Journal of Microbiological Methods 162 (2019) 28–37

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Review

Probiotics as antifungal agents: Experimental confirmation and future prospects

Janet Cheruiyot Kosgey[a,b,d], Lina Jia[a,b], Yong Fang[a,b], Jianxun Yang[b,c], Lei Gao[a,g], Jielin Wang[a], Rose Nyamao[a,b], Martin Cheteu[a,b], Dandan Tong[a], Vitalis Wekesa[d,f], Natalia Vasilyeva[e], Fengmin Zhang[a,b,*]

[a] Department of Microbiology, Harbin Medical University, Harbin 150081, China
[b] WU Lien-Teh Institute, Harbin Medical University, Harbin 150081, China
[c] Department of Dermatology, The 2nd Hospital of Harbin Medical University, Harbin 150081, China
[d] School of biological and life sciences, Technical University of Kenya, 52428-00200, Kenya
[e] Kashkin Research Institute of Medical Mycology, Department of Microbiology, North-Western State Medical University named after Machnikov, Saint Petersburg, Russia
[f] Flamingo Horticulture, Dudutech Division, P.O Box 1927, 20117, Naivasha, Kenya
[g] Electron Microscopy Center, Basic Medical Science College, Harbin Medical University, China

ARTICLE INFO

Keywords:
Probiotics
Dermatophytes
Candida albicans
Aspergillus spp.
Methods

ABSTRACT

Fungal burden throughout the world is very high and it keeps escalating due to increasing numbers of immunocompromised individuals. In contrast, the drugs used in management of fungal infections are so few some with high toxicity. Furthermore, highly resistant fungal pathogens are emerging for example *Candida auris*, *Candida glabrata*, *Candida gullemondii* and *Aspergillus* species among others. Thus now, more than ever, there is a need for combined efforts and an all round search for possible solutions to curb these problems. Therefore, the role of probiotics in management of fungal infections is indispensable. In fact, the antimicrobial activity of probiotics has been screened with promising results against microbial pathogens. Although, recent reports indicated that probiotics may also contribute to protect against fungal infections, the research done in checking antifungal activity of probiotics has used varied technology. This calls for harmonization of the methods used to screen and confirm the antimicrobial activity of probiotics and other candidate microorganisms. We therefore sought to address issues of disparity in probiotic research and their outcomes. Thus this paper is in order as it comprehensively reviews' publications, provides a summary of the methods and future prospects of probiotics as antifungal agents.

1. Introduction

The introduction covers the application of probiotics against selected pathogens which includes; the need for antifungal agents, mechanisms, benefits, side-effects of probiotics and the combinative use of probiotic microorganisms with antifungal drugs.

1.1. The need for antifungal agents

There is a dire need for new antifungal treatment considering that fungal infections have tremendously escalated in clinical field as well as emergence of antifungal resistant fungal pathogens whilst there are few antifungal drugs. It is estimated that dermatophytes affect between 10 and 20% (Pfaller et al., 2006) and 20–25% (Wu et al., 2011) of people worldwide. Candida is the fourth leading cause of nosocomial infections (Pfaller et al., 2006) and the most isolated microorganism (40–70%) from clinical specimens. In fact, the leading cause of healthcare-associated bloodstream infections in US hospitals is Candida (Magill et al., 2014). Currently, *Candida albicans* is linked to increased risk of carcinogenesis and metastasis (Ramirez-Garcia et al., 2016). Furthermore, CDC statistics show that 7% of Candida species isolated from blood are resistant to fluconazole, 70% of which are *C*andida glabrata and *Candida krusei species* (Lockhart et al., 2012; Vallabhaneni et al., 2015). Echinocandins are the first-line treatment for *Candida glabrata*, however, it is of concern to note that 3% of *Candida glabrata* isolates are resistant to echinocandins (Vallabhaneni et al., 2015). Moreover, it is estimated that candidemia results in an additional 3 to 13 days of hospitalization and high healthcare costs which is estimated at $6000

* Corresponding author at: Department of Microbiology, Harbin Medical University, 157 Baojian road, Nangang District, Harbin 150081, China.
E-mail address: fengminzhang@ems.hrbmu.edu.cn (F. Zhang).

https://doi.org/10.1016/j.mimet.2019.05.001
Received 14 March 2019; Received in revised form 30 April 2019; Accepted 2 May 2019
Available online 06 May 2019

to $29,000 (Morgan et al., 2005). Almost all of the samples of *C. auris* sent to CDC are resistant to fluconazole, and up to one-third are resistant to amphotericin B, which is reserved as a last-resort treatment (Lockhart et al., 2016). Most *C. auris* isolates are susceptible to echinocandins (Lockhart et al., 2016). Although, most antifungal resistance occurs in Candida, resistance in other fungal pathogens is equally important. *Aspergillus* the leading cause of invasive mold infections is estimated to cause 300,000 infections worldwide every year (Brown et al., 2012). Additionally, it is estimated that about 12% of *Aspergillus* infections are resistant to antifungal medications (Rivero-Menendez et al., 2016). Besides, in US up to 7% of *Aspergillus* species from patients with stem cell and organ transplants are resistant to antifungal drugs (Baddley et al., 2009; Kontoyiannis et al., 2010; Pappas et al., 2010). Of interest, is that *Aspergillus* species resistance has been traced to environmental sources especially from agricultural use of azole fungicides (Mortensen et al., 2010; Verweij et al., 2009). These factors emphasize the need for new antifungal agents. Probiotics have been demonstrated to have antifungal activity against *C. albicans* (Chew et al., 2015a,b; Song and Lee, 2017; Zhao et al., 2016), *C. glabrata* (Chew et al., 2015a,b), dermatophytes (Guo et al., 2011, 2012) and *Aspergillus* spp. (Crowley et al., 2013) thus probiotics are promising antifungal agents.

1.2. Mechanism, benefits and side-effects of probiotics

Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Gibson et al., 2017). Prebiotics refers to substrates that are selectively utilized by host microorganisms conferring a health benefit (Gibson et al., 2017; Matsubara et al., 2016c; Ohshima et al., 2016). Furthermore, biogenics involve the use of beneficial bioactive substances produced by probiotic bacteria, whose activities are independent from the viability of probiotic bacteria (Ohshima et al., 2016). The term synbiotics is where prebiotics and probiotics are utilized (Kojima et al., 2016; Ohshima et al., 2016).

The mechanisms in which probiotics protect against infection include (1) they lower pH, (2) antagonism by producing antimicrobial compounds for example bacteriocins and or other metabolic products, (3) compete with the pathogen for binding sites and receptors sites, nutrients and growth factors, (4) stimulate immunomodulatory cells, (5) production of enzymes (6) improve barrier function of intestinal mucosa, (7) modulate inflammatory responses, (8) aggregate with pathogens, (9) produce hydrogen peroxide (H_2O_2), (10) produce organic acids (Amara and Shibl, 2015; Deidda et al., 2016; Guo et al., 2015; Matsubara et al., 2016a,b,c; Shehata et al., 2016).

The probiotics are regarded as generally safe (Sulik-Tyszka et al., 2018). But theoretical side effects may exist such as conferring drug resistance genes to pathogens, thus the need to always screen for their antibiotic susceptibility and presence of drug resistance plasmids. They can also cause sepsis to critically ill or preterm babies; these properties though should be noted as species and sometimes strain specific. For instance *Saccharomyces boulardii* has been documented to cause fungemia, *Bacillus subtilis* and *Lactobacillus rhamnosus* (LGG) can cause bacteremia in specific individuals with predisposing factors (Matsubara et al., 2016b).

1.3. Combinative usage of probiotics/CFS with conventional drugs

There are many cases in which the probiotics are administered together with conventional drugs clinically. The results from these studies are promising and include faster healing, half dose of conventional drug needed and synergy of the drug (Lau et al., 2016; Rishi et al., 2011; Russo et al., 2018; Shah et al., 2013) (Table 1).

2. Areas in the used technology that need harmonization

To obtain reproducible and conclusive results, standardization of

Table 1
Comparative usage of probiotics and conventional drugs.

Condition	Probiotic	Drug	Outcome	Reference
Vulvovaginal candidiasis	*Lactobacillus acidophilus GLA-14, Lactobacillus rhamnosus HN001*	Clotrimazole	Reduced symptoms and recurrence of the disease	(Russo et al., 2018)
Vulvovaginal candidiasis	*Lactobacillus rhamnosus* GR-1 *and Lactobacillus reuteri* RC-14	Fluconazole	The probiotic increased the effectiveness of an anti-fungal pharmaceutical agent in curing disease	(Fu et al., 2017)
Aggressive peritonitis	Inersan®	Doxycycline	decrease of plaque index and gingivial index within a period of two months	(Degnan, 2012)
Vulvovaginal candidiasis	*Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum*	Fluconazole	Significant reduction in disease recurrence	(Denkova et al., 2013)

Table 2
Synopsis of the probiotic, test fungus, preparation and the inoculums size.

