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Humulus lupulus L. - Yeasts found on wild-growing hop plants and preliminary insights in their brewing characteristics

This study focuses on the microfungal community found on hops, and because little data has been available until now, also seeks to increase the body of knowledge on these microfungal communities. Additional benefits may also be gained for brewers who use fresh hops in dry hopping and for beer styles that extensively rely on the technique of dry hopping by providing brewers with insights into which yeast species can enter the beer through hop cones, whether desired or undesired. In this context, the following considerations also come into play: Industrial production of beer requires reproducibility and consistent quality. Wild yeast species are usually undesirable and are considered contaminants. Conventional brewing uses only a few brewer's yeast strains that have been selectively bred over decades. Despite this, the so-called craft beer revolution has led to the spread of spontaneously fermented beers, for which the natural yeast diversity plays a crucial role. For brewers of such beers, the regional microbiome represents an invaluable resource. The biodiversity of the yeast microbiome that naturally occurs on brewing materials, such as hop cones, as well as its fermentation properties, can provide a competitive advantage. The identified yeast and microfungi species which were found in this study on wild hop plants were: *Rhodotorula mucilaginosa*, *Papiliotrema flavescens*, *Moesziomyces aphidis*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Fusarium sporotrichioides*, *Meira spp.*, *Wickerhamomyces spp.* and *Starmerella spp.*

Descriptors: wild yeast species; hop microbiology; hop-associated micromycetes; microbiology of dry hopping; co-fermentation

1 Introduction

In biodynamic wine making, naturally occurring yeast species are part of the natural microbiome of the vines but also significantly influence and control the microbiome of wine cellars and are a highly valued means of production with unique sensory characteristics. Regarding this, Guzzon et al. (2011) identified 12 different yeast species in the process of biodynamic wine making, that contribute to the final product. Of these, *Saccharomyces cerevisiae* is a species only dominant in the wine cellar and not the most prevalent one in the vineyard or on the grapes [1]. Hence, these wild yeast species, due to their peculiar and region-related metabolome may constitute a unique selling proposition to the winemaker, equal to a microbiological certificate of origin [1–5]. According to the production of beer, at a first glance this principle seems only applicable to spontaneously fermented beers like Belgian Lambic, Berliner Weisse or modern reinterpretations of these beer styles such as various wild ales [6, 7]. For example, certain non-*Saccharomycetes* species from the genera *Kloeckera*, *Candida*, *Cryptococcus*, *Torulopsis*, *Pichia* and *Brettanomyces* are characteristic of specific regions of the valley of the Senne river and, in their interaction together with a tremendous variety of bacteria, significantly shape the aroma profile of the spontaneously fermented Lambic beers brewed there [8]. The regional yeast microbiome is therefore of immense importance not only for wines produced biodynamically. For spontaneously fermented beers, this regional yeast microbiome is thus equally essential. By contrast, it is clearly undesirable in conventionally produced beers. However, the establishment of new and unusual beers with high consumer acceptance, such as New England India Pale Ale (NEIPA) has also led to an increased interest in new yeast species with unique aroma profiles for brewing applications in recent years. The ability of the yeast for biotransformation, the metabolisation, transformation and production of unique aroma compounds, is a particularly sought-after trait in this context [9]. As a consequence, this has also led to an intensified screening for new yeast species [10–12]. The screening for new yeast species is even more relevant for a new generation of non-alcoholic beers, which are often produced by using maltose-negative yeasts with

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Table 1 **Description and GPS coordinates of the sites in southern Styria where cones of wild *H. lupulus* were collected**

Short Area Description	Description of Sample Sites	GPS coordinates
Urban Area 1	Overgrown back yard	46°42'46.7" N 15°46'26.7" E
Urban Area 2	Fence next to a railroad, a grain silo and to a weighbridge for trucks	46°42'39.4" N 15°46'19.1" E
Forest 1	Forest next to the riverside of the Mur river	46°42'51.2" N 15°47'45.4" E
Forest 2	Forest next to the riverside, downstream along the Mur river	46°42'59.9" N 15°48'12.8" E
Vineyard	Area of a biodynamic vineyard	46°46'59.5" N 15°44'55.5" E

