Yeasts are able to inhibit growth of disease-associated fungi

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Research article

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Abstract

Background Fungal sepsis is often caused by non-albicans Candida or other species. These disease-associated species have strong virulence and often show resistance to the commonly used antifungal treatments. Therefore, finding new inhibitory agents nowadays is increasingly urgent.

Results Our screening revealed that although the pathogenic fungi were much more tolerant to yeast-produced bioactive agents than the non-disease-associated yeasts, growth of Kodamaea ohmeri and Candida tropicalis could be inhibited by Metschnikowia andauensis , while Cryptococcus albidus can be controlled by Pichia anomala and Candida tropicalis. The size of the inhibitory zone formed by yeasts was depended on media, pH and temperature. However, extensive studies were carried out, we failed to find inhibitory yeast against Pichia kudriavzevii, suggesting that it must have high natural resistance.

Conclusions Certain yeast species can contribute to the future solutions of problems caused by fungal resistance and can be good candidates for finding new bioactive agents which inhibit growth of disease-associated fungi.

Background

Fungaemia is associated with substantial morbidity and mortality of immuno-compromised persons. Studies have demonstrated that fungal sepsis can quite often be caused by non- *albicans Candida* species. *Pichia kudriavzevii* (is the teleomorph of the *Candida krusei*) was isolated from neonates and hospitalized patients [1, 2, reviewed in 3]. It is supposed to be the fifth most common cause of candidemia. *Kodamaea ohmeri* cells (is the teleomorph of *Candida guillermondii*) were isolated from infant and neonate or wound lesions and blood in several cases [reviewed in 3 and 4, 5, 6]. *Candida tropicalis* is one of the most common colonizer in tropical countries. Its infections involve gastrointestinal invasions or arthritis [reviewed in 7], while *Cryptococcus albidus* was isolated from transplant recipient and lesion [8, 9].

Successful infection of the mentioned above species can be in connection with their dimorphisms (ability to morphological switch), polymorphisms of their virulence-related genes and possibly with their resistance to the commonly used antifungal agents [5, 10, 11, 12, 13, 14].

Because of these problems, we wanted to investigate whether cell division of the disease-associated species mentioned above can be inhibited by bioactive agents produced by yeasts or not. Well-known antagonistic species and species not studied for biological control were equally tested. Our screening revealed the species that were able to inhibit cell division of infectious fungi and shed light on that size of the inhibitory zones produced by the yeasts, strongly depended on media, pH and temperature. Our data suggested that *Pichia kudriavzevii* must have strong inherited resistance to the yeast-produced antifungal agents.

Results

Metschnikowia andauensis, Pichia anomala, Candida tropicalis were able to inhibit growth of disease-associated fungi

In order to find yeast species which are able to inhibit cell division of the disease-associated species, such as *Pichia kudriavzevii, Kodamaea ohmeri, Candida tropicalis, Cryptococcus albidus* [1, 2, 3, 4, 5, 6, 7, 8], several yeasts were investigated. The test yeast strains were divided into two groups. Species with known biocontrol capacity, such as *Pichia anomala*, *Metschnikowia* species, *Saccharomyces cerevisiae* belonged to the first group [10, 17, 18, 19, 20, 21 and in review 22]. Members of the second test group (*Candida stigmatis*, *Hanseniaspora thailandica*, *Candida ethanolica*, *Pichia dorogensis*, *Cryptococcus flavescens*, *Candida verbasci, Wickerhamomyces orientalis*) were randomly selected from those yeasts whose biocontrol capacity were not earlier investigated.

As the Table 1 shows, growth of *Kodamaea ohmeri* (11-466) (Fig.1a) and *Candida tropicalis* (11-471) could be inhibited by *M. andauensis* cells (11-1120), while *Cryptococcus albidus* (2-1365) was controlled by *P. anomala* (11-502) and *C. tropicalis* (11-476). Other test species were not able to form inhibitory zone on the lawns of disease-associated species, in turn they were effective in the case of non-disease-associated yeast lawns, which were used as control (Table 1). Among the non-disease-related species, the *Saccharomycopsis crataegensis* (11-463) and *Wickerhamomyces orientalis* (11-461) cells were especially sensitive, because almost all test strains were able to inhibit their growth (Table 1). Interestingly, in some cases, growth stimulation around of the lawn (indicated with S in the Table 1, Fig.1b) or co-occurence of inhibitory- and stimulation zones could also be observed (indicated with I-S in the Table 1, Fig.1c).