Probiotic				Indicator fungal pathogen				Reference
Name	Inoculum size	Incubation temp (°C)	Incubation time	Name	Inoculum size	Incubation temp (°C)	Incubation time	
L. johnsonii PV016	OD 600 nm of 1	37		*C. albicans*	OD 600 nm of 0.01	30	24 h	(Kohler et al., 2012)
L. acidophilus A2 *L. acidophilus* Ac *L. delbrueckii* spp. *bulgaricus* *Bifidobacteria* sp. Bif 4	10^8 cfu/cm^3	37 ± 1	72 h	*C. albicans*	10^8cfu/cm^3	37 ± 1	72 h	(Denkova et al., 2013)
L. brevis ATCC367 *L. delbrueckii* spp. *bulgaricus* ATCC 9645 *L. fermentum* ATCC 23271 *L. paracasei* ATCC 335 *L. plantarum* ATCC 8014 *L. rhamnosus* ATCC 9595	10^7 cfu/ml	37	24 h	*C. albicans*	1.5×10^6 cfu/ml	37	24–48 h	(Kohler et al., 2012)
L. reuteri R2	10^4 cfu/ml	37	5 days	*Trichophyton tonsurans*	3×10^3 cfu/ml	30	15 days	(Guo et al., 2011)
L. plantarum 16	–	30	24–48 h	*Aspergillus fumigatus Af293*	10^5/ml	30	7 days	(Crowley et al., 2013)
L. casei PTCC1608	10^2 cfu/ml	37	24 h	*T. rubrum* *T. verocosum* *M. canis* *M. gypseum*	λMax580 nm 0.15 & 0.17 nm- Well 5×10^6 cfu/ml-MIC	22	7 days	(Mehdi-Alamdarloo et al., 2016)
L. arizonensis R13 &R14	10^4	37	48 h	*M. canis* *M. gypseum* *E. flocossum*	$1–3 \times 10^5$ cfu/ml	30	15 days	(Martinez et al., 2009)
L. brevis JJ2P & NL *L. casei* R4 & R21 *L. reuteri* ee1p & M13	10^4	37	5 days	*M. canis* *M. gypseum* *E. flocossum*	$1–3 \times 10^5$ cfu/ml	30	15 days	
L. rhamnosus GR1 *L. leuteri* RC-14	OD 600 nm 1	37	48 h	*C. glabrata*	OD 600 nm 1	37	24 h	(Tao et al., 2014)
	OD 600 nm 1	37	48 h	*C. glabrata*	OD 600 nm 1	37	24 h	
Lactobacillus rham-nosus, Lactobacillus delbrueckii subsp. *Bulgaricus, Lactococcus lactic* subsp. *Lactis, Lactobacillus paracasei, Lactobacillus gasseri*	10^9cell/ml	37	24 h	*C. albicans*	2×10^7cell/ml	37	24 h	(Shehata et al., 2016)

protocols is important. This section reviews the choice of Indicator fungal pathogen, probiotic microorganism, inoculum size, Incubation time and conditions and technique of production of probiotics cell free supernatant (CFS): Biogenics/spent media used in previous research and the need for harmonization are discussed.

2.1. The choice of Indicator fungal pathogen

This has mainly relied on the target disease that the probiotic is thought to treat. Thus vulvovaginal candidiasis pathogens predominantly *Candida albicans* has been chosen by many researchers who seek to use probiotics to manage diseases (Denkova et al., 2013; Kohler et al., 2012), although *Candida glabrata* has also been screened (Chew et al., 2015a,b; Tan et al., 2018). However, while screening new probiotic microorganisms for general antifungal activity, major classes of fungi of medical importance should be representatively screened (Panda, 2012). For example studies should include at least a dermatophyte, non-dermatophyte and a yeast. Furthermore, include also clinical, typed microorganism and drug resistant strains due to emerging resistance (Matsubara et al., 2016a,b,c) (Table 2).

2.2. The choice of probiotic microorganism and CFS

This choice has been inspired mainly by the ability of the microorganism to adhere to epithelial cells, resist stomach acidity, bile salts and enzymes, produce antimicrobial compounds, persistence within the system, susceptibility to antibiotics, genome stability or inability to confer resistance and ability to stabilize the normal microbiota among others (Aarti et al., 2018; Matsubara et al., 2016a,b,c). It should be noted that probiotic activity is strain specific (Tables 2, 3 and 4).

2.3. Inoculum size

The actual number of viable indicator fungal pathogens in the inoculum size directly influences the outcome of the results since too heavy inoculum may give a false negative result while too little may lead to false positive results (CLSI, 2008; Panda, 2012). A basis for the choice of inoculum size can be that recommended by CLSI (Fridkin and Jarvis, 1996) (Table 2). Different authors have used different inoculum size, incubation temperature and time for both probiotic and indicator fungal pathogen (Table 2). The same case applies to *in vivo* and clinical studies as also attested by Matsubara (Matsubara et al., 2016a) in which there were varied dose, duration and frequency of probiotic intake. We propose that the specific dose and viability of probiotic microorganism used (also in production of CFS), be established by dose dependent experiments or at least mention the concentration of probiotic used.

2.4. Incubation time and conditions

Lactic acid bacteria and Bifidobacteria are fastidious hence the media chosen should have specific ingredients for example; growth factors to enhance their growth, most researchers use MRS which is an

Table 3
Comparison of the preparation of CFS by different researchers.

Probiotic	Inoculum size	Media	Time	Temp (°C)	Centrifugation speed & time	Filter size	Used & storage temp	Reference
L. arizonensis R13 &R14 *L. brevis* JJ2P & NL *L. casei* R4 & R21 *L. reuteri* ee1p & M13	10^4 cell/ml	mMRS	5 days	37	2 × 3000*g* 15 min	–	Freeze dried powder CFS 4 °C	(Martinez et al., 2009)
L. reuteri R2	10^4 cell/ml	mMRS	5 days	37	2 × 3000*g* 15 min	–	Freeze dried powder CFS 4	(J. Guo et al., 2011)
L. casei PTCC1608	10^2 cell/ml	MRS	24	37	2 × 3000 rpm 15 min	0.22 μm	CFS 4 °C	(Mehdi-Alamdarloo et al., 2016)
L. rhamnosus GR1 *L. leuteri* RC-14	OD600nm 1	MRS	48	37	11,000*g* 15 min	0.22 μm	CFS −20 °C	(Tao et al., 2014)
L. plantarum 16	–	MRS	48 h	30	4400*g* 15 min	0.45 μm	Freeze dried and concentrated ×20 cCFS 4 °C	(Crowley et al., 2013)
Lactobacillus rhamnosus, Lactobacillus delbrueckii subsp. Bulgaricus, Lactococcus lactic subsp. Lactis, Lactobacillus paracasei, Lactobacillus gasseri	10^9cell/ml	MRS	24	37	9000*g* 15 min	–	CFS 4 °C	(Shehata et al., 2016)

Table 4
Précis of *in vivo* animal models for dermatophytoses and candidiasis.

Disease	Animal	Route of infection	Target organ	Reference
Dermatomycosis				
Dermatomycosis	Guinea pig	Skin abrasion	Skin localized infection	(Hau and Schapiro, 2010)
Dermatomycosis	Guinea pig	Intravenous	Cutaneous disseminated infection	(Hau and Schapiro, 2010)
Candidiasis				
Systemic infection	BALB/c mice	Lateral tail vein	Kidney	(Hebecker et al., 2016; Jacobsen et al., 2014; Pierce et al., 2015)
Systemic infection	*Caenorhabditis elegans*	Skin	Media	(de Barros et al., 2018)
Systemic infection	Pregnant mice	Intravascular	Placenta	(Hau and Schapiro, 2010)
Systemic infection	*Galleria mellonella*	Injection	Systemic	(Rossoni et al., 2018; Vilela et al., 2015)
Systemic infection	Zebra fish	Microinjection	Disseminated infection	(Bergeron et al., 2017)
Chronic vaginitis	Rats; oophectomised and kept permanently in pseudoestrous- weekly injection of estrogen	Intravaginal with blastospores	Vaginal swabs	(Gabrielli et al., 2018; Hau and Schapiro, 2010)
Localized oral candidiasis (thrush)	Rats and several avian species	Peroral challenge with blastospores; favoured by carbohydrate rich diet, antibiotic treatment and use of germ free or specific pathogen free animals	Mouth swabs	(Hau and Schapiro, 2010; Leao et al., 2018)
Oral candidiasis	Mice induced immunosuppressed	Oral inoculation	Mouth swabs	(Ishijima et al., 2012; Leao et al., 2018)

