMADDM), incorporated in dental materials inhibited the growth and hyphal development of *C. albicans*. However, how the quaternary ammonium salts inhibited the fungal pathogens and whether the oral condition, such as salivary pH variation under different diseases, can affect the antimicrobial capacity of quaternary ammonium salts is unknown. This study evaluated the antifungal effects of DMADDM at different pH *in vitro* and in *vivo*. A pH-dependent antifungal effect of DMADDM was observed in planktonic and biofilm growth. DMADDM enhanced antifungal activity at alkaline pH. Two pH regulated genes (*PHR1/PHR2*) of *C. albicans* were correlated with the pH-dependent antifungal effects of DMADDM. The *PHR1/PHR2* genes and pH values regulated the zeta-potential of *C. albicans*, which then influenced the binding between *C. albicans* cells and DMADDM. The pH-dependent antifungal activity of DMADDM was then substantiated in a murine oropharyngeal candidiasis model. We directly demonstrated that the antifungal abilities of quaternary ammonium salts relied on the cell zeta-potential which affected the binding between fungal cells and quaternary ammonium salts. These findings suggest a new antifungal mechanism of quaternary ammonium under different pH and that DMADDM can be a potential antifungal agent applied in dental materials and stomatitis therapy.

Key Points

1. DMADDM has stronger antifungal activity in alkaline than in acidic pH conditions.
2. The pH values and pH regulated genes can affect the zeta-potential of fungal cells.
3. Zeta-potential of fungal cells directly affect the binding between DMADDM and cells.

Keywords

Fungal infection; biofilm; quaternary ammoniums; salivary pH variation; surface electrical charge; pH-dependent antifungal activity.

Introduction

Oral candidiasis is the most common oral fungal infection disease (Bandara and Samaranayake, 2019). As an opportunistic infection of the oral cavity (Ghannoum et al., 2010; Iliev and Leonardi, 2017), oral candidiasis generally occurs in patients with HIV/AIDS (Brown et al., 2012), Sjogren's syndrome (Rhodus et al., 1997), diabetes mellitus (Willis et al., 1999) and head and neck cancers (Redding et al., 1999; Lalla et al., 2010). *Candida albicans* is the most common fungal pathogen that can cause candidiasis (Iacopino and Wathen, 1992; Ramage et al., 2009; Gendreau and Loewy, 2011; Nobile and Johnson, 2015).

The variation of pH in oral cavity was a risk factor for oral *Candida* colonization and infection (Sun et al., 2016). For example, the whole or local oral cavity pH became acidic after radiotherapy because of the injury of salivary gland. Then *C. albicans* infection was significantly increased and this type of infection even significantly reduced the 5-year survival rates of oral cancer patients (Spolidorio et al., 2001; Jensen et al., 2003; Jain et al., 2016). The oral colonization and infection of *C. albicans* were also enhanced in the denture wearers who exposed to sucrose, which reduced the salivary pH (Cavazana et al., 2018). These suggested that the acidic pH elevated the *C. albicans* infections. In contrast, maintaining a relatively alkaline oral environment may help to treat *C. albicans* infections. The application of alkaline mouthwash, which caused a relatively higher oral pH value, can enhance the antifungal treatment in the population with oral candidiasis (Ghalichebaf et al., 1982; Rudd et al., 1984). The infectious abilities of *C. albicans* and antifungal treatment under different pH values

suggested a close relationship among pH variations and *C. albicans* infection and treatment (Grotz et al., 2003).

C. albicans has developed smart pathways to adapt a wide range of pH at different host niches (Calderon et al., 2010). The Phr1 and Phr2 proteins belong to the GH72 family of β-(1,3)-glucanosyltransferases and play a crucial role in cell wall assembly and in the adaptive response to the pH changes (Kovacova et al., 2015). *In vitro*, *PHR1* is expressed at pH above 5.5, and deletion of *PHR1* results in growth and morphological defects at neutral to alkaline pHs. Conversely, *PHR2* is expressed at pH 5.5 or lower, and the growth and morphology of the *PHR2* null mutant is compromised below this pH (Muhlschlegel and Fonzi, 1997; Kovacova et al., 2015).

Quaternary ammonium salt (QAS) is widely applied to resist oral bacteria, such as Clearfil Protect Bond (Kuraray Medical, Tokyo, Japan), which has witnessed a strong antibacterial activity based upon QAS antibacterial monomer 12-methacryloyloxydodecylpyridinium bromide (MDPB) (Imazato et al., 2006). In our previous study, a type of QAS, dimethylaminododecyl methacrylate (DMADDM), was added to acrylic resin as a non-releasing antifungal agent (Chen et al., 2017). Our results showed that this QAS modified resin maintained acceptable mechanical properties and antifungal activity, which effectively inhibited *C. albicans* hyphal and biofilm development for the first time. However, the antifungal mechanism of DMADDM has remained unclear. It is generally believed that QAS has a high positive charge density which exerts a strong electrostatic interaction with negatively-charged bacteria (Nikitina et al., 2016). Nevertheless, how the microbial surface charges affect the

binding between QAS and cells is still unknown. Meanwhile, the antibacterial and antifungal activities of QAS were usually conducted at neural pH (Chen et al., 2016; Zhou et al., 2016; Chen et al., 2017; Cheng et al., 2017; Li et al., 2017; Zhou et al., 2019). It is still unknown that whether the different pH values will affect the antifungal efficiency of QAS since the pH value of oral cavity can be various. The aim of this study was to evaluate how the cell charges affect the binding between QAS and fungal cells and the antifungal properties of DMADDM against *C. albicans* at different pH conditions *in vitro* and *in vivo*.

Methods

Synthesis of Antibacterial Monomer DMADDM

Dimethylaminododecyl methacrylate (DMADDM) was synthesized according to a previously described process (Chen et al., 2017; Jiang et al., 2017). Briefly, 10 mmol of 2-(dimethylamino) ethyl methacrylate (DMAEMA), 10 mmol of 1-bromododecane (BDD), and 3 g of ethanol were mixed in a vial by capping and stirring at 70 oC for 24 hours. The ethanol was evaporated after the reaction was completed. The clear liquid remaining in the vial was DMADDM, which was verified by Fourier transform infrared spectroscopy.