low fermentation efficiency and low alcohol tolerance [13–15]. In the case of NEIPAs, another element comes into play: NEIPAs are brewed with large amounts of hops, which are, unlike in the brewing of other beer styles, not added during the wort boiling process. The so-called wort is an aqueous solution of sugars, proteins, amino acids and other compounds. This solution then is boiled to adjust the sugar concentration, to reduce the microbial load and to precipitate excess amounts of protein. During this boiling process, hop is added to isomerise the otherwise insoluble phenolic hop compounds, so-called α-acids, contribute to the bitter taste of beer, and which also have bacteriostatic effects [16]. The boiling of the hops inactivates potential beer-spoiling microorganisms on the plant material, including wild yeasts. After the wort and the contained hops are boiled, the wort is cooled and the brewing yeast then is added to start the ethanolic fermentation [17]. In certain beer styles, like India Pale Ale, and even more in New England India Pale Ales, hops are not only boiled during the production of the wort, but additionally added to the fermenting beer, meaning they do not undergo any thermal treatment beyond the drying of the hop cones after the harvest. In certain cases, even fresh hops are used for this purpose, where the cones do not undergo a thermal treatment at all [18]. Both of these brewing applications present significant microbiological and biochemical challenges in the production of these beers [19, 20]. The technique of adding hops to the fermenting young beer is known as dry hopping and can cause the so-called hop creep, which is attributed to dextrin hydrolysing enzymes contained in the hops. These biochemical effects have been studied comprehensively, whereas the microbial load of the hops and the composition of the microorganisms inhabiting hop cones have gained far less attention [20, 21]. Andrew et al. (2019) identified a variety of species of bacteria on the Inflorescences of *Humulus lupulus* L., with *Proteobacteria* representing the dominating taxonomic phylum, and a high prevalence of *Pseudomonas* and *Sphingomonas*. Cottrell (2023) investigated the microbial load of 6 commercially used hop varieties, namely Azacca®, Cascade, Centennial, Citra®, Galaxy® and Mosaic®, and was able to identify a variety of bacteria and fungi on these hop varieties, which are widely used for dry hopping, among them the fungi genera *Alternaria* and *Penicillium* and the Gram-negative bacterial genus *Klebsiella* [22]. Guinard et al. (1990) also report the presence of the genera *Saccharomyces*, *Candida*, and *Cryptococcus* on hop cultivars including Cascade, Chinook and Willamette [23]. However, both studies investigated hop pellets from domesticated cultivars, but there is little data on yeast or microfungi species populating wild hops [21]. This is particularly interesting because exactly those sought-after yeast species which are capable of biotransformation of hop compounds may probably occur on hop plants. It is worth mentioning in this context that *Humulus lupulus* L. is a dioecious plant species and that only the cones of the female plants are used in brewing. In this work, hop cones from female wild hop plants from five different sites were collected. Yeasts and other microfungi species were isolated on Yeast Extract Glucose Chloramphenicol Agar (YGC), and the colonies were sent to the Research Center Weihenstephan for Brewing and Food Quality (85354 Freising, Germany) for identification and were partially also identified at the University of Applied Sciences FH JOANEUM, Graz. The following species were identified: *Rhodotorula mucilaginosa*, *Papiliotrema flavescens*, *Moesziomyces aphidis*, *Debaryomyces hansenii*, *Hanseniaspora uvarum*, *Fusarium sporotrichioides*. The genera *Starmerella* (order: Saccharomycetales, family: *Incertae sedis*), *Meira* (Brachybasidiaceae) and *Wickerhamomyces* (Wickerhamomycetaceae) were also isolated from three different sampling sites. In addition, two Gram-negative bacteria species growing on YGC were also identified: *Pseudomonas corrugata* and *Pseudomonas extremorientalis*.

Table 2 **Weather data set for 2023 for the municipality of Bad Radkersburg (Austria), the area and the year, when the hop cones for the investigations were harvested, according to the Office of the Styrian Government [24]**

Month	Mean of Temperatures in °C	Air-temperature per month in °C		Mean of precipitation in mm/h	Snow depth in cm
		Highest	Lowest		
January	3.0	15.4	-4.2	32	None
February	2.7	19.6	-8.5	37	
March	7.4	21.9	-4.1	51	
April	9.6	22.9	-2.6	53	
May	15.2	27.3	5.3	76	
June	20.1	33	11.2	107	
July	22.0	33.4	10.2	100	
August	21	34.2	10.5	101	
September (month of sample collection)	18.6	30.1	10.6	89	
October	14.2	28.1	0.7	64	
November	5.4	17.9	-4.2	62	
December	1.6	15.7	-7.2	54	

2 Material and methods

2.1 Sample sites and collection

Hop cones were sampled in September 2023 from wild hop plants growing at five different sites in southern Styria (Austria), all of them located in the political district of Southeast Styria. A short description of the sites and the GPS coordinates are given in table 1.

The samples were collected in September 2023. In the previous three months, June, July, and August, the region was affected by heavy rainfall and flooding. The weather dataset regarding the year 2023 is added in table 2. This data are according to the Office of the Styrian Government [24].

A total of nine hop cones per plant were collected in 50 ml sterile falcon tubes, using a sanitised and flamed laboratory anatomy scissor. Three cones of each plant were stored in sterile falcon tubes. Samples which were not processed immediately afterwards were stored at 4 °C in a refrigeration cell.