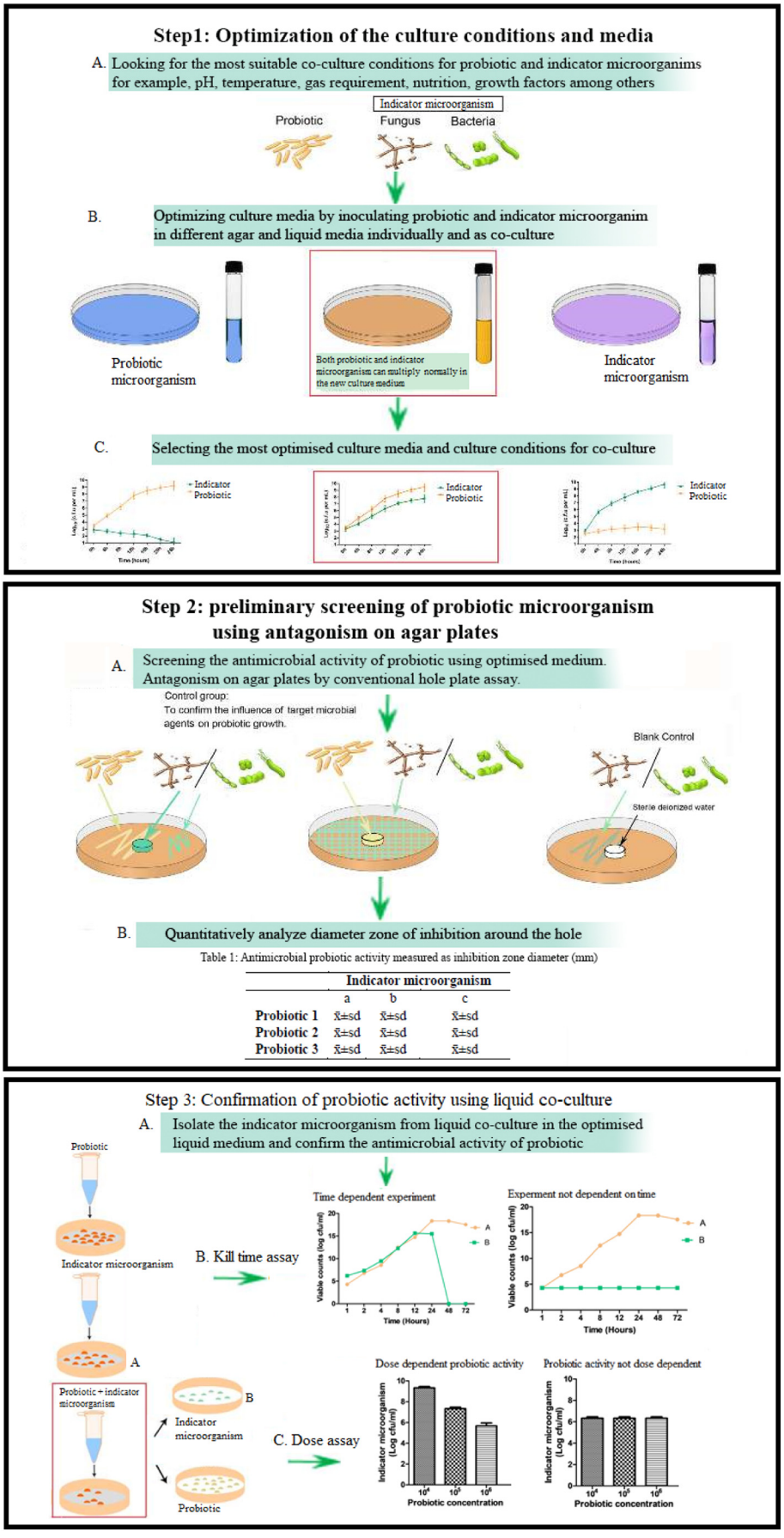

Fig. 1. Detailed proposed method for conclusively screening probiotic antimicrobial activity. First part entails choice of optimal media and growth conditions, second part is the preliminary screening on agar and last part is the confirmation of probiotic antimicrobial activity in liquid co-cultures.

appropriate media for the growth of probiotic microorganisms. MRS is both an enriched and a selective medium for the isolation and growth of only lactic acid bacteria and a few other bacteria. Therefore, if the indicator pathogen cannot be supported by this medium for example in dermatophytes (Guo et al., 2011), growth factors can be incorporated in any media of choice such as nutrient agar (NA) , potato dextrose agar (PDA) and sabouraud dextrose agar (SDA) to favour the growth of both the probiotic microorganism and the indicator fungal pathogen. Proper choice of media and its modifications is important (Guo et al., 2011; Martinez et al., 2009). The media supplemented with growth factors should be tested if it supports the growth of both probiotic microorganism and indicator fungal pathogen (Table 2 and Fig. 1). We propose that the specific incubation conditions such as time and oxygen requirements for both probiotic microorganism and indicator fungal pathogen be ascertained and confirmed by growth curve of individual microorganism (Fig. 1). Furthermore, fresh media should always be used for reproductive results as this can influence disc diffusion results.

2.5. Technique of production of probiotics cell free supernatant (CFS): Biogenics/spent media

The preparation of CFS entails the following; the probiotic microorganism is inoculated in broth media and incubated in incushaker (Tao et al., 2014). Cells are then separated by centrifuging to obtain the CFS (Crowley et al., 2013; Guo et al., 2011; Mehdi-Alamdarloo et al., 2016; Tao et al., 2014). The supernatant obtained can then be stored in a refrigerator and screened for antimicrobial activity (Tao et al., 2014) or the supernatant is further filter sterilized using a micrometer pore size syringe filter to obtain CFS (Crowley et al., 2013; Tao et al., 2014). The CFS is then used to screen for microbial activity or the resulting CFS is freeze dried (Crowley et al., 2013). The freeze dried CFS can be used or can be further concentrated to obtain concentrated CFS (Lara-Hidalgo et al., 2018) (cCFS) example 20 times its original volume (Crowley et al., 2013). Table 3 shows how different researchers have prepared the CFS and calls for harmonization of these methods.

The advantage of using biogenics/CFS is that the properties of the active component can be inferred. For example, to ascertain if it is proteinaceous in nature, heat treatment and enzymes are used. The results for both treatments are compared with non-treated CFS. If the activity is reduced or is lost it implies that the antifungal agent is proteinaceous in nature (Guo et al., 2011; Lara-Hidalgo et al., 2018). It can also be buffered and ascertained if activity is pH related (Aarti et al., 2018).

3. Technology for investigation and confirmation of the antifungal activity of probiotics

3.1. Experiments for investigation of antifungal potential of probiotics in vitro

Subsequent section is a review of each *In vitro* method for checking probiotic antifungal activity with their advantages and disadvantages. Included are antagonism on agar plates and liquid co-culture. In antagonism on agar plate's methods, probiotic and indicator microorganism are introduced in the same plate. The only difference is the sequence and manner of inoculation of either probiotic or the indicator fungal pathogen. Incubation is then done and diameter zone of inhibition which is the clear zones around the inoculated area is then read in millimeters.

3.1.1. Simple spot on lawn assay

Used to screen probiotic microorganism or CFS. The indicator fungal pathogen is inoculated first then probiotic microorganism is spotted at specific points on solid media simultaneously (Mehdi-Alamdarloo et al., 2016). Modification to this method entails spotting probiotic microorganisms as two parallel lines (Guo et al., 2011). The advantages include (a) media can be modified (Guo et al., 2011; Martinez et al., 2009), (b) different incubation conditions can be used that is, probiotic microorganism incubation conditions are optimized first then followed by optimizations for dermatophytes which need longer incubation time.

3.1.2. Agar spot assay

First described by Schilinger and Ducke (Pfaller et al., 2006). Probiotic microorganisms are spotted on agar media and incubated. Soft agar is then prepared and at around 45 °C and 50 °C indicator fungal pathogen is added and poured to the previously prepared plate spotted with probiotic microorganism (Kohler et al., 2012; Tao et al., 2014). The advantage of agar spot test is that it can allow the use of two different media that is for spotting and overlaid soft agar. Each microorganism is also grown at separate times, meaning incubation conditions can be adjusted. The disadvantage is that indicator microorganism is introduced when the soft agar is between 45 °C and 50 °C, which might kill heat labile indicator microorganisms. The strict aerobes may not grow well in this method due to pour plate method.

3.1.3. Spot on lawn assay with wells also referred to as agar well diffusion assay or as conventional whole plate method

The wells are dug and indicator fungal pathogen is inoculated then CFS/Probiotic is dispensed in it (Fijan, 2016; Mehdi-Alamdarloo et al., 2016). Unlike the former simple spot on lawn assay method, the probiotic microorganism can be allowed to grow first before the introduction of the indicator microorganisms (Figs. 1 & 2).

3.1.4. Paper disc assay

The supernatant/probiotic is dispensed in the paper discs and placed strategically on the inoculated media. It is suitable for simultaneous inoculation of both indicator fungal pathogen and probiotic microorganism.

3.1.5. Cross streak assay

Entails streaking the probiotic microorganism as 3 parallel lines on MRS media. A perpendicular line of indicator fungal pathogen is then streaked. Growth inhibition is examined at the interception point (Fijan, 2016).

3.1.6. Radial method

The probiotic microorganism is inoculated as a circle in the middle of the plate. Indicator fungal pathogen is then streaked as radial lines from the edge of the petri dish to the centre and growth inhibition is measured (Coman et al., 2014). Another method is done by cutting the media with the probiotic microorganisms and placed on top of the indicator fungal pathogen inoculated on the plate.

