The effect of isabelin, a sesquiterpene lactone from *Ambrosia artemisiifolia*, on soil microorganisms and human pathogens.

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Keywords: Ambrosia artemisiifolia, isabelin, sesquiterpene, soil microorganism, human pathogens, antimicrobial activity.

Abstract

*Ambrosia artemisiifolia* L. (common ragweed) is an invasive weed, well-known for the strong allergenic effect of its pollen, as well as for its invasiveness and impact in crop fields (e.g. causing yield losses). This
species produces a broad range of sesquiterpenoids. In recent years, new bioactive molecules have been discovered in this plant, e.g. isabelin, a sesquiterpene dilactone. The bioactivity of isabelin has been already demonstrated on allergy-related receptors and its inhibitory effect on seeds of various plant species. Isabelin was tested for potential antimicrobial effects by using a selection of soil-borne bacteria and fungi and three human pathogens as model organisms. For the majority of microorganisms tested, no antimicrobial activity of isabelin was observed. However, isabelin revealed strong antimicrobial activity against the Gram-positive soil bacterium Paenibacillus sp. and against the Gram-positive, multi-drug-resistant Staphylococcus aureus (MRSA). The observed inhibitory activity of isabelin can enlighten the importance to study similar compounds for their effect on human pathogens and on soil and rhizosphere microorganisms.

INTRODUCTION

Ambrosia artemisiifolia L. (Asteraceae) is an highly invasive weed, indigenous in North America and nowadays widespread through Europe and Asia (Bullock et al., 2010; Kong et al., 2007). The SMARTER European COST action (www.ragweed.eu) was initiated in the past years focusing on the sustainable management of this species, in particular due to its impact on human health. Problems caused by common ragweed are mainly related to strong allergenic responses to its abundant pollen, as well as interference in crops and invasiveness in urban areas (Bullock et al., 2010; Burbach et al., 2009; Casarini, 2002; Chapman et al., 2016; Genton et al., 2005; Kong et al., 2007; Sang et al., 2011). The Asteraceae are the main plants producing sesquiterpenoids, which are vast and diverse class of compounds with different functions, including the inhibition of other plant species and influence on the microbial community (Chadwick et al., 2013). A. artemisiifolia produces a broad range of these molecules, and new compounds such as artemisiifolinic acid and isoartemisiifolinic acid have been reported in recent years (Ding et al., 2015; Taglialetela-Scafati et al., 2012).
Metabolites of *A. artemisiifolia* have been already studied for their inhibitory potential on crops and weeds, (Kong et al., 2007; Molinaro et al., 2016; Vidotto et al., 2013) and on different bacterial strains (Solujic et al., 2008) including *Staphylococcus aureus*. Isabelin (Supplementary fig 1) is a germacranolide sesquiterpene dilactone present in common ragweed extract (Seaman, F.C., 1982). Its inhibitory activity as a pure compound on germination of seeds of different plant species has been recently observed (Molinaro et al., 2016). Its structure, first identified in 1969 from *Ambrosia psilostachya* (Yoshioka, H. and Mabry, T. J., 1969), presents a lactone ring and a α-methylene-γ-lactone ring on the typical germacrene backbone. The presence of two different electrophilic Michael acceptor sites on C-13 and C-5, demonstrated to provide a higher reactivity compared to costunolide in previous studies about allergy-related receptors (Avonto et al., 2011; Taglialetela-Scafati et al., 2012).

*A. artemisiifolia* has been studied for its invasiveness and escape attitude, showing an important effect on the soil biota by promotion and inhibition of growth, due to the previous presence of the same species (MacKay and Kotanen, 2008). The morphological and physiological traits of invasive plant species have shown to alter soil nutrient cycles. However, these changes cannot occur without concomitant responses of the microbial community that reside in the rhizosphere (Hawkes et al., 2005). Compounds released by the plant may affect soil microorganisms such as bacteria and fungi. However, very little is known about the direct effect and antimicrobial activity of compounds produced by *A. artemisiifolia*, such as isabelin, on soil- microorganisms.

In the past decades the number of bacteria resistant to front-line antibiotics increased dramatically, in comparison to the stagnating number of new antimicrobial compounds discovered for clinical use (Spellberg et al., 2008). Plant extracts and essential oils, contain many different compounds like for example sesquiterpenoids, which can exhibit antimicrobial activity. These sesquiterpenoid compounds
can be important in the fight against increasing antimicrobial resistance in human pathogens (Hristova et al., 2015; Solujic et al., 2008).