Strains and media

All strains used in this study are listed in **Table S1**. The *C. albicans* wildtype (WT) is identical to SC5314 also known as ATCC MYA-2876 (Gillum et al., 1984) except that ura3 is deleted (Fonzi and Irwin, 1993). Strains *phr1Δ/Δ* and *phr2Δ/Δ* each lack specific glycosidases (Muhlschlegel and Fonzi, 1997; Calderon et al., 2010). The null

mutant *phr1Δ/Δ* showed growth defects at pH higher than 5.5, conversely, the growth of the null mutant *phr2Δ/Δ* is compromised below this pH value. All strains (**Table S1**) were maintained on YPD plates (1% yeast extract, 2% peptone, 2% glucose, and 2% agar) and a single colony was chosen and inoculated into liquid YPD medium at 37 °C overnight unless specified. The different pH values of the media were adjusted by the pH buffer containing different concentrations of Na_2HPO_4 and citric acid ($C_6H_8O_7$) (**Table S2**).

Fungal Growth Curve

To generate the fungal growth curve, 200 μg/ml of prepared *C. albicans* solution (final concentration: 1×10^5 cells/ml in YPD liquid medium) was added to a 96-well plate with or without different concentrations of DMADDM. The plates were incubated in a Multiscan Spectrum (Chro Mate1, Awareness Technology, Palm City, FL, USA) at 37 °C. The OD^{530} was measured every 60 minutes. Absorbance at different time-points were plotted to generate the growth curve.

Antifungal susceptibility test

DMADDM susceptibility measurements were carried out in flat bottom, 96-well microtiter plates (Greiner, Frickenhausen, Germany) as described previously (Ren et al., 2014; Zhou et al., 2018; Lu et al., 2019). Briefly, colonies from overnight cultures were chosen to prepare a strain suspension in RPMI 1640 medium at 1×10^4 CFU/ml. Overall, 2μl of the DMADDM stock solution was added to 96-well plates, followed by an additional 198μl of the strain suspension. The maximal DMADDM concentration was 250 μg/ml, which was serially diluted with strain suspension to 3.8 μg/ml. The test

plates were incubated at 35 °C for 16 hours. Minimal inhibition concentrations (MICs) were determined by measuring and comparing the optical densities of the blank control and test wells. All experiments were performed in triplicate.

Biomass detection conducted by crystal violet experiment

Briefly, colonies from overnight cultures were chosen to prepare a strain suspension in RPMI 1640 medium with certain pH at 1×10^4 CFU/ml. 2ul DMADDM solution was added to 96-well plates, followed by an additional 198μl of the strain suspension. The test plates were incubated at 35 °C for 24 or 48 hours. Then the biomass on the bottom of the 96-well plate was detected. For the detection step, firstly, the medium upon biofilm was sucked out, and 200μl sterile PBS buffer was added to each well to remove the planktonic fungi for 3 times. 100ul methanol was added to each well for 15min, then sucked out the methanol and dried naturally. 100ul 1% crystal violet solution was added to each well and stained at room temperature for 5min. After sucking out the crystal violet dye in each well, rinsing the excess dye with running water and dried naturally. 100ul 33% glacial acetic acid solution was added to each well to dissolve crystal violet at 37℃ for 30min. Finally, OD value of the solution in each well was determined by the enzyme marker under 590nm. All experiments were performed in triplicate.

Relative quantification of differentially expressed genes by real-time PCR

The strains (wild type, *phr1*△/△, *phr2*△/△), treated or untreated with DMADDM were harvested for the determination of genes expression. All primer sequences are listed in **Table S3**. RNA isolation, complementary DNA synthesis, and

polymerase chain reaction (PCR) amplification was carried out by using the LightCycler® 480 (Roche, Basel, Switzerland) (Zhou et al., 2018). The gene expression was quantitated relative to the calibrator and was expressed as $2^{-\Delta\Delta CT}$. Each experiment was repeated three times.

Zeta potential measurement

Briefly, 24-hour fungal cultures were harvested by centrifugation at 4000 r/min at 4 ºC for 5 minutes and cells were washed twice with phosphate buffer solution. Then, the strains (wild type, *phr1△/△* or *phr2△/△*) were resuspended to a concentration of 10^6 cells/ml at a specific pH and uniformly dispersed by sonication and vortex (Fisher Scientific, Pittsburgh, PA, USA). A syringe was used to remove 1 ml of suspension and inject it into a "U-type" Potential Pool (Malvern Panalytical Ltd, Malvern WR14 1XZ, UK). Then, the pool was put into the measuring tank of a zeta potentiometer (Malvern Panalytical Ltd, Malvern WR14 1XZ, UK) at the following setting: temperature, 25 ºC; equilibrium time, 2 min; and number of sub-tests, 40. Finally, the zeta potential value was recorded. All samples were analyzed in triplicate.

Adherence of *C. albicans* cells to DMADDM-coated composite resin

Briefly, an equivalent amount of composite resin (3M, Maplewood, MN, USA) was weighed and shaped into a thin disk with a 1cm diameter and 1 mm thickness, and then coated with DMADDM on both sides. A curing light (DT, Beijing, China) was used to solidify the disk. After immersion in distilled water at 37 ºC for 24 h, the disks were sterilized in an ethylene oxide sterilizer (Anprolene AN74i, Andersen, Haw River, NC, USA).

The sterilized disks were put into 24-well plates and immersed in 2 ml of strain (wild type, *phr1*△/△ or *phr2*△/△) suspension (final concentration: 1×10^8 cells/ml in saline) for 15 minutes. The disks were removed and gently washed by saline. Then the disks were transferred into tubes with 5 ml saline. The fungal cells on each disk were harvested by sonication and vortex (Fisher Scientific, Pittsburgh, PA, USA) and serially diluted in saline. The final diluted cell suspension (100 μL) was spread on YPD agar plates and incubated at 37 ºC for 24 h to recover the viable cells and the colony-forming units (CFU) were counted.