3.1.7. Liquid co-culture method

The probiotic and indicator fungal pathogen are both introduced to broth culture media (MRS broth), then incubated. Samples are collected intermittently and viability (cfu/ml) of fungal pathogen is determined. It shows if probiotic effect is static or cidal (Deidda et al., 2016; Denkova et al., 2013), it may also be used to elucidate the mechanism in which the probiotic bacteria exert its antifungal activity (Tao et al., 2014). Microtitre assay is used to determine minimum inhibitory concentration (MIC) of CFS using macroserial dilution, microdillution method or conventional kill time assay (Kheradmand et al., 2014; Tao et al., 2014). It is recommended as a confirmatory test (Figs. 1 & 2).

3.1.8. Antagonism assay

Antagonism assay on agar plates has the main advantage of being a simple and fast way of screening probiotic microorganisms. The main disadvantage is that it does not facilitate direct interaction of the probiotic microorganism and indicator fungal pathogen. Thus, the probiotic microorganism should produce sufficient antimicrobial agent

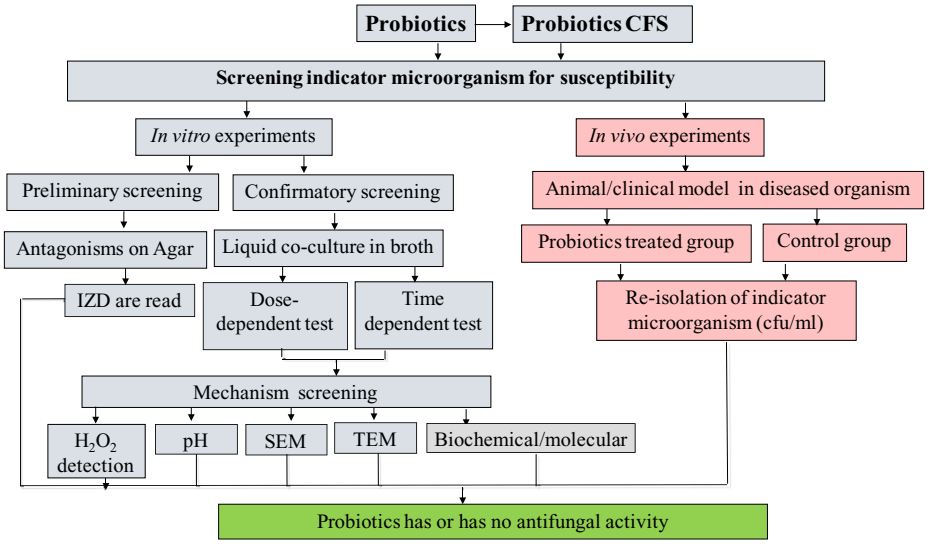

Fig. 2. Abridgement of methods involved in determining probiotic microorganisms and the properties of antimicrobial agents both *in vitro* and *in vivo*.

that should have the potential of diffusing through media in terms of size and spatial centrifugation (Santos et al., 2016). Therefore, for this reason these methods should not be used as the only method of ascertaining antimicrobial activity of probiotic microorganisms. Hence, we recommend the use of antagonism on agar plates and liquid co-culture to ascertain the antifungal activity of probiotic microorganism and CFS (Figs. 1 & 2).

3.2. Experiments for the discovery of antifungal mechanisms of probiotics

The methods used to ascertain probiotic microorganism antifungal activity mechanisms include; the ability to inhibit virulent factors, alteration of cell morphology and apoptosis.

3.2.1. Ability to inhibit virulence factors

For example the virulent factors in *Candida* include secretion of hydrolases, yeast to hypha transition, contact sensing, thigmotropism, biofilm formation, phenotypic switching and range of fitness attributes (Mehdi-Alamdarloo et al., 2016; Tan et al., 2018). Thus the probiotic microorganism can act against one or more of these factors, therefore the levels of expression of specific genes controlling these factors can be ascertained when checking for probiotic activity (Crowley et al., 2013; de Barros et al., 2018; Gabrielli et al., 2018; Guo et al., 2015; Kohler et al., 2012; Oliveira et al., 2016; Tao et al., 2014). Other factors used to ascertain the antifungal activity include aggregation assay using spectrophotometric autoaggregation and coaggregation (Chew et al., 2015a, 2015b; Lara-Hidalgo et al., 2018) (Figs. 1 & 2). The morphological transition of *C. albicans*, represented by germ tube formation contributes to enhance the adherence and invasion to the host tissue and increased virulence (Henriques et al., 2006; Lu et al., 2014). Lactobacilli have been demonstrated to build co-aggregates with Candida cells, and this process neutralizes germ tube growth (Mason et al., 2012). The co-aggregation can also prevent access of pathogens to cell receptors, thus inhibiting pathogen adhesion which is a prerequisite for colonization and the subsequent development of disease (Chew et al., 2015a,b; Martín et al., 2012; Santos et al., 2016).

3.2.2. Germ tube and hyphal growth inhibition method

Pelleted spores of dermatophytes and dimorphic pathogenic fungus are allowed to develop germ tubes and hyphae. CFS is then added and incubated. Growth is determined by examination of germ tube and hyphae at ×400 or ×1000 magnification (Crowley et al., 2013; Guo et al., 2012).

3.2.3. Spore germination inhibition assay of CFS

Pelleted mycelia and CFS are added to media and incubated. Samples are withdrawn and examined under ×400 or ×1000. Percentage spore germination is calculated by the following formula (Crowley et al., 2013; Guo et al., 2011, 2012):

$$\%\text{spore germination} = [\text{Numbers of germinated spores} / \text{Numbers of total spores}] \times 100$$

3.2.4. Scanning electron microscopy

Scanning electron microscopy is used to check cell integrity which include morphological distortion, adherence, biofilm or apoptosis (Shu Yih Chew et al., 2015; Tan et al., 2018; Zhao et al., 2016).

3.2.5. Confocal laser scanning microscopy

Live or dead cells are counted and their metabolic activity ascertained (Chew et al., 2015a,b; Tan et al., 2018). Live/dead can also be confirmed by subcultures and counting cfu/ml in time kill assays.

3.2.6. Mechanism of cell death

Apoptosis is outlined by a sequence of unique morphological changes which include; visible cell shrinkage and chromatin condensation, extensive plasma membrane blebbing which is followed by nuclear fragmentation, formation of apoptotic bodies and terminates with decomposition of apoptotic bodies within the phagosome and complete recycling of the components (Eisenberg-Lerner et al., 2009; Elmore, 2007). ROS accumulation and decreased membrane potential are well known biochemical and cytological responses of programmed cell death (PCD) such as apoptosis (Susan, 2007), or at very high concentrations induce necrosis, (Avery, 2011). These changes can be used to ascertain the integrity of the cell. Among cytological and biochemical methods used to check fungal pathogen cell integrity after treatment with probiotics include but are not limited to; nuclear fragmentation using Tunnel/ DAPI (Hao et al., 2013; Hwang et al., 2014; Khan et al., 2014; Ribeiro et al., 2006; Seong and Lee, 2018; Yun et al., 2017); DNA laddering using pulsed field gel electrophoresis (Ribeiro et al., 2006; Seong and Lee, 2018); *in situ* ligation assay (Ribeiro et al., 2006); cell membrane externalization of Annexin V/PI (Hao et al., 2013; Hwang et al., 2012; Khan et al., 2014; Lee et al., 2016; Ma et al., 2016; Yun et al., 2017); mitochondrial depolarization using mitochondria membrane potential detection kits for example JC fluorescent probes (Hao et al., 2013; Hwang et al., 2012; Hwang et al., 2014; Lee et al., 2016; Lee and Lee, 2015; Ma et al., 2016; Seong and Lee, 2018; Yun et al.,

2017); cytosolic & mitochondrial calcium (Lee et al., 2016; Lee and Lee, 2015; Seong and Lee, 2018; Yun et al., 2017); accumulation of reactive oxygen species (ROS) (Lee et al., 2016) (Lee and Lee, 2015; Ma et al., 2016; Seong and Lee, 2018; Yun et al., 2017); detecting cytochrome *c* in cytoplasm using color metric kits or western blotting (Hwang et al., 2012; Hwang et al., 2014; Lee et al., 2016; Lee and Lee, 2015; Seong and Lee, 2018; Yun and Lee, 2016; Yun et al., 2017); detecting metacaspace activation using detecting kits for example CaspACE FITC-VAD-FMK *in situ* Marker (Hwang et al., 2012; Hwang et al., 2014; Lee et al., 2016; Yun et al., 2017); lipid peroxidation (Lee et al., 2016; Yun et al., 2017); potassium release (Yun et al., 2017) and mitochondria/cytosol intracellular glutathione (Lee et al., 2016; Yun et al., 2017). A combination of a number of these methods can attest to the integrity of the indicator fungal pathogen and can be used to assess the antifungal activity of a probiotic microorganism. In these methods careful choice of positive (example antifungal drug) and negative (untreated) controls are important for interpretation of the results.