Hence, the aims of this study were to test isabelin for antimicrobial activity 1) on a set of soil-borne fungi and bacteria and 2) on three human pathogenic model organisms.

**MATERIAL AND METHODS**

**Bacteria and culture conditions**

To test the effect of isabelin on soil bacteria, ten phylogenetically different soil bacterial strains isolated from soils and sandy dune soils in The Netherlands were used: *Achromobacter denitrificans* sp. MH58.1, *Arthrobacter* sp. V13, *Burkholderia* sp. AD24, *Collimonas* sp. Ter91, *Ochrobactrum* sp. 44, *Paenibacillus* sp. AD87, *Pseudomonas fluorescens* sp. AD21, *Serratia plymuthica* PGPRI-I, *Stenotrophomonas maltophilia* sp. MH58.27, *Xanthomonas campestris* sp. V79 (Czajkowski et al. 2011, Garbeva et al., 2014, Tyc et al., 2017). The soil bacteria were pre-cultured from -80°C glycerol stocks on 1/10th Tryptone Soya Broth agar (Garbeva and de Boer, 2009) and incubated for three days at 24°C prior application. To test the effect of isabelin on human pathogenic model bacterial strains two indicator bacteria namely *Escherichia coli* WA321 (DSMZ # 4509) and *Staphylococcus aureus* 533R4 (DSMZ # 20231) were used (Meyer and Schleifer, 1978; Tyc et al., 2014). The indicator bacteria were pre-cultured from -80°C glycerol stocks on LB-A medium (LB-Medium Lennox, Carl Roth GmbH + Co. KG, 20 gL-1 Merck Agar) (Sambrook and Russell, 2001) and incubated overnight at 37°C prior application. All bacterial strains used in this study are listed in table 1.
Eukaryotic target organisms and culture conditions

To detect antifungal activity of isabelin, eight fungal model organisms isolated from a sandy dune soil in The Netherlands were used: *Chaetomium* sp., *Fusarium culmorum* PV, *Fusarium oxysporum*, *Fusarium solani*, *Mucor hiemalis*, *Rhizoctonia solani* AG2.2 IIIB, *Trichoderma harzianum*, *Verticillium albo-atrum* and one oomycete: *Pythium ultimum* P17 (de Rooij-van der Goes et al., 1995; Garbeva et al., 2014). The fungi and the oomycete were pre-cultured on 1/5th Potato Dextrose Agar (PDA) (29 g·L⁻¹ Oxoid CM 139) (Fiddaman and Rossall, 1993) and incubated at 24°C for 5 days prior application. As a model organism for yeast-like fungi the yeast *Candida albicans* BSMY 212 (DSMZ # 10697) (Schmidt, 1996) was used. The yeast was pre-cultured from -80°C glycerol stocks on YEPD plates (20 g·L⁻¹ Merck Dextrose, 20.0 g·L⁻¹ BACTO™ Peptone, 10.0 g·L⁻¹ BACTO™ Yeast extract, 20 g·L⁻¹ Merck Agar) and incubated at 37°C. All used model organisms are listed in table 1.

Isabelin purification and preparation of stock solution

All used reagents were analytical or LC-MS grade and were obtained from Sigma-Aldrich, Milan, Italy. *A. artemisiifolia* plant material was collected in University of Turin Campus (Agriculture, Forest and Food Science Department, Grugliasco (TO), 45°03'58.8"N 7°35'36.3"E) from invasive colonies next to maize and soybean fields in July 2016. For the methanol extraction, fractionation of the *A. artemisiifolia* extract and the preparation of pure isabelin, the previously developed semi preparative LC method was used (Molinaro et al., 2016). For each preparative injection, 1 mL of methanol concentrated extract was diluted with 1 mL of MilliQ water and the total 2 mL were injected using a 2 mL loop on a Rheodyne® injection valve. Pure isabelin fractions were obtained using a semi-preparative LC column (GL Sciences C18, 10x150mm, Milan, Italy) in isocratic method with 70% H₂O, 30% acetonitrile, and a total flow of 4 mL/min. In the expected retention time (20-25 min), 1 minute fractions (4 mL each one) were collected.
Each fraction was checked through LC-MS in order to assure the purity of isabelin. The LC-MS system was a Varian MS-310 triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source, and 212 LC pump (Agilent, Milan, Italy). Separation was performed on a Kinetex RP C18 column, 5 µm particle size, 50 x 2.0 mm (Phenomenex, Torrance, CA, USA). The mobile phase was (A) water and (B) acetonitrile, both containing 0.1% (v/v) acetic acid. The mobile phase gradient was from 90% to 10% A in 10 min (0.2 mL/min flow rate). ESI conditions used in negative polarization mode for the full-scan method were: needle potential -3500V, shield -500V, and capillary -30V. Gas conditions were set with 20.0 psi for nebulizing gas and 25.0 psi of N₂ at 300ºC as drying gas. The fractions containing pure isabelin were reunited and the solvent was evaporated to dryness in order to obtain pure isabelin. The 1000 µg/mL stock solution was prepared by weighing isabelin and dissolving it in the corresponding amount of solvent (75% v/v Acetonitrile or sterile demi water).