Murine oropharyngeal candidiasis model

A murine model of oropharyngeal candidiasis was constructed according to a previous report (Zhou et al., 2018). To simulate the pH variation in oral cavity, the mice were fed water at different pHs (pH 4, 7, 9). Since it is difficult to maintain a particular pH in the mouth of mice, we just simulated the clinical pathological oral cavity in a relatively acidic/alkaline environment. Briefly, female BALB/c mice were injected subcutaneously with 3 mg cortisone acetate per mouse (in 200μL PBS with 0.5 % Tween 80) on the day before and post-infection. The second day after injection, the mice were anesthetized for at least 75 min with an intraperitoneal injection of 5 % chloral hydrate ($10ml \cdot kg^{-1}$). Then a swab soaked in a 1×10^7 CFU/ml of *C. albicans* yeast in sterile saline was placed on the tongue. To monitor the efficacy of DMADDM treatment, a small cotton swab soaked with DMADDM/NaCl solution at a specific pH was placed on the tongue for 20 minutes every day under anesthesia after the mice were infected. After two days, the mice were euthanized. The tongue was removed and

divided longitudinally into two pieces. One half was homogenized and cultured to quantify the amount of *Candida* and the other half was processed for histopathology analysis (Zhou et al., 2018).

Immunohistochemistry of murine tissue

The *C. albicans*-infected murine tongues were fixed in 10% (v/v) formaldehyde before embedding and processing in paraffin wax using standard protocols. For each tongue, 5-μm sections were prepared using a Leica microtome and silane-coated slides. The sections were dewaxed using xylene. Then, *C. albicans* and infiltrating inflammatory cells were visualized by staining with Periodic Acid-Schiff (PAS) stain and hematoxylin and eosin (HE) stain. The sections were then examined by light microscopy. Histological quantification of infection was performed by measuring the area of infected epithelium and expressing the area as a percentage relative to the entire epithelial area.

Statistics

One-way analysis of variance (ANOVA) was performed to detect the significant effects of the variables. Mann-Whitney test was used for data of different variances. A p-value < 0.05 was considered statistically significant.

Results

DMADDM inhibited *C. albicans* planktonic growth and biofilm in a pH-dependent manner

First, we synthesized DMADDM (**Fig. 1A**). Next, we tested the antifungal activity of DMADDM under different pH conditions. As shown in **Fig. 1B**, the MIC of

DMADDM against *C. albicans* was 62.5μg/ml at pH 3 and gradually reduced in a pH-dependent manner to a final MIC of 15.625μg/ml at pH 9. The MIC at pH 9 was 4-fold lower than that at pH 3. To better characterize this phenomenon, *C. albicans* was incubated with different concentrations of DMADDM at specific pH values. The growth curves (**Fig. 1C**) were consistent with the results shown in **Fig. 1B**. At pH 3, there was no antifungal effect at any of the four concentrations. At pH 4-6, only 31.25μg/ml of DMADDM inhibited the growth of *C. albican*s. However, at pH 7-10, both 31.25μg/ml and 15.625μg/ml DMADDM exhibited inhibitory activities.

Then we tested that if DMADDM had the same pH-dependent inhibitory effect on *C. albicans* biofilm. As expected, except for 62.5μg/ml DMADDM group in 48h, the DMADDM showed better anti-biofilm formation activities at pH 7 and 9 compared to the pH 4 (**Fig. 1D**).

***PHR1* and *PHR2* were correlated with the antifungal activities of DMADDM**

The different antifungal activities of DMADDM at different pH values lead us to suppose that the *PHR1* and *PHR2* genes were involved in this phenomenon since these two genes are responsible for the adaption of different pH values. Then *phr1*△/△ and *phr2*△/△ strains were employed for further testing. Interestingly, DMADDM exhibited different antifungal activities against wild type (WT), *phr1*△/△ and *phr2*△/△ **(Fig. 2 A and B)**. At pH 3, wild type and *phr1*△/△ shared the same MIC. At 3 **<** pH **<** 5.5 , the MIC of *phr1*△/△ was higher than that of wild type. At pH 5.5, wild type and *phr1*△/△ shared the same MIC. At 5.5 **<** pH **<** 8 , the MIC of *phr1*△/△ was lower than that of wild type. Interestingly, the MIC of *phr2*△/△ was always lower

than that of wild type at pH ranging from 5.5 to 9.

PHR1 is expressed at pH above 5.5 and *PHR2* is expressed at pH 5.5 or lower (Muhlschlegel and Fonzi, 1997; Kovacova et al., 2015). To further prove our hypothesis, we chose several typical pH conditions to test the relative expression of *PHR1/PHR2*. At pH 4 and 5.5, the expression of *PHR2* was significantly reduced in wild type treated with DMADDM, whereas there was no obvious difference between the treated and untreated groups in the *phr1△/△* strain (**Fig. 2C**). However, at pH 7 and 9, the expression of *PHR1* was significantly reduced in both the wild type and *phr2△/△* strains treated with DMADDM (**Fig. 2D**).

The pH values and *PHR1/PHR2* genes affected the cell zeta-potential of *C. albicans*

We furtherly tested whether the pH values and expression of *PHR1/PHR2* genes affected the cell surface charges since the positively charged QAS can attract negative charged cells (Oblak et al., 2014; Deryabin et al., 2015; Halder et al., 2015). The *C. albicans* wild type was negatively charged, with zeta potentials ranging from -12.1 to -25.7 mv under different pH values (**Fig. 3A**). The zeta potential of *phr2△/△* was more negative than wild type at pH 5.5 - 9 (**Fig. 3B**). While *phr1△/△* was less negative at pH 4 and 5, however, it was more negative at pH 5.5-7 (**Fig. 3C**). The potential variation of the wild type, *phr1△/△*, and *phr2△/△* strains was consistent with the variations from their MICs against DMADDM (**Fig. 2B**), suggesting that the more negative zeta potential of fungal cells, the lower the MIC values (**Fig. 3** and **Fig. 2B**). The results proved that the pH values and the expression of *PHR1/PHR2* influenced the zeta potential of *C. albicans*.