3.3. Experiments that confirm the antifungal activity of probiotics in vivo

In vitro studies can provide information about susceptibility responses (Zak, 1991), optimal concentrations and exposure times required (Craig, 2014) of antimicrobial agents. However, it has they have their limitations for instance, the majority of antimicrobial agents that are active *in vitro*, lack significant activity *in vivo* and the opposite occurs at times (Zak, 1991). Animal models have an additional advantage in that antimicrobial efficacy can be determined at specific body sites for example thigh, peritoneum, lung, endocarditis, and meningitis can be evaluated (Craig, 2014). Furthermore, in animal model antimicrobial agents are altered by host factors such as metabolism and immune system (Craig, 2014). Therefore, animal model bridge the gap between *in vitro* and clinical trials (Zak, 1991) and are indispensable for validation of probiotic antifungal activity. In brief, *in vivo* animal models and clinical studies are an absolute requirement to provide proof of beneficial activities of probiotic antifungal activity. Dermatophytoses and candidiasis *in vivo* models will be discussed in this review.

3.3.1. Animal models

The infection route of dermatophytes is strictly dependent on the goal of the study which reflects the animal, fungus and disease model applied. Examples of geophilic and anthrophilic dermatophytes are *Microsporum gypseum* and *Trichophyton rubrum* which are difficult to establish infections in laboratory animals' thus zoophilic dermatophytes especially *Trichophyton metangrophytes* var. *mentagrophtes*, var. *quinckeanum* and var. *granulae*, *Trichophyton verrucosum* and *Microsporum canis* are used. The most recommended animal model for dermatophytoses is hairless guinea pigs because the infection resembles infections in humans and topical treatment is suitable. Mouse, rat, hamster and dog have the disadvantage of defecating, licking additionally, and they also bite itching or irritating lesions intensively (Hau and Schapiro, 2010).

C. albicans and *Candida tropicalis* have high virulence in systemically induced mice (Hebecker et al., 2016; Jacobsen, 2014; Jacobsen et al., 2014; Pierce et al., 2015). Zebra fish (Bergeron et al., 2017), pregnant mice (Hau and Schapiro, 2010) and *Caenorhabditis elegans* (de Barros et al., 2018) have also been utilized in disseminated systemic models. *Candida metapsilosis* is virulent in vaginal mouse model (Németh et al., 2013). Additionally, oophectomised rats can be used for chronic vaginitis (Gabrielli et al., 2018; Hau and Schapiro, 2010). However, *C. glabrata*, *C. parapsilosis* and *C. krusei* do not induce mortality in mice (Jacobsen, 2014). Furthermore, *C. albicans* (Hamamoto et al., 2004), *C. tropicalis, C. parapsilosis* complex (*C. parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*) are virulent in the invertebrate model *Galleria monella* (Jacobsen, 2014). Murine oral candidiasis model of choice is induced immunosuppressed mice. The immunosuppressed condition is induced with prednisolone 100 mg per kg (Ishijima et al., 2012) or ketamine:xylazine 90–100 mg/kg and 10 mg/kg respectively (Leao et al., 2018) of body weight injected subcutaneously 24 h before inoculation with Candida orally. Additionally, rats and avian species have been used as oral candidiasis models (Hau and Schapiro, 2010; Leao et al., 2018), a summary of these *in vivo* models is given in Table 4.

3.3.2. Clinical trials

Clinical trials have been conducted after *in vitro* and/or *in vivo* animal model experiments with promising results. Few clinical trials on confirmation of antifungal effect of probiotics have been reported so far, but they been considered as the final confirmative experiment. Furthermore, due to the safety of the probiotics (Deidda et al., 2016; Sulik-Tyszka et al., 2018) many researchers skip this important step. This is seen in the case where many commercially marketed probiotics have pending clinical experiments or clinical studies (Degnan, 2012). Randomized placebo controlled clinical trials is the most recommended (Matsubara et al., 2016a,b,c). Probiotics have been tested clinically for the management of fungal pathogens of oral (Keller and Kragelund, 2018; Matsubara et al., 2016a,b,c; Mishra et al., 2016; Santos et al., 2009; Shah et al., 2013), uro-genital infections (Davar et al., 2016; Fu et al., 2017; Mezzasalma et al., 2017; Reid, 2016; Russo et al., 2018) and gastrointestinal systems (Zuo and Ng, 2018) with promising results thus, supporting some probiotics as potential antifungal agents. For example out of the 17 randomized controlled studies summarized by Matubara (Matsubara et al., 2016a,b,c) 13 showed a significant decrease in candida infection. The inactivity in the studies that showed no significant difference on candida were attributed to short administration times of probiotics (Pirotta et al., 2004), low sample size and species specificity of probiotics (Manzoni et al., 2006; Sutula et al., 2012, 2013). Areas that need urgent attention in clinical studies include actual probiotic viability, dosage, duration and frequency of administration of probiotics and route of administration.

4. Summary and future prospects of probiotics as antifungal agents

With the increase in the number of immunocompromised persons, an increase in the number of cases with fungal infections has occurred with probiotics offering an alternative means of treatment. However, there is a need for detailed conclusive research on *in vitro, in vivo* and clinical trials of both formulated drugs from probiotic and biogenic (CFS) administration which clearly demonstrates the benefits and side effects of each. More emphasis needs to be placed on the choice of probiotic used, methods and experimental designs. Research findings have demonstrated the fact that probiotics, sometimes of a particular strain, may have antifungal activity against one pathogen and not another (Guo et al., 2015; Matsubara et al., 2016a,b,c). This has been attributed to the ability of the particular pathogens to form biofilms, encode resistance genes in their genome and induce inflammatory responses (Benjamin et al., 2006; Mehdi-Alamdarloo et al., 2016; Shetty et al., 2005). Martinez et al. demonstrated that *Lactobacillus reuteri* RC14 alone or in combination with *Lactobacillus rhamnosus* GR1 can decrease the yeast population recovered from infected vaginal cells *In vitro* (Martinez et al., 2009). These are among the few studies done on synergism of probiotic microorganisms with repeat experiments required (Amara and Shibl, 2015). In addition, the synergism between probiotic microorganisms and conventional drugs also needs to be outlined. It has also been reported that some probiotics do not show any antibacterial activity *in vitro* but show better results *in vivo* and therefore there is need for thorough screening of probiotics before antimicrobial activity is or is not confirmed.

Acknowledgements

This research was supported by National Natural Science Foundation of China (No.81301703), the Natural Science Foundation of Heilongjiang Province, China (No.QC2013C078). The Chinese

Government (CSC) Scholarship (No. 2016BSZ783) and Government of Kenya under The Technical University of Kenya financially supported JCK. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Aarti, C., Khusro, A., Varghese, R., Arasu, M.V., Agastian, P., Al-Dhabi, N.A., ... Choi, K.C., 2018. In vitro investigation on probiotic, anti-Candida, and antibiofilm properties of Lactobacillus pentosus strain LAP1. Arch. Oral Biol. 89, 99–106. https://doi.org/10.1016/j.archoralbio.2018.02.014.

Amara, A.A., Shibl, A., 2015. Role of probiotics in health improvement, infection control and disease treatment and management. Saudi Pharm. J. 23 (2), 107–114. https://doi.org/10.1016/j.jsps.2013.07.001.

Avery, S.V., 2011. Molecular targets of oxidative stress. Biochem J 434 (2), 201–210. https://doi.org/10.1042/bj20101695.

Baddley, J.W., Marr, K.A., Andes, D.R., Walsh, T.J., Kauffman, C.A., Kontoyiannis, D.P, Moser, S.A., 2009. Patterns of susceptibility of Aspergillus isolates recovered from patients enrolled in the transplant-associated infection surveillance network. J. Clin. Microbiol. 47 (10), 3271–3275.

Benjamin, D.K., Stoll, B.J., Fanaroff, A.A., McDonald, S.A., 2006. National Institute of Child Health and Human Development neonatal research network. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. Pediatrics 117. https://doi.org/10.1542/peds.2004-2292.

Bergeron, A.C., Seman, B.G., Hammond, J.H., Archambault, L.S., Hogan, D.A., Wheeler, R.T., 2017. *Candida albicans* and *Pseudomonas* interact to enhance virulence of mucosal infection in transparent Zebrafish. Infect. Immun. 85 (11), e00417–e00475. https://doi.org/10.1128/IAI.00475-17.

Brown, G.D., Denning, D.W., Gow, N.A.R., Levitz, S.M., Netea, M.G., White, T.C., 2012. Hidden killers: human fungal infections. Sci. Transl. Med. 4 (165), 165rv113.

Chew, S.Y., Cheah, Y.K., Seow, H.F., Sandai, D., Than, L.T., 2015a. Probiotic *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exhibit strong antifungal effects against vulvovaginal candidiasis-causing *Candida glabrata* isolates. J. Appl. Microbiol. 118 (5), 1180–1190. https://doi.org/10.1111/jam.12772.

Chew, S.Y., Cheah, Y.K., Seow, H.F., Sandai, D., Than, L.T.L., 2015b. In vitro modulation of probiotic bacteria on the biofilm of *Candida glabrata*. Anaerobe 34, 132–138.