**Antibacterial and anti-yeast assays based on agar-diffusion test**

To test for antimicrobial activity against bacteria and the yeast, agar disk-diffusion tests were performed in 12-well plates (Balouiri et al., 2016). Single colonies of *E. coli* WA321, *S. aureus* 533R4 and *C. albicans* BSMY 212 were picked from plates and grown overnight in liquid LB or YEPD media at 37°C, 220 rpm. Fresh LB- agar or YEPD-agar (1.0% Merck Agar) was prepared and cooled down to ~45°C. The target organisms were added to a final OD₆₀₀ of 0.002 corresponding to 6.0 x 10^5 CFU/mL (*E. coli* WA321), 4.0 x 10^5 CFU/mL (*S. aureus* 533R4) or 1.6 x 10^4 Cells/mL (*C. albicans* BSMY 212). A volume of 1 mL of seeded agar was added to each well of the 12 well plates (Greiner bio-one, Cat# 665180). After solidification, a filter paper (diameter ~5,5 mm) (Whatman™, Cat# 1003-150, 6 µm pore size) was added on the top of the agar surface and a volume of 5 µl of 1000 µg/mL isabelin (=5 µg) was added to the filter papers. As control 5 µl of the solvent (75% Acetonitrile) was added. Additionally, for positive assay
control, 5 µl of appropriate antibiotic (Ampicillin 100 mg/mL for *E. coli* WA321, Tetracycline 15 mg/mL for *S. aureus* 533R4 or Cycloheximide 25 mg/mL for *C. albicans* BSMY 212) was used. As negative assay control filter papers without added antibiotics or isabelin were applied. The plates were incubated overnight at 37°C and on the next day plates were examined for visible zones of inhibition (ZOI) around the filter papers. Digital photographs were taken and analyzed using the AXIO VISION v4.8 imaging Software (Carl Zeiss Imaging Solutions GmbH) for surface-area determination (in pixel^2). All treatments were performed in quadruplicates.

**Bioassays to test for antifungal/anti oomycetal activity based on agar-diffusion test**

To test the effect of isabelin on fungal and oomycetal growth (mycelial extension), the following fungi/oomycetes were used: *Chaetomium* sp., *Fusarium culmorum PV*, *Fusarium oxysporum*, *Fusarium solani*, *Mucor hiemalis*, *Phytophthora ultimum* P17, *Rhizoctonia solani* AG2.2 IIIB, *Trichoderma harzianum*, *Verticillium albo-atrum*. The assays were performed in 12 well plates (Greiner bio-one, Cat# 665180). For testing the effect of isabelin on mycelial growth, fresh PD- agar (29 g L^{-1} Oxoid CM 139) was prepared and a volume of 1 mL PD- agar was added to each well. After solidification the target fungi/oomycete were added by placing a 5 mm diameter plug of each fungus/oomycete at the top edge of each well. A filter paper with a diameter of ~5.5 mm (Whatman™, Cat# 1003-150, 6 µm pore size) was placed on the agar surface at the lower edge of each compartment and a volume of 5µl stock solution (corresponding to 5 µg) isabelin was added to each filter paper. For control, 5 µl of the solvent (75% v/v acetonitrile) was used. Plates were incubated at 24 °C for 4 days. After incubation plates were examined for fungal growth (=mycelial extension) and digital photographs were taken. The digital images were analyzed using the
AXIO VISION v4.8 imaging Software (Carl Zeiss Imaging Solutions GmbH) to compare the growth of the treatments to the controls without added isabelin.