2.2 Isolation of yeasts

30 ml of Buffered Peptone Water (bioMérieux Austria GmbH, 1100 Vienna) was subsequently added to each sample tube under laminar flow. The tubes then were closed again and vortexed for 2 minutes. Yeast Glucose Chloramphenicol medium (YGC; derived from Carl Roth GmbH + Co. KG, 76231 Karlsruhe, Germany) was prepared according to the manufacturer's instructions as follows: 40.2 g of medium were dissolved in 1 litre of distilled water and then autoclaved for 15 minutes at 121 °C. Then the plates were poured using the pour plate method. From each sample tube, 100 µl of the suspension was pipetted on one Petri dish prepared with YGC and then spread with a Drigalski spatula. This was repeated three times for each sample tube. Afterwards, the Petri dishes were incubated for 96 hours at 30 °C in a GFL 3032 incubator (GFL Gesellschaft für Labortechnik mbH; 30938 Burgwedel, Germany). After 96 h single colonies were preselected and isolated using an inoculation loop. The preselection was based on the colony morphology and the growth rate of the colony as well as on light microscopy, according to Martins et al. (2022), Voordeckers et al. (2012) and Back and Back (2005) [25–27]. Moulds or colony forms suggesting filamentous fungi were not isolated from these plates, nor were cultures whose colony morphology and microbiological appearance under the light microscope indicated bacteria. Light microscopy was performed using an EVOS cell imaging system M5000 (Thermo Fisher Scientific Inc., 02451 Waltham, U.S.A.). Each single colony was spread on a total of 12 plates of YGC and the plates then were incubated at 30 °C for 96 h again. The following fermentation experiments were performed as follows: Six wide-neck 500 ml Erlenmeyer flasks were prepared with 300 ml Malt Extract Broth (Carl Roth GmbH + Co. KG, 76231 Karlsruhe, Germany) adjusted to 8 % extract (w/w) and to 20 international bittering units using pre-isomerised hop-extract, 6 % α-acid, derived from Hopfen und mehr GmbH (88099 Neukirch, Germany). Next, 19.2 g of medium was dissolved in 1 L of distilled water and then autoclaved for 15 minutes at 115 °C according to the manufacturer's instructions. Regarding this, it is noteworthy to mention that one international bittering unit (IBU) equals a concentration of 1 mg iso- α-acid per litre of wort or beer. This modified Malt Extract Broth was produced to mimic the properties of a diluted beer wort, which in breweries is used to prepare starter cultures of brewing yeast for the fermentation of larger volumes of beer wort. Each Erlenmeyer flask was then inoculated with the harvested colonies of three plates of the respective yeast species and then incubated for 96 h at 30 °C in an orbital shaker-incubator (GFL 3032 incubator, set to 75 rpm). These initial steps of the isolation and the incubation of the yeast species from the hop cones are illustrated in figure 1.

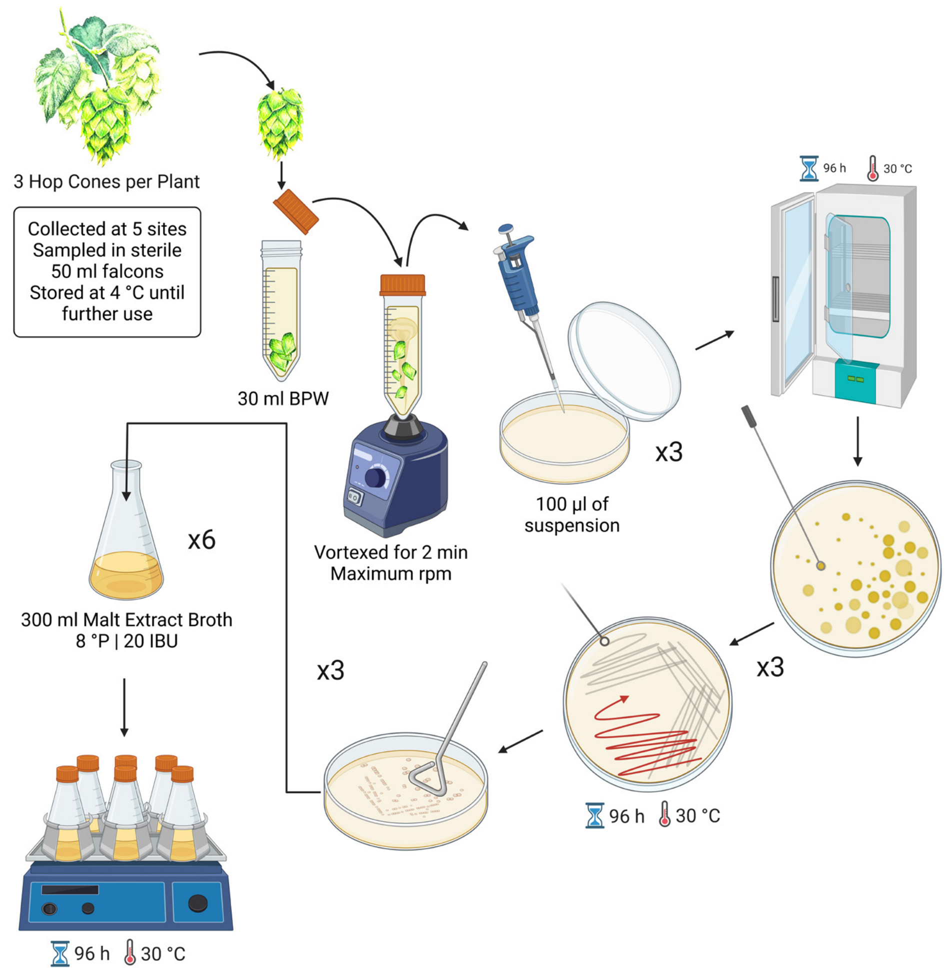

Fig. 1 **Initial steps and procedures used to isolate yeast species from cones of *Humulus lupulus* L. Created in BioRender. Rehorska, R. (2024) https://BioRender.com/o47o013**