CLSI, 2008. M38-A2 reference method for broth dilution antifungal susceptibility testing of filamentous Fungi. In: Approved Standard, 2nd edition. . http://shop.clsi.org/site/Sample_pdf/M38A2_sample.pdf.

Coman, M.M., Verdenelli, M.C., Cecchini, C., Silvi, S., Orpianesi, C., Boyko, N., Cresci, A., 2014. In vitro evaluation of antimicrobial activity of *Lactobacillus rhamnosus* IMC 501((R)), *Lactobacillus paracasei* IMC 502((R)) and SYNBIO((R)) against pathogens. J. Appl. Microbiol. 117 (2), 518–527. https://doi.org/10.1111/jam.12544.

Craig, W.A., 2014. In vitro and animal PK/PD models. In: Fundamentals of Antimicrobial Pharmacokinetics and Pharmacodynamics. Springer, pp. 23–44.

Crowley, S., Mahony, J., Morrissey, J.P., van Sinderen, D., 2013. Transcriptomic and morphological profiling of *Aspergillus fumigatus* Af293 in response to antifungal activity produced by *Lactobacillus plantarum* 16. Microbiology 159 (Pt 10), 2014–2024. https://doi.org/10.1099/mic.0.068742-0.

Davar, R., Nokhostin, F., Eftekhar, M., Sekhavat, L., Bashiri, M.Z., Shamsi, F., 2016. Comparing the recurrence of vulvovaginal candidiasis in patients undergoing prophylactic treatment with probiotic and placebo during the 6 months. Prob. Antimicrob. Proteins 8 (3), 130–133.

de Barros, P.P., Scorzoni, L., Ribeiro, F.C., Fugisaki, L.R.O., Fuchs, B.B., Mylonakis, E., ... Rossoni, R.D., 2018. *Lactobacillus paracasei* 28.4 reduces in vitro hyphae formation of *Candida albicans* and prevents the filamentation in an experimental model of *Caenorhabditis elegans*. Microb. Pathog. 117, 80–87. https://doi.org/10.1016/j.micpath.2018.02.019.

Degnan, F.H., 2012. Clinical studies involving probiotics: when FDA's investigational new drug rubric applies—and when it may not. Gut Microbes 3 (6), 485–489. https://doi.org/10.4161/gmic.22158.

Deidda, F., Amoruso, A., Allesina, S., Pane, M., Graziano, T., Del Piano, M., Mogna, L., 2016. In vitro activity of *Lactobacillus fermentum* LF5 against different Candida species and *Gardnerella vaginalis*: a new perspective to approach mixed vaginal infections? J. Clin. Gastroenterol. 50 (Suppl. 2), S168–S170. https://doi.org/10.1097/MCG.0000000000000692. *Proceedings from the 8th Probiotics, Prebiotics & New Foods for Microbiota and Human Health meeting held in Rome, Italy on September 13-15, 2015.*

Denkova, R.V., Yanakieva, Z., Denkova, V., Radeva, V., Nikolova, V., 2013. In vitro inhibitory activity of Bifidobacterium and Lactobacillus strains against *Candida albicans*. Bulgarian J. Veterinary Med. 16 (3), 186–197.

Eisenberg-Lerner, A., Bialik, S., Hans-Uwe, S., Kimchi, A., 2009. Life and death partners: apoptosis, autophagy and the cross-talk between them. Cell Death Differ. 16 (7), 966.

Fijan, S., 2016. Antimicrobial effect of probiotics against common pathogens. In: Rao, V., Rao, L.G. (Eds.), Probiotics and Prebiotics in Human Nutrition and Health. InTech, Rijeka pp. Ch. 10.

Fridkin, S.K., Jarvis, W.R., 1996. Epidemiology of nosocomial fungal infections. Clin. Microbiol. Rev. 9 (4), 499–511.

Fu, J., Ding, Y., Wei, B., Wang, L., Xu, S., Qin, P., ... Jiang, L., 2017. Epidemiology of *Candida albicans* and non-*C. albicans* of neonatal candidemia at a tertiary care hospital in western China. BMC Infect. Dis. 17 (1), 329. https://doi.org/10.1186/s12879-017-2423-8.

Gabrielli, E., Pericolini, E., Ballet, N., Roselletti, E., Sabbatini, S., Mosci, P., Vecchiarelli, A., 2018. *Saccharomyces cerevisiae*-based probiotic as novel anti-fungal and anti-inflammatory agent for therapy of vaginal candidiasis. Benefic. Microbes 9 (2), 219–230. https://doi.org/10.3920/bm2017.0099.

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S.L., Reimer, R.A., Salminen, S.J, Cani, P.D., 2017. The international scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat. Rev. Gastroenterol. Hepatol. 14 (8), 491–502.

Guo, J., Mauch, A., Galle, S., Murphy, P., Arendt, E.K., Coffey, A., 2011. Inhibition of growth of *Trichophyton tonsurans* by *Lactobacillus reuteri*. J. Appl. Microbiol. 111 (2), 474–483. https://doi.org/10.1111/j.1365-2672.2011.05032.x.

Guo, J., Brosnan, B., Furey, A., Arendt, E., Murphy, P., Coffey, A., 2012. Antifungal activity of Lactobacillus against *Microsporum canis*, *Microsporum gypseum* and *Epidermophyton floccosum*. Bioeng Bugs 3 (2), 104–113. https://doi.org/10.4161/bbug.19624.

Guo, Y., Wei, C., Liu, C., Li, D., Sun, J., Huang, H., Zhou, H., 2015. Inhibitory effects of oral Actinomyces on the proliferation, virulence and biofilm formation of *Candida albicans*. Arch. Oral Biol. 60, 1368–1374. https://doi.org/10.1016/j.archoralbio.2015.06.015.

Hamamoto, H., Kurokawa, K., Kaito, C., Kamura, K., Razanajatovo, I.M., Kusuhara, H., ... Sekimizu, K., 2004. Quantitative evaluation of the therapeutic effects of antibiotics using silkworms infected with human pathogenic microorganisms. Antimicrob. Agents Chemother. 48 (3), 774–779.

Hao, B., Cheng, S., Clancy, C.J., Nguyen, M.H., 2013. Caspofungin kills *Candida albicans* by causing both cellular apoptosis and necrosis. Antimicrob. Agents Chemother. 57 (1), 326–332. https://doi.org/10.1128/aac.01366-12.

Hau, J., Schapiro, S.J., 2010. Handbook of laboratory animal science. In: Essential Principles and Practices. vol. I CRC press.

Hebecker, B., Vlaic, S., Conrad, T., Bauer, M., Brunke, S., Kapitan, M., ... Jacobsen, I.D., 2016. Dual-species transcriptional profiling during systemic candidiasis reveals organ-specific host-pathogen interactions. Sci. Rep. 6, 36055.

Henriques, M., Azeredo, J., Oliveira, R., 2006. *Candida albicans* and *Candida dubliniensis*: comparison of biofilm formation in terms of biomass and activity. Br. J. Biomed. Sci. 63 (1), 5–11.

Hwang, I., Lee, J., Hwang, J.H., Kim, K.J., Lee, D.G., 2012. Silver nanoparticles induce apoptotic cell death in *Candida albicans* through the increase of hydroxyl radicals. FEBS J. 279 (7), 1327–1338.

Hwang, J.H., Choi, H., Kim, A.R., Yun, J.W., Yu, R., Woo, E.-R., Lee, D.G., 2014. Hibicuslide C-induced cell death in *Candida albicans* involves apoptosis mechanism. J. Appl. Microbiol. 117 (5), 1400–1411.

Ishijima, S.A., Hayama, K., Burton, J.P., Reid, G., Okada, M., Matsushita, Y., Abe, S., 2012. Effect of *Streptococcus salivarius* K12 on the in vitro growth of *Candida albicans* and its protective effect in an oral candidiasis model. Appl. Environ. Microbiol. 78 (7), 2190–2199. https://doi.org/10.1128/aem.07055-11.

Jacobsen, I.D., 2014. *Galleria mellonella* as a model host to study virulence of Candida. Virulence 5 (2), 237–239.

Jacobsen, I.D., Lüttich, A., Kurzai, O., Hube, B., Brock, M., 2014. In vivo imaging of disseminated murine *Candida albicans* infection reveals unexpected host sites of fungal persistence during antifungal therapy. J. Antimicrob. Chemother. 69 (10), 2785–2796.

Keller, M.K., Kragelund, C., 2018. Randomized pilot study on probiotic effects on recurrent candidiasis in oral lichen planus patients. Oral Dis. 24 (6), 1107–1114. https://doi.org/10.1111/odi.12858.

Khan, A., Ahmad, A., Khan, L.A., Manzoor, N., 2014. *Ocimum sanctum* (L.) essential oil and its lead molecules induce apoptosis in *Candida albicans*. Res. Microbiol. 165 (6), 411–419.

Kheradmand, E., Rafii, F., Yazdi, M.H., Sepahi, A.A., Shahverdi, A.R., Oveisi, M.R., 2014. The antimicrobial effects of selenium nanoparticle-enriched probiotics and their fermented broth against *Candida albicans*. DARU J. Pharm. Sci. 22 (1), 48. https://doi.org/10.1186/2008-2231-22-48.