**In vitro test of growth inhibition on soil bacteria in liquid media**

For the determination of antimicrobial activity of isabelin in liquid media optical density, (absorbance) measurements and counting of colony forming units (CFU) were performed in 96-well plates (Greiner bio-one, Cat# 655180). For the *in vitro* test, six phylogenetically different soil bacteria were used: *Achromobacter denitrificans* sp. MH58.1, *Pseudomonas fluorescences* sp. AD21, *Serratia plymuthica* sp. PGPRI-1, *Stenotrophomonas maltophilia* sp. MH58.27, *Xanthomonas campestris* sp. V79, *Paenibacillus* sp. AD87. Single colonies of each bacterial isolate were picked from plates and grown in 20 mL 1/10th Tryptic Soy Broth (TSB) overnight at 24°C, 200 rpm. For the absorbance measurement 20 µl of 1000 µg/mL isabelin dissolved in water (10% v/v) (=20 µg) was dispensed in 180 µl 1/10th TSB containing the soil bacterial isolates diluted to OD$_{600}$ of 0.05. As positive assay control 5 µl of 15 mg/mL of Tetracycline (=75 µg) (Sigma-Aldrich product T-7660) was applied. The growth rates were monitored in the presence of isabelin and were compared to the growth rates of the controls (soil bacteria in the absence of isabelin) using a BioTek Synergy™ HT Multi-Mode Microplate Reader (Beun de Ronde Life Sciences, The Netherlands). The absorbance was measured using the following settings: measure absorbance at 600 nm, 24 hours of measurement with an interval of 1 hour, delay between each sample measurement was set to 50 ms, shaking intensity was set to one and the duration to 15 min. before each measurement. The entire assay was performed at 24°C for 24 h. All treatments were measured in three replicates. The measured values were normalized against the mean absorbance values of the used culture media.
**In vitro test of growth inhibition on human pathogenic bacteria and yeast model organism**

The effect of pure isabelin was tested on three human pathogenic model organisms. For this, optical density measurements and counting of colony forming units (CFU) were performed. Single colonies of each bacterial target organism, *E. coli* WA321, *S. aureus* 533R4 and of the yeast like fungi *C. albicans* BSMY 212, were picked from plates and grown overnight in 20 mL LB broth or YEPD broth at 37 °C, 220 rpm. Fresh LB- or YEPD growth media was supplemented with the target organisms at a final OD$_{600}$ of 0.002 corresponding to 6.0 x 10$^5$ CFU/mL (*E. coli* WA321), 4.0 x 10$^5$ CFU/mL (*S. aureus* 533R4) or 1.6 x 10$^4$ Cells/mL (*C. albicans* BSMY 212). The assay was performed in 96-well plates (Greiner bio-one, Cat# 655180). For the measurement, 20 µl of 1000 ppm (equivalent to 20 µg) isabelin were dispensed in 180 µl 1/10$^{th}$ LB or YEPD media containing the target organisms. As positive assay control, 5 µl of 15 mg/mL (=75 µg) of Tetracycline (Sigma-Aldrich product T-7660) or 20 µl of 200 mg/mL (= 4 mg) Cycloheximide (Sigma-Aldrich) was applied. The growth rate of the model organisms was monitored in the presence of isabelin and was compared to the growth rates of the controls (in the absence of isabelin). The absorbance measurements were performed on a BioTek Synergy™ HT Multi-Mode Microplate Reader (Beun de Ronde Life Sciences, The Netherlands) with the following settings: measure absorbance at 600 nm, 24 hours of measurement with 1 hour interval, delay between each sample measurement 50 ms, shaking intensity one and 15 min between each measurement. The entire assay was performed at 37°C for 24 h. All treatments were measured in three replicates. The measured values were normalized against the mean absorbance values of the used culture media.
**Enumeration of target organism growth**

The growth of the target organisms grown in liquid was evaluated by plate counting. After 24 hours of incubation and the optical density measurements, a sample of 100 µl of each well was taken and added to 900 µl of 10 mM phosphate buffer (KH₂PO₄, pH 6.5). Dilution series of each treatment were prepared in triplicates. A volume of 100 µl of each serial dilution was plated in three replicates with a disposable Drigalski spatula on 1/10th TSBA plates (soil bacteria), LB- agar plates (human pathogenic model bacteria) or on YEPD- agar plates (yeast model organism). The plates were incubated for three days at 24°C or overnight at 37°C and CFU enumeration was carried out on an aCOlyte Colony Counter (Don Whitley Scientific, Meintrup DWS Laborgeräte GmbH, Germany).