Each inoculum was then centrifuged with an Eppendorf Centrifuge 5910 ri

(Eppendorf Vertrieb Deutschland GmbH, 50389 Wesseling-Berzdorf, Germany) at 23 °C and 3600 × g for 5 minutes in preweighed, sterile 175 ml centrifuge tubes. The supernatant was discarded, and the cell pellet was gently resuspended in sterile BPW. This process created a cell suspension with 100 mg of cell mass per milliliter of BPW to ensure that each fermentation setup could be inoculated with the same cell mass.

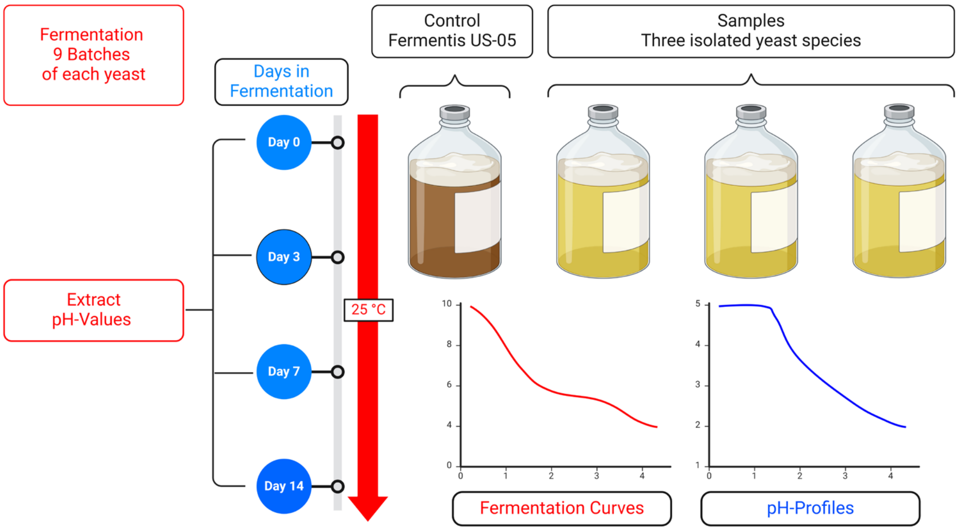

Fig. 2 **Scheme of the experimental design of the fermentation experiments with isolated yeasts from *Humulus lupulus* L. Created in BioRender. Rehorska, R. (2024) https://BioRender.com/y59u702**

2.3 Fermentation experiments

Nine autoclaved 4 L glass fermentation flasks were then prepared with 3 L of beer wort each, consisting of tap water and liquid malt extract (Weyermann® Bavarian Pilsner, Mich. Weyermann® GmbH & Co. KG, 96052 Bamberg, Germany). The so-produced wort was adjusted to 10 % extract (w/w) and to a total of 20 IBU. 20 ml of suspended yeast-slurry was drawn from the incubated Erlenmeyer flasks to inoculate each fermentation flask, using a serological pipette controller (Powerpette Plus, from VWR International, 1100 Vienna, Austria) and sterile pipet tips. The nine fermentation flasks were incubated for a total of 14 days at 25 °C in a KBF-S 720 constant climate chamber (Binder GmbH 78532 Tuttlingen, Germany). An aliquot of the prepared wort was not inoculated and used as negative control. Extract degradation, pH-values and temperature were measured over the course of the fermentation on day 0, 1, 7 and 14. The samples were drawn with a sterile pipet and degassed before the measurements. The extract degradation was measured with a DMA 35 oscillating u-tube (Anton Paar GmbH, 8054 Graz, Austria). The pH-values were measured using a WTW pH 3110 pH meter (VWR International). In total, 4 different yeast species were tested. Fermentis SafAle US-05, a widely used top fermenting brewing yeast strain of *Saccharomyces cerevisiae* was used as positive control. The experimental design for the fermentation experiments is given in figure 2.

Based on the results of the fermentation experiments, a repeated measures ANOVA (RM-ANOVA) was conducted with DATAtab [28]. This was done to evaluate whether the fermentation characteristics of the three isolated yeast strains differed significantly from those of the control strain. The data consisted of the extract measurements collected at four time points across four groups: Control, *Hanseniaspora uvarum*, *Debaryomyces hansenii*, and *Papiliotrema flavescens*. Each group included nine fermentation replicates in total. Post-hoc comparisons were performed using Bonferroni correction to identify pairwise differences between groups and to control for type I error. Differences between the mean extract levels were evaluated at a significance threshold of $p < 0.05$.

3 Results and discussion

In total, eight species of yeasts and yeast-like fungi and two species of bacteria were isolated from cones of wild female hop plants growing on five different sites. Regarding the observed colony growth on YGC, a significantly larger microbial diversity, consisting of microfungi species with filamentous growth, moulds and even of chloramphenicol-resistant bacteria such as Gram-negative *Pseudomonas* species was found on the cones of female *Humulus lupulus* plants. The isolated species, related to the respective sample sites, as well as short descriptions are given in table 3.