Kohler, G.A., Assefa, S., Reid, G., 2012. Probiotic interference of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 with the opportunistic fungal pathogen *Candida albicans*. Infect. Dis. Obstet. Gynecol. 2012, 636474. https://doi.org/10.1155/2012/636474.

Kojima, Y., Ohshima, T., Seneviratne, C.J., Maeda, N., 2016. Combining prebiotics and probiotics to develop novel synbiotics that suppress oral pathogens. J. Oral Biosci. 58 (1), 27–32.

Kontoyiannis, D.P., Marr, K.A., Park, B.J., Alexander, B.D., Anaissie, E.J., Walsh, T.J., Brown, J.M., 2010. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the transplant-associated infection surveillance network (TRANSNET) database. Clin. Infect. Dis. 50 (8), 1091–1100.

Lara-Hidalgo, C.E., Dorantes-Alvarez, L., Hernandez-Sanchez, H., Santoyo-Tepole, F., Martinez-Torres, A., Villa-Tanaca, L., Hernandez-Rodriguez, C., 2018. Isolation of yeasts from Guajillo pepper (Capsicum annuum L.) fermentation and study of some probiotic characteristics. Prob. Antimicrob. Proteins. https://doi.org/10.1007/s12602-018-9415-x.

Lau, C.S.M., Ward, A., Chamberlain, R.S., 2016. Probiotics improve the efficacy of standard triple therapy in the eradication of Helicobacter pylori: a meta-analysis. Infect. Drug Res. 9, 275–289. https://doi.org/10.2147/IDR.S117886.

Leao, M.V.P., Tavares, T.A.A., Goncalves, E., Silva, C.R., Dos Santos, S.S.F., Junqueira, J.C., de Oliveira, L.D., Jorge, A.O.C., 2018. Lactobacillus rhamnosus intake can prevent the development of candidiasis. Clin. Oral Investig. 22 (7), 2511–2518. https://doi.org/10.1007/s00784-018-2347-8.

Lee, J., Lee, D.G., 2015. Novel antifungal mechanism of resveratrol: apoptosis inducer in Candida albicans. Curr. Microbiol. 70 (3), 383–389.

Lee, H., Hwang, J.S., Lee, D.G., 2016. Scolopendin 2 leads to cellular stress response in Candida albicans. Apoptosis 21 (7), 856–865.

Lockhart, S.R., Iqbal, N., Cleveland, A.A., Farley, M.M., Harrison, L.H., Bolden, C.B., Park, B.J., 2012. Species identification and antifungal susceptibility testing of Candida bloodstream isolates from population-based surveillance studies in two US cities from 2008 to 2011. J. Clin. Microbiol. 50 (11), 3435–3442.

Lockhart, S.R., Etienne, K.A., Vallabhaneni, S., Farooqi, J., Chowdhary, A., Govender, N.P, Desjardins, C.A., 2016. Simultaneous emergence of multidrug-resistant *Candida auris* on 3 continents confirmed by whole-genome sequencing and epidemiological analyses. Clin. Infect. Dis. 64 (2), 134–140.