Pichia kudriavzevii was highly resistant

Our screening suggested that *Pichia kudriavzevii* (11-462, 11-460) can have strong resistance against yeasts (Table 1). To learn whether it is true or not, further test strains belonging to different species and originated from different regions of the World were investigated on the *Pichia kudriavzevii* (11-460, 11-462) lawns. Our data confirmed the strong resistance of *Pichia kudriavzevii* (Table 2), since altogether 50 strains belonging 35 species were not able to inhibit its growth on complete and minimal media (Table 2). In contrast, *Saccharomycopsis crataegensis* cells (11-463) (used as control) could be inhibited by several yeast species (Table 2).

Size of inhibitory zone can strongly depend on media, pH and temperature

Our earlier data suggested that medium and culture conditions can have strong impact on biocontrol activity (see *Saccharomycopsis crataegensis*-Table 2). Thus, we repeated our experiments with one of the disease-associated species (*Cryptococcus albidus*) (2-1365) applying minimal (EMMA) and complete (YPA) media, different pH and temperature and using further test strains. Our data confirmed that culture conditions can strongly influence antagonistic effect of the test strains (Table 3). Consequently, modifying of the culture conditions could lead to finding further antagonistic species, such as e.g. *Candida insectorum* (11-1193) against *Cryptococcus albidus* (Table 3).

Discussion

Non-albicans *Candida* or other species including *Pichia kudriavzevii*, *Kodamaea ohmeri Candida tropicalis* or *Cryptococcus albidus* have been more frequently isolated from hospitalized patients [1, 2, 3, 4, 7, 9]. These species seem to be very virulent and often show resistance to the commonly used antifungal treatments [5, 7, 13, 14]. Thus, consequences of these fungal infections can be very serious, especially in children, neonates or immune-compromised patients. Accordingly, finding new inhibitory agents is increasingly urgent.

In order to identify yeast species which can have inhibitory effect against disease-associated fungi, screening of yeasts on *Pichia kudriavzevii, Kodamaea ohmeri, Candida tropicalis, Cryptococcus albidus* lawns were carried out. Our data showed that growth of *Kodamaea ohmeri* and *Candida tropicalis* could be inhibited by *Metschnikowia andauensis*, while *Cryptococcus albidus* can be controlled by *Pichia anomala* and *Candida tropicalis* (Table 1, Fig.1a). It means that bioactive agents of these inhibitory test strains well worth examining and yeasts can be attractive possibilities in the future solution of fungal resistance problems. Although, certain enzymes and proteins produced by these yeasts are partly known (11, 17, 23, 24, 25), we do not know exactly, which inhibitory agent was effective against the disease-associated strains mentioned above. To identify them precisely, further studies are required. Our tests shed also light that pathogenic fungi are much more tolerant to bioactive agents than the non-disease-associated yeast, such as e.g. *Saccharomycopsis crataegensis* and *Wickerhamomyces orientalis* (Table 1). The antagonistic effects were often dependent on media, pH and temperature (Table 3), similarly to others observations obtained in different species [26, 27, 28, 29]. The influencing factors could be species specific and their identification can lead to finding new inhibitory yeasts. Consequently, tests carried out under different culture conditions were very useful, since thereby further inhibitory yeasts (*Pichia dorogensis, Wickerhamomyces orientalis, Trichosporon asahii, Sporidiobolus ruineniae, Candida insectorum*) could be identified against *Cryptococcus albidus* (Table 3). In contrast, application of minimal and complex media and 50 different test strains (belonging to 35 species) did not lead to success in the case of *Pichia kudriavzevii*, because we failed to find inhibitory yeast against it (Table 2). Causes of its high resistance are not known and require further studies. We suppose that it can be an inherited species-specific feature of *Pichia kudriavzevii*, because our strains (11-460, 11-462) were isolated from nature and did not meet earlier with antifungal medicaments. Its high tolerance is in good agreement with multidrug resistance of the clinical isolates [2].