3.1 Description of the isolated species

The following paragraphs elaborate on the isolated species in more detail and highlight their potential for brewing applications based on literature data.

Rhodotorula mucilagionsa and *Starmerella spp.* were found on two different sample sites whereas *Hanseniaspora uvarum* at least suggests a nearby vineyard and indicates, that *H. uvarum* may also opportunistically occur on hop cones, although it could also have been due to contamination by aerosols or insects from the nearby winery [29, 30]. *R. mucilaginosa* is a very versatile and common yeast, colonies show rapid growth on a wide range of media and are of a distinct red colour due to the production of carotinoids [31]. Hence, this trait is common to many species of the genus *Rhodotorula*, their occurrence can be assumed early in incubation. *Hanseniaspora uvarum*, *Debaryomyces hansenii* and certain species of the genus *Starmerella* are mentioned frequently in context with ethanolic fermentation and wine making [29, 32–35]. *Rhodotorula mucilaginosa*, *Papiliotrema flavescens* and *Moesziomyces aphidis* are microfungi which may also show filamentous growth, and which are considered potential human pathogens, causing opportunistic infections [36–38].

Meira spp. is a basidiomycetous, yeast-like mite-associated microfungi and a promising fungal biocontrol agent against mites [39, 40]. *Fusarium sporotrichioides* (Nectriaceae) is a mycotoxin-producing plant pathogen, which severely affects seedlings of cereals[41]. Although it was not the aim of the conducted investigation, two bacterial species were also identified by circumstance: *Pseudomonas*

Table 3 **Identified yeast and bacteria species isolated from the collected cones of *H. lupulus* plants, growing on the sampled sites in Southeast Styria (Austria), providing short descriptions of the isolated species and their potential for brewing applications, based on the results of the fermentation experiments and according to the literature review**

Sample Site & Description	Isolated Species	Short Description	Potential for Brewing Applications
Urban Area 1 Overgrown back yard	*Rhodotorula mucilagionsa*	Basidiomycetous yeast, ubiquitous in soils and on plants	uncertain
	Fusarium sporotrichioides	Mycotoxin-producing plant pathogen associated with cereals	negative
	Pseudomonas corrugata	Gram-negative bacterial plant pathogen, found on tomato plants	negative
Urban Area 2 Vicinity of train station and grain silo	*Rhodotorula mucilagionsa*	see above	See above
	Papiliotrema flavescens	Basidiomycetous yeast, former Cryptococcus flavescens, associated with grapes	negative
	Pseudomonas extremorientalis	Gram-negative bacterium, salt-tolerant	negative
	Meira spp.	Basidiomycetous, yeast-like mite-associated microfungi	uncertain
Forest 1 Forest next to the riverside of the Mur river	*Starmerella spp.*	Order: Saccharomycetales, associated with various plant families	promising
	Debaryomyces hansenii	Family: Saccharomycetaceae; xero-, osmo- and halotolerant yeast	uncertain
	Wickerhamomyces spp.	Family: Wickerhamomycetaceae; ferments wine and rice-based beverages	promising
Forest 2 Forest next to the riverside, downstream along the Mur river	*Starmerella spp.*	see above	See above
Vineyard Area of a biodynamic vineyard	*Hanseniaspora uvarum*	Family: Saccharomycodaceae, found on grapes, in musts and wine	promising
	Moesziomyces aphidis	Family: Ustilaginaceae, ubiquitous species, associated with plants and aphids	negative

corrugate and *Pseudomonas extremorientalis*.

Pseudomonas corrugata is a plant pathogen known to infect tomato plants and cause tomato pith necrosis, whereas *Pseudomonas extremorientalis*, a less well studied species, is reported to show salt tolerance. This bacterial species was isolated for the first time by Ivanova et al. (2002) from a drinking water reservoir near Vladivostok [42–44]. Table 2 lists the sampled sites and the yeasts and bacteria which have been identified on hop plants growing there.

Rhodotorula mucilaginosa is a basidiomycetous yeast with the ability to form biofilms. The species is considered ubiquitous in soils and on plants but can also be found in waste waters. It can be a commensal yeast of the human microbiome, where it is considered an opportunistic pathogen [36, 45]. Kurtzman et al. (2010) list it as a yeast species that is not capable of the fermentation of the sugars glucose, galactose, sucrose, maltose, lactose, raffinose and trehalose [46]. Hence, although this yeast may potentially be used for the fermentative production of carotenoids based on agricultural by-products, it is likely unsuitable for brewing applications due to these properties [47].

Papiliotrema flavescens is a not very well-studied yeast species. Kurtzman et al. (2010) do list a lack of data on its fermentation capabilities as well on its growth reactions and other characteristics. [46]. This species was formerly known as *Cryptococcus flavescens,* a basidiomycetous yeast, and it is interestingly also associated with wine grapes. *P. flavescens* excretes viscous exopolysaccharides which are investigated for potential biotechnological applications [48]. Aside from that, this yeast can also act as an opportunistic human pathogen and it is reported to promote the growth of plants and to induce systemic resistance [37, 49]. It was decided to test this yeast in the fermentation experiments due to a lack of data on its fermentation characteristics.