The zeta potential of *C. albicans* affected the binding to DMADDM resin tablet

Since the mechanism of QAS was assumed to be the contacting-inhibition against bacteria, we supposed that the variations of zeta potential of *C. albicans* can affected the binding to DMADDM. Then with the immersion of the resin tablet coating with DMADDM, the fungal cells bond to the tablet surface and the amount of adsorbed fungal cells were measured by CFU. As shown in **Fig. 4A**, the binding capacity of wild type was increased in a pH-dependent manner in line with the increase of negative zeta potential. The resin tablet in control group (uncoated discs) showed significantly lower binding capacity in all pHs (**Fig. 4B-E**). Compared to WT, the *phr1△/△* was not grow at pH 9 and exhibited weaker binding capacity at pH 4 (**Fig. 4B**), but no difference at pH 5.5 (**Fig. 4C**) and stronger at pH 7 (**Fig. 4D**), consistent with its increased negative zeta potentials (**Fig. 3C**). The *phr2△/△* strain always exhibited stronger binding capacity at pH 5.5, pH 7 and pH 9 compared to the WT (**Fig. 4C-E**), in line with its more negative zeta potentials (**Fig. 3B**).

DMADDM treated oropharyngeal candidiasis at different pH values

We next assessed the pH-dependent antifungal effect of DMADDM in a murine model of oropharyngeal candidiasis. To simulate the salivary pH variation in oral cavity, the mice were fed water at different pH values. As expected, the mice from acidic group (pH 4) exhibited typical pseudomembranous lesions on the lingual surface, whereas the neutral (pH 7) and alkaline (pH 9) groups formed fewer lesions (**Fig. 5A**). DMADDM significantly reduced the epithelial infectious area, while it reduced more at alkaline pH compared to the neutral and acidic groups (**Fig. 5B**). DMADDM can inhibit the *C.*

albicans colonization at different pH values, and it increased the inhibition at neutral and alkaline groups (**Fig. 5C, D**). After the treatment with DMADDM, the inflammatory cells were reduced **(Fig. 5E, black arrows)**, indicating the strong activity of DMADDM in treating oropharyngeal candidiasis. In addition, after treating with DMADDM, there was much less hypha at pH 7 and pH 9 than pH 4 **(Fig. 5E, red arrows)**.

Discussion

This study is the first investigation of the antifungal activities of QAS at various pH conditions. A pH-dependent antifungal effects of DMADDM were observed, where stronger antifungal effects were seen at higher pH conditions both *in vitro* and *in vivo*. This phenomenon was proved to be related to two genes (*PHR1*/*PHR2*), whose expression was reduced by DMADDM. At pH 4, the expression of *PHR2* was reduced by DMADDM in WT, while no significant change in *phr1Δ/Δ*, thus WT was more sensitive to DMADDM compared to *phr1Δ/Δ* tolerant at pH 4 as the reduced *PHR2* by DMADDM in WT resulted in the weak growth adaption under acidic pH. For the similar reason, at pH 7 and pH 9, the expression of *PHR1* was reduced in WT and *phr2Δ/Δ* by DMADDM, then both WT and *phr2Δ/Δ* was sensitive to DMADDM at pH 7 and pH 9.Furtherly, we revealed that the pH values and *PHR1*/*PHR2* expression caused zeta potential alterations in the fungal cells, then significantly affected the bindings between fungal cells and DMADDM. The results of this study showed that "more negative *C. albicans* surface charges, better bonding to DMADDM". Particularly, *C. albicans* had more negative surface charges and bound with more DMADDM at

higher pH, leading to the stronger antifungal activities at higher pH conditions *in vitro* and *in vivo*.

Quaternary ammonium salt was thought to interact with negative charged bacteria due to its positive charge density, then break the cell wall and membrane to cause the cell death (Halder et al., 2015; Nikitina et al., 2016). Here we directly demonstrated that DMADDM (a type of QAS) can bind to fungal cells and the binding abilities were correlated with the cell zeta potential, which can be regulated by some pathways (such as the *PHR1/PHR2* genes in *C. albicans*). This result indicated that the microbial cells may regulated their interaction with QAS through the activation of some pathways. Our results may also explain the reason that the different antimicrobial activities of QAS against various microbes. The next investigation of our team will focus on how different oral microbes regulate their membrane charges and how these pathways respond to QAS antimicrobial agents.

In our study, we discovered that *C. albicans* exhibited different zeta potential at different pH values, which then affected antifungal activities of DMADDM for the first time. This mechanism indicated that we can combine DMADDM with the agents which can alter the negative zeta potential of microbial cells to achieve even better antimicrobial activities. An earlier study found that cationic agents (cetyl trimethyl ammonium bromide and polymyxin B) altered zeta potential of *Escherichia coli* and *Staphylococcus aureus* to increased cell surface permeability (Halder et al., 2015). Arakha et al. also proved that ZnONPs can interact with *Bacillus subtilis* and *Escherichia coli* by altering their surface zeta potential (Arakha et al., 2015).

DMADDM may synergize with these compounds to against various microbial infections.

Denture stomatitis is a common infection manifested by inflammation of the oral mucous membrane beneath a denture (Offenbacher et al., 2019). We previously developed a new and biocompatible double-decker denture resin containing DMADDM which can inhibit *C. albicans* and its biofilm effectively (Zhang et al., 2016; Chen et al., 2017). According to our current results, the denture resin containing DMADDM can even elevate its antifungal effect if using an alkaline mouthwash simultaneously which provides a relatively higher oral pH condition. Meanwhile, DMADDM modified denture resin is a non-release antimicrobial material, therefore, it can also provide long-term antimicrobial treatment and prevent recolonization of the denture reservoir with fungal or bacterial pathogens. Ghalichebaf et al. found that immersion-type cleansers containing sodium hypochlorite at pH 11.0 were the most effective in removing denture plaque (Ghalichebaf et al., 1982). Rudd et al. also demonstrated the sterilizing effect of sodium hypochlorite as a denture soak, while alkaline hypochlorite has been shown to eliminate denture plaque effectively *in vitro* (Rudd et al., 1984). Thus, the combination of sodium hypochlorite and DMADDM dentures can be an effective way to eliminate all of the denture plaque even the mixed infection of fungi and bacteria. More importantly, our results also suggested the clinical application of QAS containing dental materials: patients administrated by QAS containing dental materials should limit the carbonated drinks and acidic food during the treatment period.