Lu, Y., Su, C., Unoje, O., Liu, H., 2014. Quorum sensing controls hyphal initiation in Candida albicans through Ubr1-mediated protein degradation. Proc. Natl. Acad. Sci. 111 (5), 1975–1980.
Ma, F., Zhang, Y., Wang, Y., Wan, Y., Miao, Y., Ma, T., ... Li, M., 2016. Role of Aif1 in regulation of cell death under environmental stress in Candida albicans. Yeast 33 (9), 493–506.
Magill, S.S., Edwards, J.R., Wendy, Bamberg, Beldavs, Z.G., Ghinwa, Dumyati, Kainer, M.A, Nadle, J., 2014. Multistate point-prevalence survey of health care–associated infections. N. Engl. J. Med. 370 (13), 1198–1208.
Manzoni, P., Mostert, M., Leonessa, M.L., Priolo, C., Farina, D., Monetti, C., ... Gomirato, G., 2006. Oral supplementation with Lactobacillus casei subspecies rhamnosus prevents enteric colonization by Candida species in preterm neonates: a randomized study. Clin. Infect. Dis. 42 (12), 1735–1742. https://doi.org/10.1086/504324.
Martín, R., Sánchez, Borja, Suárez, Juan Evaristo, Urdaci, María C., 2012. Characterization of the adherence properties of human lactobacilli strains to be used as vaginal probiotics. FEMS Microbiol. Lett. 328 (2), 166–173.
Martinez, R.C., Seney, S.L., Summers, K.L., Nomizo, A., De Martinis, E.C., Reid, G., 2009. Effect of Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the ability of Candida albicans to infect cells and induce inflammation. Microbiol. Immunol. 53 (9), 487–495. https://doi.org/10.1111/j.1348-0421.2009.00154.x.
Mason, K.L., Downward, J.RE., Mason, K.D., Falkowski, N.R., Eaton, K.A., Kao, J.Y., Huffnagle, G.B., 2012. Candida albicans and bacterial microbiota interactions in the cecum during recolonization following broad-spectrum antibiotic therapy. Infect. Immun. 80 (10), 3371–3380.
Matsubara, V.H., Bandara, H.M., Ishikawa, K.H., Mayer, M.P., Samaranayake, L.P., 2016a. The role of probiotic bacteria in managing periodontal disease: a systematic review. Expert Rev. Anti-Infect. Ther. 14 (7), 643–655. https://doi.org/10.1080/14787210.2016.1194198.
Matsubara, V.H., Bandara, H.M., Mayer, M.P., Samaranayake, L.P., 2016b. Probiotics as antifungals in mucosal candidiasis. Clin. Infect. Dis. 62 (9), 1143–1153. https://doi.org/10.1093/cid/ciw038.
Matsubara, V.H., Wang, Y., Bandara, H.M., Mayer, M.P., Samaranayake, L.P., 2016c. Probiotic lactobacilli inhibit early stages of *Candida albicans* biofilm development by reducing their growth, cell adhesion, and filamentation. Appl. Microbiol. Biotechnol. 100 (14), 6415–6426. https://doi.org/10.1007/s00253-016-7527-3.
Mehdi-Alamdarloo, S., Ameri, A., Moghimipour, E., Gholipour, S., Saadatzadeh, A., 2016. Formulation development of a topical probiotic gel for Antidermatophytosis effect. Jundishapur J. Nat. Pharm. Prod. 11 (3), e35893. https://doi.org/10.17795/jjnpp-35893.
Mezzasalma, V., Manfrini, E., Ferri, E., Boccarusso, M., Di Gennaro, P., Schiano, I., ... Labra, M., 2017. Orally administered multispecies probiotic formulations to prevent uro-genital infections: a randomized placebo-controlled pilot study. Arch. Gynecol. Obstet. 295 (1), 163–172.
Mishra, R., Tandon, S., Rathore, M., Banerjee, M., 2016. Antimicrobial efficacy of probiotic and herbal oral rinses against Candida albicans in children: a randomized clinical trial. Int. J. Clin. Pediatric. Den. 9 (1), 25.
Morgan, J., Meltzer, M.I., Plikaytis, B.D., Sofair, A.N., Huie-White, S., Wilcox, S., ... Teutsch, S.M., 2005. Excess mortality, hospital stay, and cost due to candidemia: a case-control study using data from population-based candidemia surveillance. Infect. Control Hosp. Epidemiol. 26 (6), 540–547.
Mortensen, K.L., Mellado, E., Lass-Flörl, C., Rodriguez-Tudela, J.L., Johansen, H.K., Arendrup, M.C., 2010. Environmental study of azole-resistant Aspergillus fumigatus and other aspergilli in Austria, Denmark, and Spain. Antimicrob. Agents Chemother. 54 (11), 4545–4549.
Németh, T., Tóth, A., Szenzenstein, J., Horváth, P., Joshua, D.N., Grózer, Z., ... Csaba, V., 2013. Characterization of virulence properties in the C. parapsilosis sensu lato species. PLoS ONE 8 (7), e68704.
Ohshima, T., Kojima, Y., Seneviratne, C.J., Maeda, N., 2016. Therapeutic application of Synbiotics, a fusion of probiotics and prebiotics, and Biogenics as a new concept for Oral Candida infections: a mini review. Front. Microbiol. 7 (10). https://doi.org/10.3389/fmicb.2016.00010.
Oliveira, V.M.C., Santos, S.S.F., Silva, C.R.G., Jorge, A.O.C., Leão, M.V.P., 2016. Lactobacillus is able to alter the virulence and the sensitivity profile of Candida albicans. J. Appl. Microbiol. 121 (6), 1737–1744.
Panda, S.K., 2012. Screening methods in the study of antimicrobial properties of medicinal plants. Int. J. Biotechnol. Res. 2 (1), 1–35.
Pappas, P.G., Alexander, B.D., Andes, D.R., Hadley, S., Kauffman, C.A., Freifeld, A., Ito, J., 2010. Invasive fungal infections among organ transplant recipients: results of the transplant-associated infection surveillance network (TRANSNET). Clin. Infect. Dis. 50 (8), 1101–1111.
Pfaller, M.A., Pappas, P.G., Wingard, J.R., 2006. Invasive fungal pathogens: current epidemiological trends. Clin. Infect. Dis. 43 (Supplement_1), S3–S14. https://doi.org/10.1086/504490.
Pierce, C.G., Chaturvedi, A.K., Lazzell, A.L., Powell, A.T., Saville, S.P., McHardy, S.F., Lopez-Ribot Jose, L., 2015. A novel small molecule inhibitor of Candida albicans biofilm formation, filamentation and virulence with low potential for the development of resistance. Biofilms Microb. 1, 15012.
Pirotta, M., Gunn, J., Chondros, P., Grover, S., 2004. Effect of lactobacillus in preventing post-antibiotic vulvovaginal candidiasis: a randomised controlled trial. BMJ 329 (7465), 548. https://doi.org/10.1136/bmj.38210.494977.DE.
Ramirez-Garcia, A., Rementeria, A., Aguirre-Urizar, J.M., Moragues, M.D., Antoran, A., Pellon, A., ... Hernando, F.L., 2016. *Candida albicans* and cancer: can this yeast induce cancer development or progression? Crit. Rev. Microbiol. 42 (2), 181–193.
Reid, G., 2016. The development of probiotics for women's health. Can. J. Microbiol. 63 (4), 269–277.
Ribeiro, G.F., Côrte-Real, M., Johansson, B., 2006. Characterization of DNA damage in yeast apoptosis induced by hydrogen peroxide, acetic acid, and hyperosmotic shock. Mol. Biol. Cell 17 (10), 4584–4591.
Rishi, P., Preet, S., Kaur, P., 2011. Effect of L. plantarum cell-free extract and co-trimoxazole against Salmonella Typhimurium: a possible adjunct therapy. Ann. Clin. Microbiol. Antimicrob. 10, 9. https://doi.org/10.1186/1476-0711-10-9.
Rivero-Menendez, O., Alastruey-Izquierdo, A., Mellado, E., Cuenca-Estrella, M., 2016. Triazole resistance in Aspergillus spp.: a worldwide problem? J. Fungi 2 (3), 21.
Rossoni, R.D., Dos Santos, V.M., Figueiredo, L.M.A., Martins, C.P., Jorge, A.O.C., Junqueira, J.C., 2018. Clinical strains of Lactobacillus reduce the filamentation of Candida albicans and protect Galleria mellonella against experimental candidiasis. Folia Microbiol. (Praha) 63 (3), 307–314. https://doi.org/10.1007/s12223-017-0569-9.
Russo, R., Superti, F., Karadja, E., De Seta, F., 2018. Randomized clinical trial in women with recurrent Vulvovaginal candidiasis: efficacy of probiotics and lactoferrin as maintenance treatment. Mycoses. https://doi.org/10.1111/myc.12883. *0*(ja).
Santos, A.L., Jorge, A.O.C., Santos, S.S.F, Leão, M.V.P., 2009. Influence of probiotics on Candida presence and IgA anti-Candida in the oral cavity. Braz. J. Microbiol. 40 (4), 960–964.
Santos, C.M.A., Pires, M.C.V., Leão, T.L., Hernández, Z.P., Rodriguez, M.L., Martins, A.K.S, Nicoli, J.R., 2016. Selection of Lactobacillus strains as potential probiotics for vaginitis treatment. Microbiology 162 (7), 1195–1207. https://doi.org/10.1099/mic.0.000302.
Seong, M., Lee, D.G., 2018. Reactive oxygen species-independent apoptotic pathway by gold nanoparticles in Candida albicans. Microbiol. Res. 207, 33–40.
Shah, M.P., Gujjari, S.K., Chandrasekhar, V.S., 2013. Evaluation of the effect of probiotic (Inersan®) alone, combination of probiotic with doxycycline and doxycycline alone on aggressive periodontitis – a clinical and microbiological study. J. Clin. Diagn. Res. 7 (3), 595–600. https://doi.org/10.7860/JCDR/2013/5225.2834.
Shehata, M.G., El Sohaimy, S.A., El-Sahn, Malak A., Youssef, M.M., 2016. Screening of isolated potential probiotic lactic acid bacteria for cholesterol lowering property and bile salt hydrolase activity. Ann. Agric. Sci. 61 (1), 65–75. https://doi.org/10.1016/j.aoas.2016.03.001.
Shetty, S.S., Harrison, L.H., Hajjeh, R.A., Taylor, T., 2005. Determining risk factors for candidemia among newborn infants from population-based surveillance: Baltimore, Maryland, 1998-2000. Pediatr. Infect. Dis. J. 24. https://doi.org/10.1097/01.inf.0000168751.11375.d6.
Song, Y.G., Lee, S.H., 2017. Inhibitory effects of Lactobacillus rhamnosus and Lactobacillus casei on Candida biofilm of denture surface. Arch. Oral Biol. 76, 1–6. https://doi.org/10.1016/j.archoralbio.2016.12.014.
Sulik-Tyszka, B., Snarski, E., Niedzwiedzka, M., Augustyniak, M., Myhre, T.N., Kacprzyk, A., ... Wroblewska, M., 2018. Experience with Saccharomyces boulardii probiotic in Oncohaematological patients. Prob. Antimicrob. Proteins 10 (2), 350–355. https://doi.org/10.1007/s12602-017-9332-4.
Susan, E., 2007. Apoptosis: a review of programmed cell death. Toxicol. Pathol. 35 (4), 495–516.
Sutula, J., Coulthwaite, L., Thomas, L., Verran, J., 2012. The effect of a commercial probiotic drink on oral microbiota in healthy complete denture wearers. Microb. Ecol. Health Dis. 23.
Sutula, J., Coulthwaite, L.A., Thomas, L.V., Verran, J., 2013. The effect of a commercial probiotic drink containing Lactobacillus casei strain Shirota on oral health in healthy dentate people. Microb. Ecol. Health Dis. 24.
Tan, Y., Leonhard, M., Moser, D., Ma, S., Schneider-Stickler, B., 2018. Inhibitory effect of probiotic lactobacilli supernatants on single and mixed non-albicans Candida species biofilm. Arch. Oral Biol. 85, 40–45. https://doi.org/10.1016/j.archoralbio.2017.10.002.
Tao, X.B., Qian, L.H., Li, Y., Wu, Q., Ruan, J.J., Cai, D.Z., Peng, H., 2014. Hospital-acquired infection rate in a tertiary care teaching hospital in China: a cross-sectional survey involving 2434 inpatients. Int. J. Infect. Dis. 27, 7–9. https://doi.org/10.1016/j.ijid.2014.05.011.
Vallabhaneni, S., Cleveland, A.A., Farley, M.M., Harrison, L.H., Schaffner, W., Beldavs, Z.G., 2015. Data from a large multisite population-based candidemia surveillance program, 2008–2014. In: Paper Presented at the Open Forum Infectious Diseases.
Verweij, P.E., Snelders, E., Kema, G.H.J., Mellado, E., Melchers, W.J.G., 2009. Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? Lancet Infect. Dis. 9 (12), 789–795.
Vilela, S.F.G., Barbosa, J.O., Rossoni, R.D., Santos, J.D., Prata, M.C.A., Anbinder, A.L, Junqueira, J.C., 2015. Lactobacillus acidophilus ATCC 4356 inhibits biofilm formation by C. albicans and attenuates the experimental candidiasis in Galleria mellonella. Virulence 6 (1), 29–39. https://doi.org/10.4161/21505594.2014.981486.
Wu, S.X., Guo, N.R., Li, X.F., Liao, W.Q., Chen, M., Zhang, Q.Q., ... Kuan, Y.Z., 2011. Human pathogenic fungi in China-emerging trends from ongoing national survey for 1986, 1996, and 2006. Mycopathologia 171 (6), 387–393. https://doi.org/10.1007/s11046-011-9398-6.
Yun, D.G., Lee, D.G., 2016. Silibinin triggers yeast apoptosis related to mitochondrial Ca2+ influx in Candida albicans. Int. J. Biochem. Cell Biol. 80, 1–9.
Yun, J., Hwang, J., Lee, D.G., 2017. The antifungal activity of the peptide, periplanetasin-2, derived from American cockroach Periplaneta americana. Biochem. J. 474 (17), 3027–3043.
Zak, O., O'Reilly, T., 1991. Animal models in the evaluation of antimicrobial agents. Antimicrob. Agents Chemother. 35 (8), 1527.
Zhao, C., Lv, X., Fu, J., He, C., Hua, H., Yan, Z., 2016. In vitro inhibitory activity of probiotic products against oral Candida species. J. Appl. Microbiol. 121 (1), 254–262. https://doi.org/10.1111/jam.13138.
Zuo, T., Ng, S.C., 2018. The gut microbiota in the pathogenesis and therapeutics of inflammatory bowel disease. Front. Microbiol. 9, 2247. https://doi.org/10.3389/fmicb.2018.02247.