Debaryomyces hansenii is a variable species regarding its fermentation capability of various sugars according to Kurtzman et al. (2010). It does not ferment lactose, but can show variable capabilities in the fermentation of glucose, galactose, sucrose, maltose, raffinose and trehalose [46]. This makes it at least interesting to subject this species to a more in-depth investigation regarding possible brewing applications. Yeasts which are identified as positive in the fermentation of maltose and raffinose are potential candidates for the fermentation of beer wort, which contains these sugars [50]. In addition, *D. hansenii* is a xero-, osmo- and halotolerant yeast which has been thoroughly investigated for potential biotechnological applications and it is also associated with the production of a variety of foods and beverages such as cheese, sausages and wine [51, 52]. *Debaryomyces hansenii* is also among the numerous non-*Saccharomyces* yeast species that have been isolated from Belgian Lambic, where this yeast is found both in the ceilings and in the air of the cellars and attics of Lambic fermentation sites [53, 54]. Methner et al. (2019) screened different strains of *D. hansenii* for possible brewing applications, identifying phenolic off-flavour

(POF) positive and negative strains, which demonstrated fermentation characteristics ranging from sulfurous notes to exotic fruits. Regarding the results of the fermentation experiments presented in this work, it is especially noteworthy that the growth of at least two strains of *Debaryomyces hansenii* was significantly inhibited by β-acid concentrations of 200 mg/L in the yeast screening experiments performed by Methner et al. (2019) [55].

Hanseniaspora uvarum is typically associated with the yeast ecosystem found on grapes, in musts, and in wine. In wine, it is the yeast species predominantly involved in the initial phase of alcoholic fermentation [56, 57]. This species is also reported to especially attract *Drosophila melanogaster* (fruit flies) due to its production of volatile compounds. This insect species is a common vector for a variety of yeast species, which may also offer an explanation why *H. uvarum* was found on hop cones. [30]. Hop cones have recently been reported to share certain aroma compounds with wine grapes, which indicates that both plant parts can attract *Drosophila melanogaster* [58]. According to Kurtzman et al. (2010) it ferments glucose, but it does not ferment the other aforementioned sugars. [46]. *Hanseniaspora uvarum* is also investigated on its properties in co-fermentation together with other conventional yeast species such as *Saccharomyces cerevisiae* [57]. It is also reported by Matraxia et al. (2021) that this species shows strong fermentation capability in beer wort, where it even exhibits a stronger growth in presence of hops and ethanol than the used control yeast did, a top-fermenting *Saccharomyces cerevisiae* yeast strain (US-05). The authors also assume a high co-fermentation potential of *H. uvarum* and *Saccharomyces cerevisae* and emphasise the positive contribution of *H. uvarum* to beer flavour [59]. Various studies conclude the same and are mentioning the promising potential of *Hanseniaspora uvarum* for brewing applications [60]. Beers brewed in co-fermentation with *H. uvarum* were characterised by higher ester and lower alcohol concentration, and an improved aromatic perception [61]. In contrast, Methner et al. (2019) describe that beer wort fermented with *Hanseniaspora uvarum* exhibited neutral to fruity flavours, but simultaneously featured an acidic aroma, making it scarcely acceptable for commercial brewing applications. Hence, the assumption that the aroma improving properties of *H. uvarum* can be directly transferred from vinification to the fermentation of beer seem questionable [55]. These findings are not surprising, since *Hanseniaspora uvarum* was also shown to be one of the most prevalent yeasts in the initial stage of Lambic fermentation, which is, in fact, determined by the enterobacterial and the acetic acid bacterial phase. Vermote at al. (2023) found *Hanseniaspora uvarum* and *S. cerevisiae* in equal cell counts, log 6.0 (CFU/mL), in the fermenting wort of Lambic barrels during the first day of fermentation [53].

Moesziomyces aphidis belongs to the family of the Ustilaginaceae. This basidiomycetous species is found worldwide, it grows on leaf surfaces of plants, but it can also be isolated from soils and aphids [62]. Ding et al. (2020) report that it can also be a plant pathogen, causing fruit stain on wine grapes [63]. There is at least one study that mentions *M. aphidis* as a human pathogen related to the infection of a premature neonate [38].

Starmerella spp. (*incertae sedis*) belongs to the order of the Saccharomycetales and was first isolated from the infloresences of *Calystegia sepium* (Larger bindweed) and an associated sap-feeding beetle species of the genus *Conotelus* (Family Nitidulidae) and described by Rosa & Lachance (1998). In addition, conjugation and forming of asci was observed [64]. At least six novel species of this genus have been recently isolated from various plant families, among the Cactaceae, Convolvulaceae and Malvaceae [65]. Within the genus, *Starmerella bacillaris* shows promising enological properties. It is reported to perform well in co-fermentation with *Saccharomyces cerevisiae* and to possess advantageous metabolic traits. It produces high amounts of glycerol, adequate amounts of ethanol and low amounts of acetic acid [33]. There are few studies addressing potential brewing applications for *Starmerella*. However, recently Schinca et al. (2024) reported a high utilisation of maltotriose and a pronounced glycerol production, at least by *Starmerella meliponinorum*, which makes this genus interesting for brewing purposes, whereas *Starmerella bombicola* was tested successfully in the production of non-alcoholic beer by Vaštík et al. (2022) [66, 67].