Author Contributions Statement

HC and YZ conceived and designed research. HC and BL conducted experiments. XZ contributed new reagents. HX and CC analyzed data. HC wrote the manuscript. LC and BR critically revised manuscript. All authors read and approved the manuscript.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical approval

All mouse experiments described in this study were conducted in strict accordance with the Principles “US Govemment Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research and Training”. The study protocol was approved by Ethics Committee of West China Hospital of Stomatology Sichuan University (license

number WCHSIRB-D-2019-157). All efforts were made to minimize suffering and ensure the highest ethical and humane standards. The mice were euthanized with an intraperitoneal injection of 5 % chloral hydrate.

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Figure Legends

Fig. 1 The antifungal activities of DMADDM at different pH values. A. Chemical structure of DMADDM. **B.** MIC of *C. albicans* at different pHs. **C.** Growth curves of *C. albicans* at different DMADDM concentrations in different pHs. **D.** DMADDM inhibited biofilm formation after 24h (left) and 48h (right). Experiments were performed 3 times. Mann-Whitney test was performed. **p < 0.01, ***p < 0.001 compared to pH 4 group.

Fig. 2 *PHR1* and *PHR2* were correlated with the antifungal activities of DMADDM. **A.** The growth status of the fungal strains. Fungal strains were subjected to two-fold serial dilutions of DMADDM in YPD at 37 °C for 24 hours. The optical densities were standardized to untreated controls (see the relative growth bar). **B.** MIC of wild type, *phr1*△/△, and *phr2*△/△ at different pHs. **C&D.** DMADDM influences gene expression in wild type, *phr1*△/△, and *phr2*△/△. Expression levels of treated (31.25μg/ml DMADDM) and untreated cells measured by qRT-PCR. The error bars represent the standard deviation between assay triplicates. Assay results are representative of biological triplicates. One-way analysis of variance (ANOVA) was performed. ***p < 0.001 compared to untreated, ns, no statistical significance; DMA, DMADDM. NG, no growth; ND, no detection.

Fig. 3 The pH value and expression of *PHR1/PHR2* affected the cell zeta-potential of *C. albicans* **A.** Zeta potential of wild type. **B.** Zeta potential of *phr2*△/△ compared to wild type. **C.** Zeta potential of *phr1*△/△ compared to wild type. The error bars

represent the standard deviation between assay triplicates. One-way analysis of variance (ANOVA) was performed. *p < 0.05, **p < 0.01, ***p < 0.001 compared to wild type. ns, no statistical significance.

Fig. 4 The number of *C. albicans* strains adsorbed on tablets coating with DMADDM. A. The number of adsorbed wild type at different pHs. **B-E.** The number of fungi of different strains absorbed at pH 4, 5.5, 7 and 9. Control group represents uncoated discs. Experiments were performed 3 times. Mann-Whitney test was performed. *p < 0.05, **p < 0.01, ***p < 0.001, ns, no statistical significance.

Fig. 5 DMADDM treated the mucosal infection *in vivo* and schematic diagram of the antifungal activities of DMADDM. **A.** Images of infected mice tongues with oral candidal leukoplakia after 2-day oropharyngeal infection with *C. albicans*. Pseudomembranous lesions on the tongue *in vivo* indicated by the white arrow. **B.** Average percentage of entire mice tongue epithelium area infected with *C. albicans*. **C.** Fungal burdens obtained from the tongues of mice after 2-day oropharyngeal infection with *C. albicans*. **D.** Growth ratio of *C. albicans* indicated by the percentage of live *C. albicans* in the experimental and control groups. **E.** HE- and PAS-stained tongues from mice 2 days post-infection with *C. albicans*. The inflammatory cells are indicated by black arrowheads and invading hyphae are shown by red arrowheads. HE, hematoxylin and eosin; PAS, Periodic Acid-Schiff. Experiments were performed 3 times. Mann-Whitney test was performed. *p < 0.05, **p < 0.01, ***p < 0.001 compared to wild type.