Our experiments shed also light on complexity of the action of bioactive agents, since growth stimulation was noticed in certain lawns (Fig.1 b) (Tables 1, 3), similarly to the previous experiences [28, 30]. Co-appearance of inhibitory- and stimulation zones was more interesting and unexpected (Fig.1 c). The latter phenomenon suggests a sophisticated mechanism of action and can indicate that effect of the bioactive agent produced by *M. andauensis* might be concentration dependent.

Conclusion

Taken together, this study demonstrates that yeasts can be good candidates for finding new bioactive agents which can inhibit growth of disease-associated fungi. These bioactive agents can contribute to the future solutions of problems of fungal resistance.

Materials And Methods

Strains

Strains used in this study were purchased from collections (*Metschnikowia andauensis* (11-1120) HA 1657, *Metschnikowia pulcherrima* 11-11 CBS 610, *Metschnikowia pulcherrima* 11-578 CBS 5833) or collected by Prof. Sipiczki from different regions of the World (Table 2).

Taxonomic position

PCR and sequencing methods were used for identification of the strains. Taxonomic position of the yeast species were identified by analysis of D1/D2 domain of 26S rDNA [15] (Table 2).

Culture media

Yeast strains were grown in YPL (1% yeast extract, 2% glucose, 2% peptone), YPA (YPL+2.5% agar) and EMMA [16].

Spot assay for growth inhibition

Cells of the overnight culture (YPL incubated at 28^{o}C) were harvested and cell suspension was prepared in sterile water (final cell density was OD_{595}=1). EMMA minimal and YPA complete media were flooded with 1mL of the cell suspension. After drying of the cell suspension in sterile box (lawn), yeast strains to be tested for antagonistic capacity (test-strain) were streaked or dropped (10 ul of cell suspension, OD_{595}=1) onto the surface of agar plates and were incubated at the indicated temperature. Appearances of inhibitory zones were investigated after 3-10 days. The results are coming from three separate experiments.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Data of this study are included in this published article.

Competing of interest

The authors declare no conflict of interest.

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Tables

Table 1. Yeast species are able to inhibit growth of disease-associated fungi.

	Test-strains	**Lawn** **diseases-associated species**				**Lawn** **non-disease-related species**		
Collection number	**Species** **with known biocontrol capacity**	11-462 *Pichia kudriavzevii/***	11-466 *Kodamaea ohmeri*	11-471 *Candida tropicalis*	2-1365 *Cryptococcus albidus*	11-465 *Candida stigmatis*	11-463 *Saccharomycopsis crataegensis*	11-467 *Starmere melipon*
11-460	*Pichia kudriavzevii*	-	-	-	-	-	+	-
11-502	*Pichia anomala*	-	-	-	+	+	+	-
11-481	*Saccharomyces cerevisiae*	-	-	-	S	-	+	nd
11-476	*Candida tropicalis*	-	-	-	+	-	+	-
11-505	*Pichia guilliermondii*	-	-	-	-	-	+	+
11-1120	*Metschnikowia andauensis*	-	+	+	-	+	I-S	+
11-578	*Metschnikowia pulcherrima*	-	-	-	S	+	S	+
11-11	*Metschnikowia pulcherrima*	-	-	-	S	+	S	-
	species with no known biocontrol capacity							
11-465	*Candida stigmatis*	-	-	-	-	-	+	-
11-472	*Hanseniaspora thailandica*	-	-	-	S	-	+	-
11-473	*Candida ethanolica*	-	-	-	-	-	+	-
11-486	*Pichia dorogensis*	-	-	-	-	-	+	-
11-489	*Cryptococcus flavescens*	-	-	-	-	-	+	-
11-1055	*Candida verbasci*	-	-	-	-	-	+	+
11-461	*Wickerhamomyces orientalis*	-	-	-	-	S	-	+

-:no inhibitory zone on EMMA (pH7) at room temperature

+: appearance of inhibitory zone on EMMA (pH7) at room temperature

S: growth stimulation

I-S: co-occurence of inhibitory- and stimulation zone

nd: not determined

**11-460 strain used as lawn gave the same results

Table 2 *Pichia. kudriavzevii* has strong resistance against yeast-produced bioactive agents.