Wickerhamomyces spp.: Species of this genus include various strains that exhibit strong antagonistic effects against different field and storage fungi, such as those from the genus *Fusarium* with *Wickerhamomyces* anomalus being proposed as biocontrol agent against Fusarium-infections in the malting process [68]. *Wickerhamomyces* anomalus is also mentioned in context with winemaking and co-fermentation with *Saccharomyces cerevisiae*, where certain strains are reported to improve the sensory qualities of Sauvignon wines [69]. Regarding beer and brewing *Wickerhamomyces anomalus* was found in Belgian Lambic [53, 54]. According to Methner et al. (2019), *W. anomalus* is presumably a strain-independent POF positive yeast, that shows a great variety within different strains regarding acceptable aroma profiles [55]. This species is also found in Nuruk, a traditional wheat and rice-based starter culture for Korean alcoholic beverages such as Makgeolli, a raw and unpasteurised rice wine. In fermentation *Wickerhamomyces anomalus* demonstrates an alcohol tolerance comparable to that of *Saccharomyces* [70]. Regarding possible brewing applications, Basso et al. (2016) concluded that *Wickerhamomyces subpelliculosus* may be suitable for the production of low alcohol beers, whereas *Wickerhamomyces anomalus* can be used in bioflavouring and in the brewing of low calorie beers [71].

3.2 Fermentation experiments – Extract degradation and pH-drop

The results of the fermentation experiments show that of the tested yeast species only *H. uvarum* evinced distinct fermentation activity in comparison to the used dry yeast strain of *Saccharomyces cerevisiae*, Fermentis SafAle™ US-05. *H. uvarum* achieved an apparent degree of fermentation (ADF) of 58 %, which is the same ADF achieved by the *Saccharomyces* strain US-05. It is noteworthy that this is a rather low ADF regarding US-05 yeast, which normally achieves an ADF above 75 %. Reasons for this may range from a fermentation temperature at the upper limits of this yeast to a possibly undetected damage of the vacuumised yeast packages. No fermentation at all could be observed with *D. hansenii* (ADF of 3 %) and *P. flavescens* (ADF of 1 %) In the conducted experiments, these isolated yeast strains were obviously not able to ferment typical wort sugars. The same observations were made regarding

Table 4 **Extract degradation of the batches inoculated with *S. cerevisiae*, *H. uvarum*, *D. hansenii* and *P. flavescens* over the course of 14 days of fermentation and the corresponding pH-values. Shown are means and standard deviations of nine inoculated batches of each tested yeast species (N = 9). RM ANOVA and Bonferroni post-hoc tests were performed with DATAtab [28]. Levels of significance: *p < 0.05; **p < 0.01**

Yeast		D0	D1	D3	D7	D14
S. Cerevisiae (control)	Extract % (w/w)	10.3 ± SD 0.00	9.5 ± SD 0.05	7.5 ± SD 0.15	5.5 ± SD 0.51	4.3 ± SD 0.56
	pH-value	6.02 ± SD 0.03	4.89 ± SD 0.05	4.45 ± SD 0.06	4.41 ± SD 0.08	4.33 ± SD 0.05
H. uvarum	Extract % (w/w)	10.3 ± SD 0.07	9.5 ± SD 0.08	8.1 ± SD 0.34	6.5 ± SD 0.45	4.3 ± SD 0.16
	pH-value	5.73 ± SD 0.00	4.85± SD 0.02	4.72 ± SD 0.04	4.90 ± SD 0.11	4.41 ± SD 0.01
* *D. hansenii*	Extract % (w/w)	10 ± SD 0.06	10 ± SD 0.03	10 ± SD 0.03	9.9 ± SD 0.05	9.7 ± SD 0.09
	pH-value	5.78 ± SD 0.31	5.58 ± SD 0.01	5.51 ± SD 0.02	5.22 ± SD 0.03	5.05 ± SD 0.02
** *P. flavescens*	Extract % (w/w)	10.3 ± SD 0.03	10.3 ± SD 0.08	10.3 ± SD 0.05	10.3 ± SD 0.08	10.2 ± SD 0.07
	pH-value	5.64 ± SD 0.02	5.68 ± SD 0.02	5.56 ± SD 0.01	5.47 ± SD 0.04	5.20 ± SD 0.12

the pH drop, that accompanies the ethanol fermentation by yeasts. Whereas the pH drops of the batches fermented with *H. uvarum* and S. cerevisae was distinct, the batches inoculated with *D. hansenii* and *P. flavescens* showed only minor changes in the pH-value over the course of the fermentation. Noteworthy was also an unexplained minor increase of the pH-value in day 1 of the batches inoculated with *Papiliotrema flavescens*, which still needs to be clarified. The results of the conducted experiments are presented in table 4. The temperature of the constant climate chamber was measured permanently. The mean temperature over the course of the trials was 25.1 °C.