Figure 1

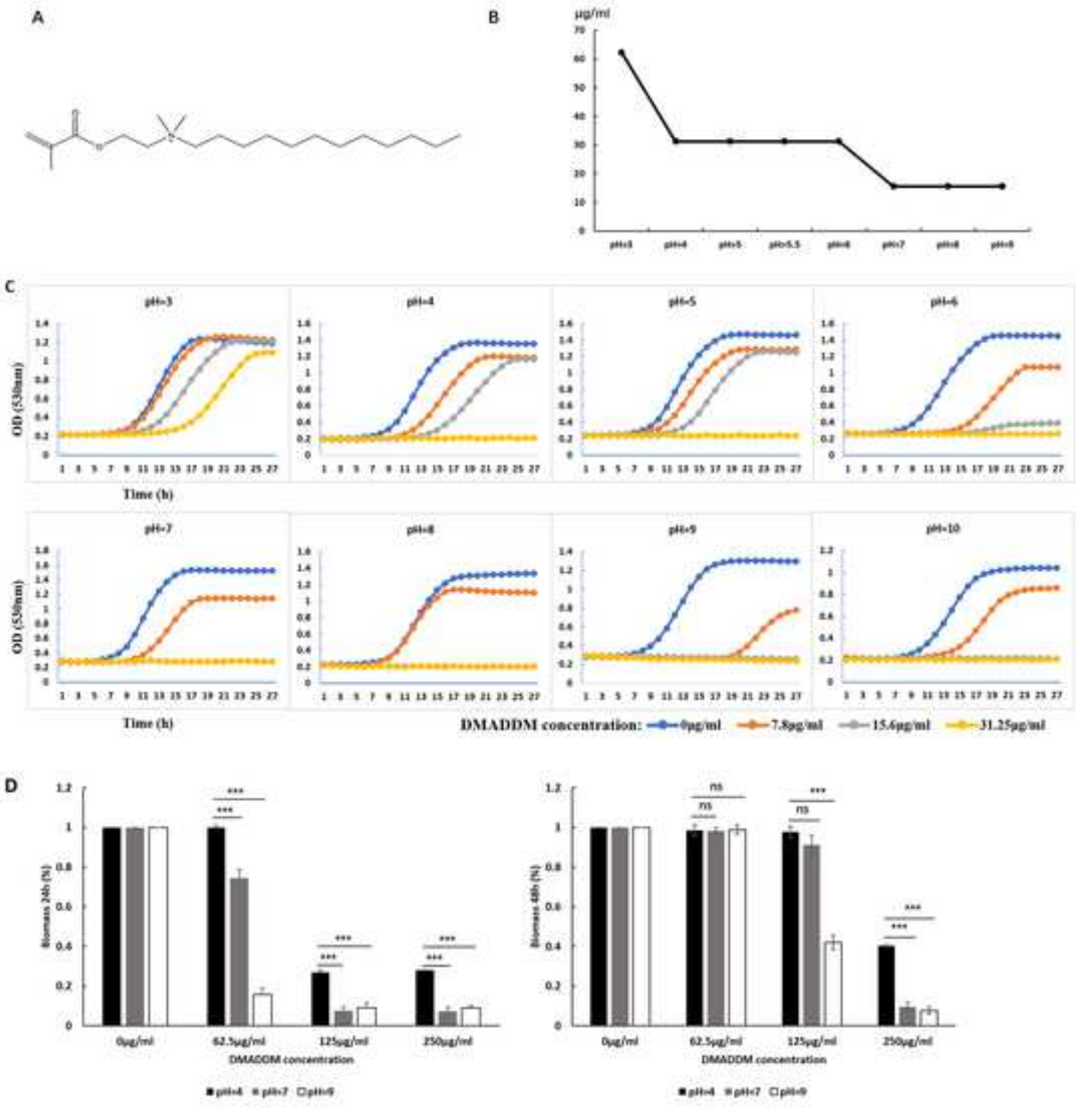

Figure 2

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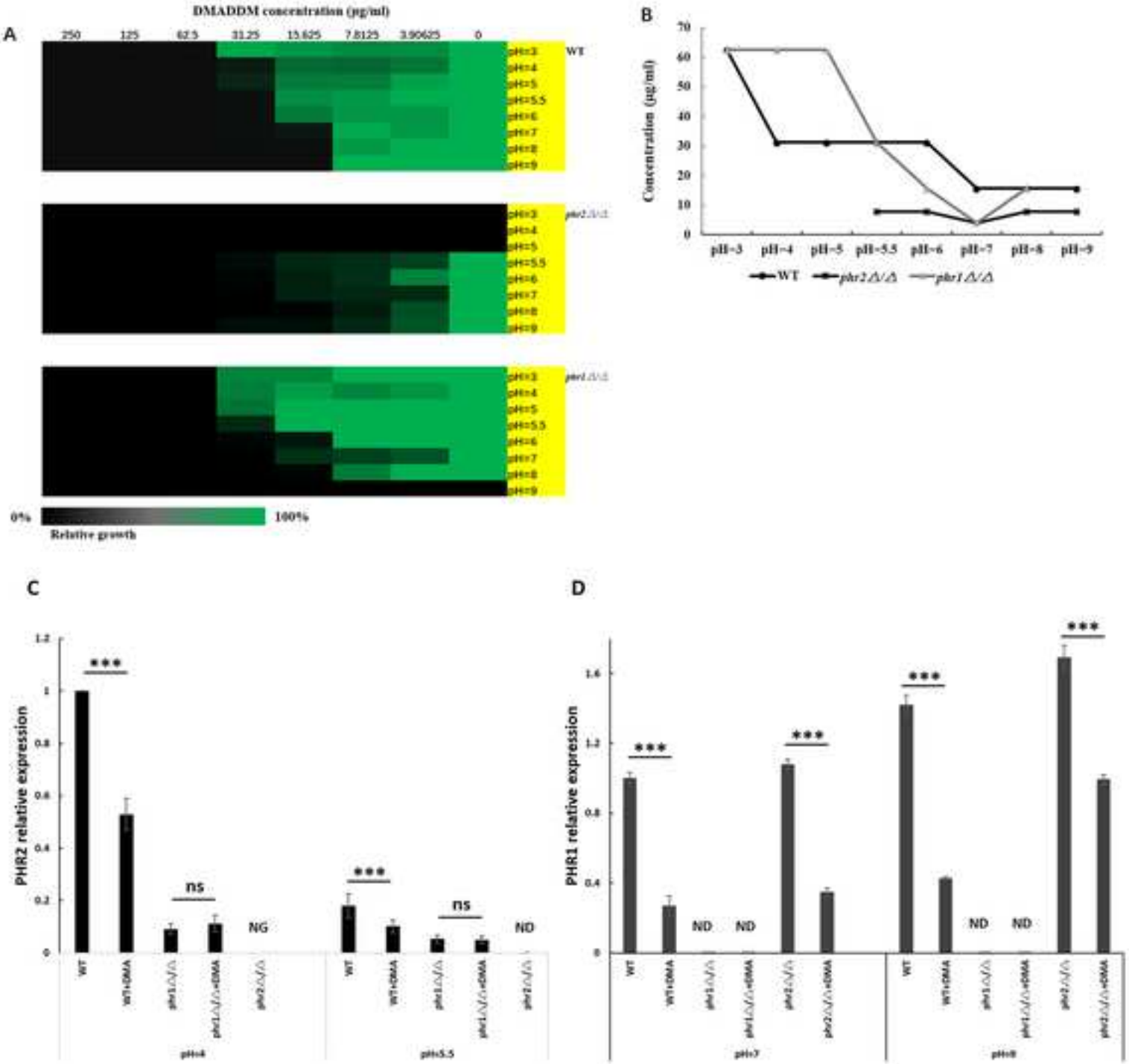