**Statistical analysis**

Statistical analyses on fungal mycelial extension, the size of the zone of inhibition (ZOI) and on viable count data (CFU) of bacteria and yeast-like fungi was performed with IBM SPSS Statistics 24 (IBM, Somers, NY, USA) using one-way ANOVA and post-hoc Tukey test (HSD) or two-tailed t-test between the data sets. Data was normalized by log transformation prior statistical analysis. The 5% level was taken as threshold for significance between the control and the treatments.

**RESULTS AND DISCUSSION**

**Effect of isabelin on soil microorganisms**

Studying how plants and soil microorganisms interact is key to understand how plants can modify the soil environment by promoting or inhibiting specific soil microorganisms. Here we performed bioassays on commonly occurring soil microorganisms (fungi and bacteria) in order to determine potential growth inhibition or growth promotion by isabelin, a germacranoide sesquiterpene dilactone present in common ragweed extract, which has phytotoxic activity (Molinaro et al., 2016). No changes in growth of
fungi or oomycete could be observed in the presence of isabelin (fig 1). All tested soil fungi and the oomycete did not show significant differences of mycelial extension of the treatments compared to the controls.

Although we did not observed any antifungal or antioomycetal activity exhibited by isabelin, which can be excreted by the invasive plant species *Ambrosia artemisiifolia*, other studies have demonstrated that invasive plant species are able to suppress arbuscular mycorrhizal fungi (Callaway et al., 2008; Klironomos, 2002; Rout and Callaway, 2012).

Bacterial enumeration of colony forming units (CFU) after 24 hours of growth in the presence of isabelin revealed the inhibition of only one of the tested soil bacteria, *Paenibacillus* sp. AD87 (Firmicutes) (fig 2). The growth of the Gram- positive *Paenibacillus* sp. AD87 was significantly inhibited (p<0.001) by reaching 2.38 * 10^6 CFU/mL in presence of 20 µg isabelin compared to 2.64 * 10^7 CFU/mL in the control (fig 2).

No effect by the presence of isabelin was observed on the other tested soil-bacteria (*Achromobacter denitrificans* sp. MH58.1, *Arthrobacter* sp. V13, *Burkholderia* sp. AD24, *Collimonas* sp. Ter91, *Ochrobactrum* sp. 44, *Pseudomonas fluorescens* sp. AD21, *Serratia plymuthica* PGPRI-I, *Stenotrophomonas maltophilia* sp. MH58.27, *Xanthomonas campestris* sp. V79), belonging to the phyla of Actinobacteria, alpha, beta and gamma –proteobacteria (Supplementary fig S2, supplementary table S1). The genus *Paenibacillus* comprises bacterial species relevant to plants, rhizosphere and the soil environment (Grady et al., 2016). Many *Paenibacillus* species can promote crop growth directly via biological nitrogen fixation, phosphate solubilization, production of the phytohormone indole-3-acetic acid (IAA), and the release of siderophores which enable iron acquisition. Furthermore, they can offer plant protection against insect herbivores and phytopathogens, including bacteria, fungi, nematodes, and viruses (Anand et al., 2013; Debois et al., 2013; Ryu et al., 2005). The observed specific effect of
isabelin on the *Paenibacillus* genus may be providing a strategy of invasive plant to (1) inhibit this particular group of plant growth promoting bacteria (PGPR) and create an unfavorable environment for competitor plant species or (2) to select specific microbiome for surrounding the roots of invasive plant species.

**Effect of isabelin on human pathogens**

Both the agar diffusion tests and liquid media bioassay on the human pathogens model organisms revealed inhibition on two of the three tested model species (fig. 3). No inhibition was observed in the assays performed with *E. coli*. On the other hand, results obtained with *S. aureus* revealed a significant growth inhibition (p<0.001) by reaching 1.33 * 10^6 CFU/mL in presence of 20 µg isabelin compared to 8.44 * 10^6 CFU/mL in the control (fig 3). Furthermore, the presence of 20 µg isabelin reduced absorbance values in OD_600 measurement. This observation confirmed the appearance of the inhibition area (ZOI) in the agar-diffusion test. This clear difference in antimicrobial activity of isabelin observed, against Gram-positive and Gram-negative bacteria is in line with other studies showing that Gram-positive bacteria are more sensitive to antimicrobial compounds than Gram-negative bacteria (Giske et al., 2008; Rice, 2006).

The yeast-model organism, *C. albicans*, showed a significant (p=0.001) decreased number of colony forming units (CFU) in the presence of 20µg isabelin. *C. albicans* reached 1.32 * 10^7 CFU/mL in presence of 20 µg isabelin compared to 5.79 * 10^7 CFU/mL in the control (fig 3). This result is in accordance with the observed reduced absorbance values in the OD_600 measurements.