Petri dishes were generally incubated at room temperature (RT)
+: there was inhibitory zone
-: no inhibitory zone
1:11-462 *Pichia kudriavzevii* strain and complete media gave similar result
2:11-460 and 11-462 were isolated from Sri Lanka, Colombo
** This species has not yet been identified. Analysis of the D1/D2 domain of its 26S ribosomal DNA suggests close relation with *Pichia minuta*. GenBank Accession number: JX515967.1

Table 3 Composition of the media, pH and temperature can influence inhibition of *Cryptococcus albidus*

- : no inhibitory zone
+: appearance of the inhibitory zone
nd : not determined,
S: stimulation
I-S: co-occurence of inhibitory- and stimulation zone

Figures

Figure 1

Yeast-produced bioactive agents can cause not only inhibition, but growth stimulation. (a) Inhibition - lawn: Kodamae ohmeri (11-466), test strain: Metschnikowia andauensis (11-1120) (C. tropicalis lawn gave similar result). (b) Growth stimulation - lawn: Candida diversa (11-470), test strain: Candida tropicalis (11-476). Co-occurence of inhibitory and stimulation zones could also be observed (c) lawn: Saccharomycopsis crataegensis (11-463), test strain: Metschnikowia andauensis (11-1120). EMMA media pH6.5 were incubated at room temperature and photographed after 5 days. White arrows show the clear inhibitory zone, where cells of the lawn could not divide.

Collection number	Test-strain	Test-strain	Lawn 11-460 [1,2] *Pichia kudriavzevii*	Lawn 11-463 *Saccharomycopsis crataegensis*
	Species	Origin	Growth inhibition	
			Media	
			EMMA	EMMA/YPA
11-483	New species similar to *Pichia minuta* **	Borneo, Brunei	-	-
11-503	*Pichia anomala*	Laos, Luang Prabang	-	-/+
11-520	*Pichia anomala*	Laos, Vientiane	-	-/+
11-522	*Pichia anomala*	Laos, Vientiane	-	-/+
11-485	*Pichia bruneiensis*	Borneo, Brunei	-	-/+
11-480	*Pichia manshurica*	Philippines, Manila	-	+/-
11-461	*Wickerhamomyces orientalis*	Sri Lanka, Galle	-	-
11-496	*Saccharomycopsis crataegensis*	Philippines, Manila	-	-
11-464	*Metschnikowia koreensis*	India, Hyderabad	-	-
11-482	*Metschnikowia koreensis*	Borneo, Brunei	-	-/+
11-524	*Metschnikowia laotica*	Laos, Luang Prabang	-	-/+
11-1062	*Metschnikowia pulcherrima*	Georgia, Tbilisi	-	-/+
11-523	*Candida glabrata*	Laos, Vientiane	-	+/-
11-484	*Candida boidinii*	Borneo, Brunei	-	-
11-471	*Candida tropicalis*	Philippines, Caticlan	-	-
11-521	*Candida tropicalis*	Laos, Vientiane	-	-/+
11-470	*Candida diversa*	Borneo, Brunei	-	+/-
11-477	*Candida californica*	Borneo, Brunei	-	-
11-478	*Candida californica*	Borneo, Brunei	-	-
11-473	*Candida ethanolica*	Borneo, Brunei	-	-
11-469	*Candida citri*	Borneo, Brunei	-	-/+
11-488	*Candida pseudointermedia*	Borneo, Brunei	-	-/+
11-479	*Candida zemplinina*	Philippines, Manila	-	-
11-487	*Candida borneonana*	Borneo, Brunei	-	-
11-504	*Candida intermedia*	Laos, Luang Prabang	-	-/+
11-506	*Candida jaroonii*	Laos, Luang Prabang	-	-/+
11-512	*Candida jaroonii*	Laos, Luang Prabang	-	-/+
11-514	*Candida jaroonii*	Laos, Luang Prabang	-	-/+
11-507	*Candida suratensis*	Laos, Luang Prabang	-	-/+
11-509	*Candida suratensis*	Laos, Luang Prabang	-	-/+
11-510	*Candida suratensis*	Laos, Luang Prabang	-	-/+
11-513	*Candida butyri*	Laos, Luang Prabang	-	-
11-517	*Candida sergipensis*	Laos, Vientiane	-	+/-
11-519	*Candida parapsilosis*	Laos, Vientiane	-	-
11-466	*Kodamaea ohmeri*	India, Hyderabad	-	-/+
11-490	*Kodamaea ohmeri*	Philippines, Manila	-	-/+
11-500	*Kodamaea ohmeri*	Philippines, Manila	-	-/+
11-467	*Starmerella meliponinorum*	India Hyderabad	-	+
11-1071	*Starmerella caucasica*	Azerbaijan, Baku	-	+
11-474	*Torulaspora delbrueckii*	Borneo, Brunei	-	+/-
11-475	*Issatchenkia terricola*	Borneo, Brunei	-	-
11-491	*Hanseniaspora thailandica*	Philippines, Manila	-	-
11-495	*Hanseniaspora thailandica*	Philippines, Manila	-	-
11-499	*Hanseniaspora thailandica*	Philippines, Manila	-	-
11-494	*Hanseniaspora uvarum*	Philippines, Manila	-	-
11-501	*Aureobasidium pullulans*	Philippines, Manila	-	-/+
11-511	*Metahyphopichia laotica*	Laos, Luang Prabang	-	-
11-516	*Metahyphopichia laotica*	Laos, Vientiane	-	-/+
11-518	*Cryptococcus heveanensis*	Laos, Vientiane	-	-
11-489	*Cryptococcus flavescens*	Philippines, Banaue	-	+