Figure 3

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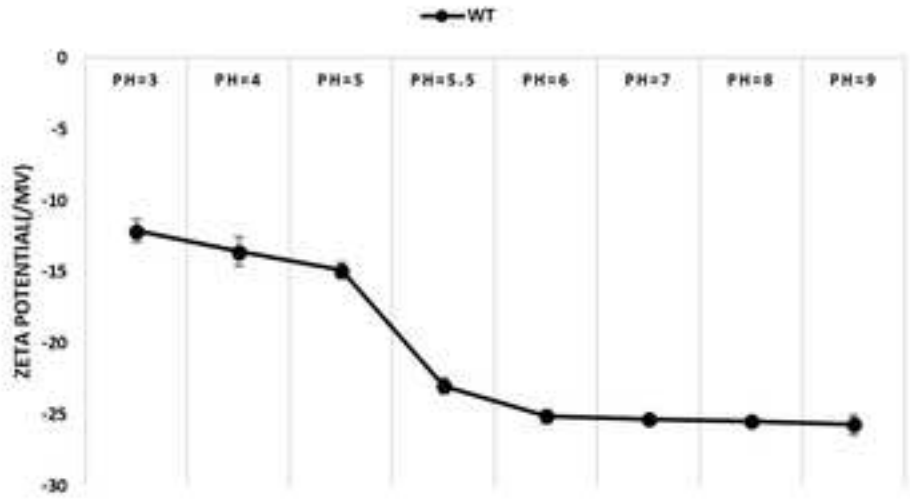