*Candida* species are ubiquitous common commensal yeasts, responsible for many nosocomial infections. *Candida albicans*, in particular, is responsible for more than half of all the candidemia cases (Lass-Flörl,
In the last decade, many *Candida* strains developed resistance to the azoles antibiotics, which are normally used for the treatment of these infections (Cleveland et al., 2015). There is an increased interest in finding new active molecules with the ability of inhibiting *Candida* strains (Bona et al., 2016).

The cell wall organization in Gram-positive bacteria and *Candida* could possibly explain the observed growth-inhibitory effects of isabelin. Another sesquiterpene lactone, cnicin, a germacranolide sesquiterpene lactone found in *Centauria* species, is a molecule presenting Michael acceptors sites. Cnicin has been able to alkylate the thiol-groups of cysteine residue of the UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) enzyme involved in the peptidoglycan biosynthesis, causing the inhibition of the biosynthesis of the cell wall (Bachelier et al., 2006). The high reactivity of isabelin with thiol groups has been already demonstrated (Taglialetela-Scafati et al., 2012) and the action on cell wall biosynthesis could be responsible for the antimicrobial activity of this compound.

**CONCLUSIONS**

This is the first study to test the antimicrobial activity of the pure sesquiterpene dilactone isabelin, produced by the invasive weed *Ambrosia artemisiifolia*, against a range of phylogenetically different soil-borne microorganisms and human pathogens. Isabelin did not reveal any antifungal or antioomycetal activity; however, it revealed strong antimicrobial activity against the Gram-positive soil bacteria *Paenibacillus* sp., the multi-drug-resistant human pathogen *Staphylococcus aureus* (MRSA), and the yeast human pathogen *Candida albicans*.

Many sesquiterpene lactones have been related to allergenic effects, however, little is known to date about the antimicrobial properties of these molecules. Our results indicate that isabelin can inhibit...
certain soil borne bacteria and hence, modify the surrounding soil microbiome. Moreover, isabelin revealed strong antimicrobial activity against important human pathogens, indicating that metabolites released by invasive plant species can be explored as a source of novel compounds needed to combat the raising multidrug resistant pathogens.

Further studies will be needed to explain the mechanisms of antimicrobial activity of isabelin as well as to examine possible link between allergenic effects and antimicrobial activity of this compound.

REFERENCES


Acknowledgements

The authors would like to thank the two anonymous reviewers for their suggestion for improving the manuscript. This is publication 6380 of the NIOO-KNAW

Figure 1: Effect of 5 µg isabelin dissolved in water (Isabelin), water control (CTRL) on the growth of soil fungi and oomycetes. Effect on mycelial extension on (a) F. culmorum, (b) R. solani, (c) M. hiemalis and (d) P. ultimum. Data represent the mean of three replicates, error bars represent standard deviation.
Figure 2: Colony forming units (CFU) of *Paenibacillus* sp. AD87: in presence of 20 µg isabelin dissolved in water (Isabelin), water control (CTRL) and 75 µg Tetracycline as inhibition control (TC). Significant differences between treatments and the control are marked with an asterisk and the respective p-value. Data represent the mean of three replicates, error bars represent standard deviation.
Figure 3: Effect of 20 µg isabelin dissolved in water on the growth of three human pathogens:

(a) *Escherichia coli* WA321, (b) *Staphylococcus aureus* 533R4. (c) *Candida albicans* BSMY212. Controls: water control (CTRL) and 75 µg Tetracycline (TC) or 4 mg Cycloheximide.
(CH) as inhibition control. Significant differences between treatments and the controls are marked with an asterisk and the respective p-value. Data represent the mean of three replicates, error bars represent standard deviation.
### Tables:

**Table 1: Bacterial, fungal and oomycetal organisms used in this study.**

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<th>Reference</th>
<th>Function</th>
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<td><em>Paenibacillus sp.</em> AD87</td>
<td>Firmicutes</td>
<td>LXQN00000000</td>
<td>Tyc et al. 2017</td>
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<td><em>Pseudomonas fluorescence</em> sp. AD21</td>
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<td>Garbeva et al. 2014</td>
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<td><em>Serratia plymuthica</em> PGPR-1</td>
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<td>Garbeva et al. 2014</td>
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<td><em>Staphylococcus aureus</em> 533R4</td>
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<td>LN681573</td>
<td>Meyer et al. 1978</td>
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<td><em>Xanthomonas campestris</em> sp. V79</td>
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