Test-strains		Lawn: 2-1365 *Cryptococcus albidus*							
Collection number	Species	pH=5 YPA 24ºC	pH=6.5 YPA 24ºC	pH=5 EMMA 24ºC	pH=6.5 EMMA 24ºC	pH=5 YPA 30ºC	pH=6.5 YPA 30ºC	pH=5 EMMA 30ºC	pH=6.5 EMMA 30ºC
11-460	*Pichia kudriavzevii*	-	-	-	-	-	-	+	-
11-1146	*Pichia kudriavzevii*	-	-	-	-	-	-	+	-
11-502	*Pichia anomala*	+	+	-	+	+	+	-	+
11-481	*Saccharomyces cerevisiae*	-	-	-	S	-	-	+	-
11-476	*Candida tropicalis*	+	+	-	+	+	+	-	+
11-505	*Pichia guilliermondi*	-	-	-	-	-	-	-	-
11-1120	*Metschnikowia andauensis*	-	-	I-S	-	+	-	+	-
11-578	*Metschnikowia pulcherrima*	-	-	S	-	-	-	S	-
11-11	*Metschnikowia pulcherrima*	-	-	S	S		-	S	-
11-465	*Candida stigmatis*	-	-	-	-	-	-	-	-
11-472	*Hanseniaspora thailandica*	-	-	-	S	-	-	-	-
11-473	*Candida ethanolica*	-	-	-	-	-	-	-	-
11-486	*Pichia dorogensis*	+	-	+	-	+	-	+	-
11-489	*Cryptococcus flavescens*	-	-	-	-	-	-	-	-
11-1055	*Candida verbasci*	-	-	-	-	-	-	-	-
11-461	*Wickerhamomyces orientalis*	+	+	-	-	-	-	-	-
11-523	*Candida glabrata*	-	-	-	-	-	-	-	-
11-1127	*Trichosporon asahii*	-	-	+	+	-	-	+	+
11-1135	*Pichia kluyveri*	-	-	-	-	-	-	-	-
11-1185	*Sporidiobolus ruineniae*	+	+	-	-	+	+	-	S
11-1193	*Candida insectorum*	+	+	+	+	+	+	+	+
2-1366	*Candida magnifica*	-	-	-	-	-	-	-	-