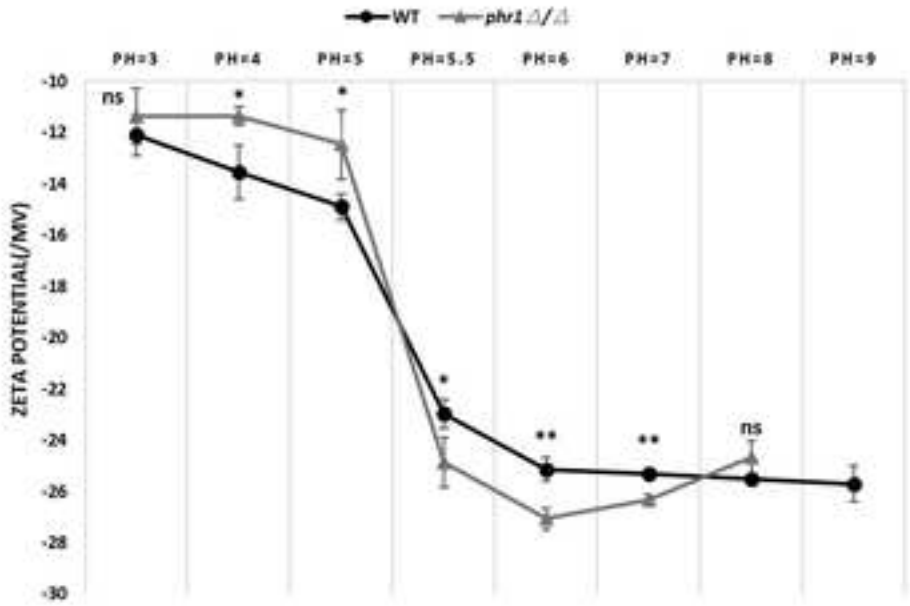

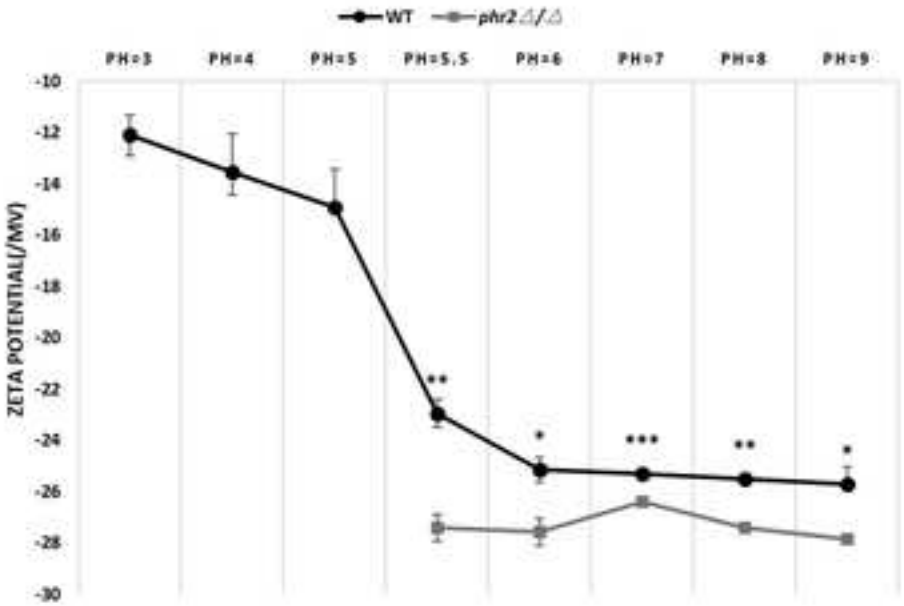

Figure 4

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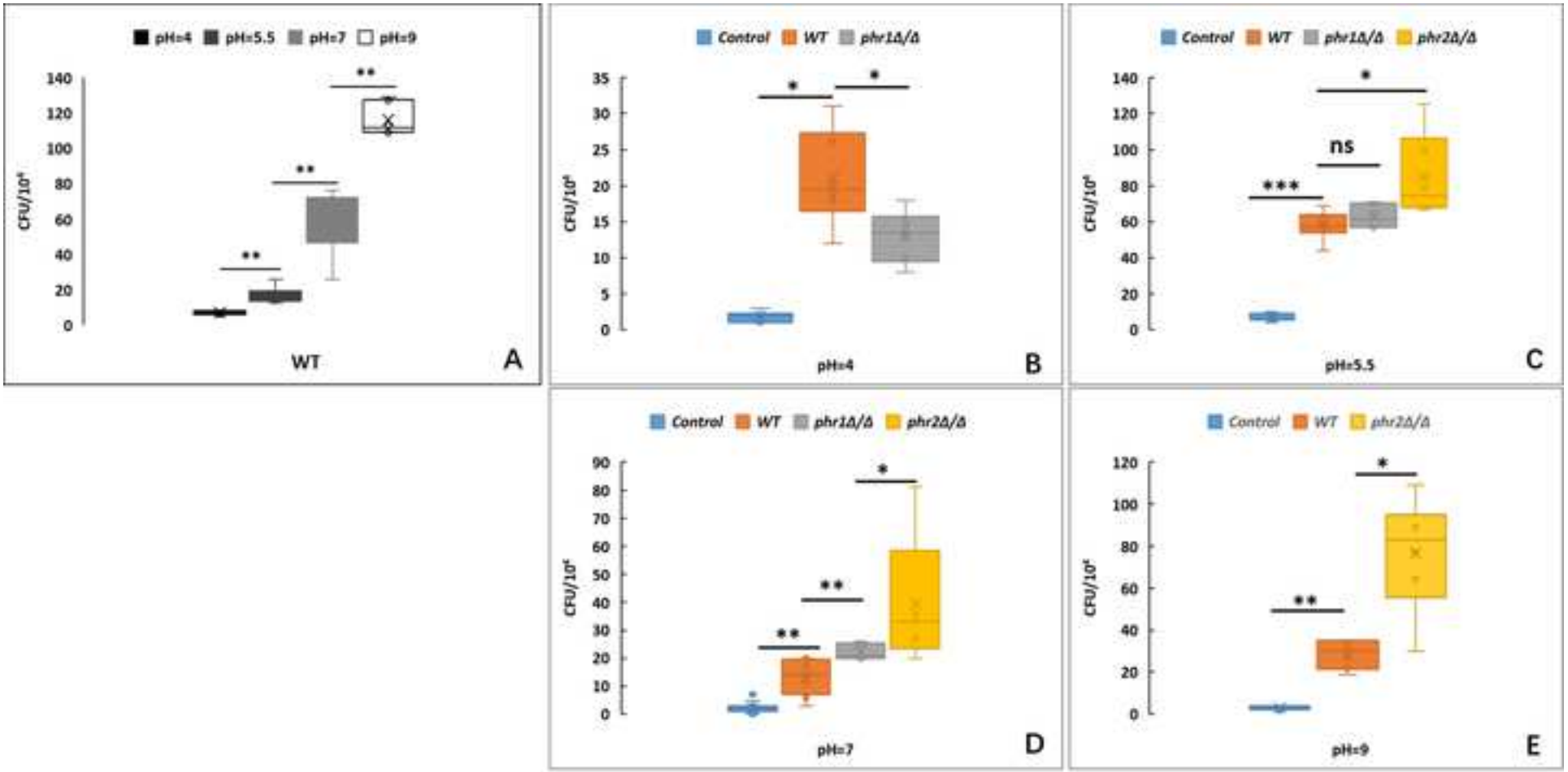

Figure 5

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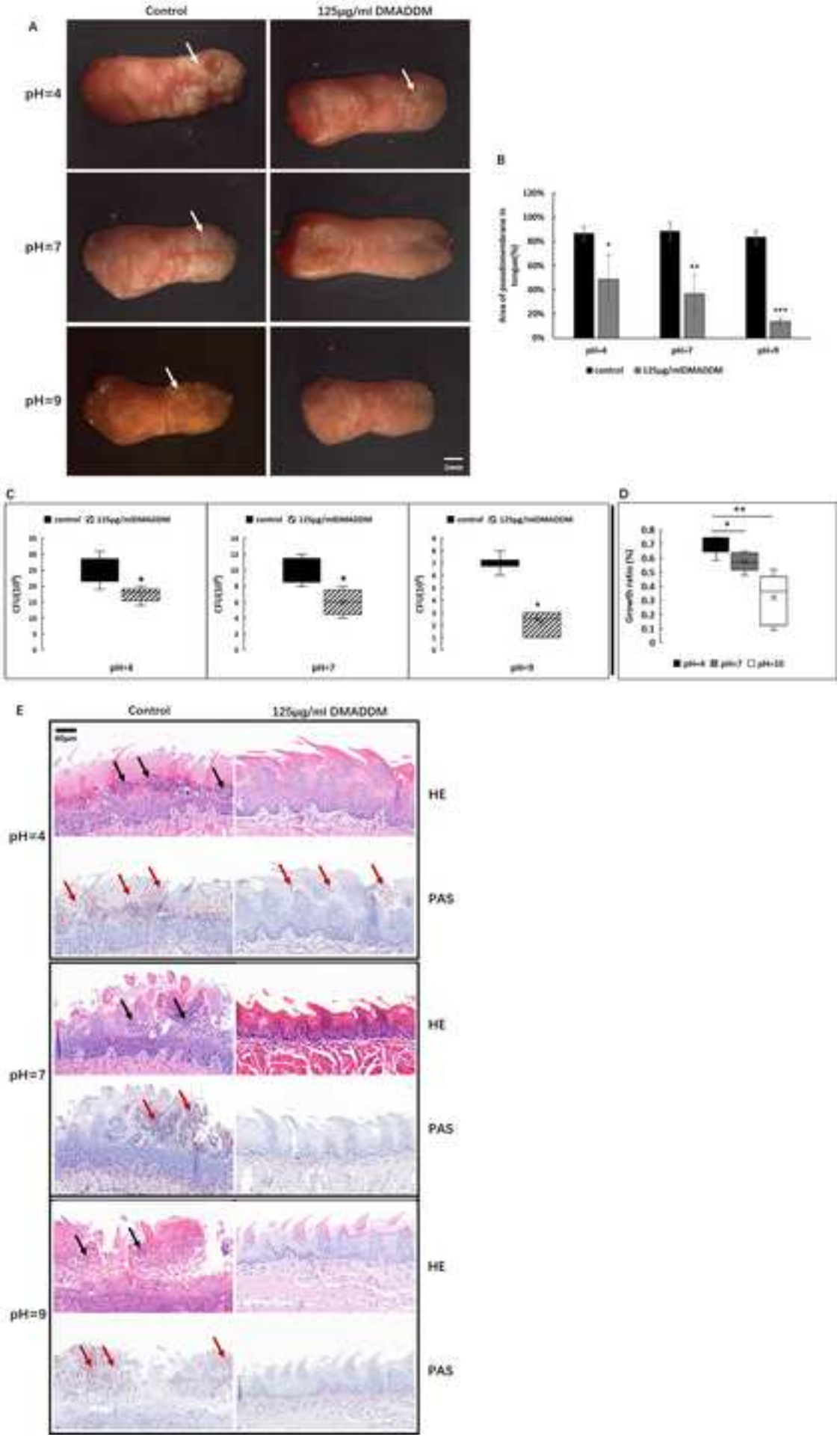

graphical abstract