The results of the repeated measures ANOVA, conducted with DATAtab (2024) indicated a significant effect of time on the extract levels (F = 63.9, $p < 0.001$), as well as a significant interaction between time and group (F = 7.3, $p < 0.001$). This indicates that the fermentation behavior differed among the yeast strains over time. The Bonferroni post-hoc tests revealed that there was no significant difference between the *S. cerevisiae* strain (control) and *Hanseniaspora uvarum* (p = 1). Significant differences were observed between *S. cerevisiae* and both *Debaryomyces hansenii* (p = 0.034) and *Papiliotrema flavescens* (p = 0.009). Both strains did not show fermentation characteristics comparable to *S. cerevisiae*.

4 Conclusion

The natural biodiversity of yeasts still receives comparatively little attention, even though the work of pioneers like Hutzler and Kurtzman highlights a very large and untapped potential for biotechnological, oenological, and brewing applications [12, 46]. The authors of this study are aware that it provides only very superficial and preliminary results concerning solely yeast species found on *Humulus lupulus* L. It should also be emphasised that the described species were only found on hop cones. This does not mean that they are hop-specific. The findings need to be compared with a more in-depth and statistically significant analysis of the surrounding microbiome in order to gain more clarity on this matter. However, these results at least justify a much greater interest in this research than has surprisingly been the case so far. The fact that five of the isolated yeast species detected on hop cones are also associated with wine suggests a potential link between certain volatile aroma compounds of both plants and corresponding vectors such as *Drosophila melanogaster* and certain sap-beetles [30, 64]. In addition, *Starmerella* is among the yeast communities associated with stingless bees, whereof certain species, such as *Tetragonisca angustula*, incorporate the resin of *Humulus lupulus* glands in their nests as defense against pathogens, which is also interesting regarding the abundance of *Starmerella* in honey and propolis [72, 73]. This is of great interest since hop plants harbour a wide range of various other arthropods that can act as vectors for wild yeasts: Gardiner et al. (2003) reported 133 different species [74]. The fact that only one of the tested yeasts, namely *Hanseniaspora uvarum*, was suitable for brewing applications with certain limitations, such as described by Methner et al. (2019), is disappointing at first glance, but similar to Saccharomycetes used in brewing, the specific strain undoubtedly plays a significant role in most cases here as well [55, 75]. The present study was conducted as part of research-oriented teaching in the Sustainable Food Management Bachelor's degree program at the FH JOANNEUM University of Applied Sciences in Graz. In the context of the accompanying course, only three of the isolated yeast species were investigated for their fermentative properties in brewing applications. For this reason, the decision was made to test only those yeasts that could be identified at the species level. However, the isolated *Starmerella spp.* would have also been a promising candidate for this purpose [33]. Regarding this, the isolated species will be interesting to investigate in a follow up study. Nonetheless, particularly in the field of brewing applications, several wine-associated yeast strains, such as *Metschnikowia pulcherrima*, are currently being investigated for their performance in co-fermentation with *Saccharomyces* yeasts, which can result in unique aroma profiles [76]. *Debaryomyces hansenii* did not ferment beer wort in the trials of this study, which partially contradicts the available literature [77]. *Debaryomyces hansenii*, one of the so-called extremophilic yeasts, would be particularly interesting for the alcoholic fermentation of worts with a very high extract content, possibly also in co-fermentation with suitable strains of *Saccharomyces cerevisiae* or *Saccharomyces pastorianus*, although the flavour profile of the resulting beers may not meet high acceptance [52, 55]. The fact that some of the isolated yeast species are potentially pathogenic to humans should not overshadow that only wild hop plants were sampled in this study. The use of fresh and untreated wild hops for dry hopping is very exceptional in brewing [78]. In contrast, industrial processing of cultivated hops involves thorough drying of the hop cones to a very low residual moisture content; additionally, the processed products are deep-frozen for long-term storage. This combination of methods effectively prevents contamination [79]. In summary, it can be said that even within the very limited scope of the present study, eight yeast species were detected on the cones of *Humulus lupulus* L., for which a wide range of potential applications is currently being investigated. These include not only oenological and brewing applications but also biotechnological ones, such as the synthesis of carotenoids and the degradation of plastic polymers by *Rhodotorula mucilaginosa*, as well as plant protection applications, such as those involving *Papiliotrema flavescens* [33, 47, 49, 80]. This study only sampled locations with significant anthropogenic influence. A future research question could include more pristine locations and highlight differences. The variety of found species undoubtedly warrants more in-depth and extensive research, for which the present study, only to be regarded as preliminary, provides compelling arguments. Future metagenomic analyses of the microbiomes of wild hop plants and of the immediate environment, contextualised with a biotope mapping, which represents an inventory of ecological, biological, and geographical factors, can thus significantly enhance the understanding of natural hop micro-biodiversity.

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