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Occurrence, diversity and characterization of effective soil yeast isolates from different agro climatic zones of Maharashtra

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Abstract
Isolation of soil yeast from diverse 150 soil samples collected from nine agro climatic region comprising 26 districts of Maharashtra state, India yielded total 34 soil yeast cultures from 17 districts. In Konkan region approximately 42.42% of locations harbor the soil yeast followed by Western Maharashtra region where 18.05% of locations harbor the soil yeast. Further, in Marathwada region 16.16% of locations harbors the soil yeast whereas in Vidarbha region 15.15% of locations harbors the soil yeast. On the basis of morphological, physiological and biochemical characteristics, the yeast isolates were identified as *Trichomonascus petatosporus*, *Candida labiduridarum*, *Candida shehatae*, *Eremothecium ashbyi* and *Eremothecium cymbalariae* and *Moniliella megachiliensis*. The yeast of the genus *Trichomonascus* was present in the soils of Kokan region and Vidarbha region of high rainfall zone. The yeast isolates which were found effective against the *Pythium* sp. were KTPD-10, WSWB-3, WNSS-9, WAJJ-11, WAAB-12, MLAA-1 and VCGN-5. The yeast of genus *Candida* was prevalent in the soils of marathwada and western Maharashtra region whereas the yeast *Moniliella* and *Eremothecium* genus were predominant in the soils of western Maharashtra region. These soil yeast species are reported from the soils of Maharashtra for the first time.

Keywords: Soil yeast, diversity, effective isolates, agro climatic zones and Maharashtra

Introduction
The increasing concern about the environmental safety and indiscriminate use of chemical pesticides and fungicides resulted in adverse effect on the environment, food items and public health all over the world. Thus, the regulatory agencies have reacted to public pressure and introduced comprehensive legislation to reduce the pesticide application. Therefore, biological control considers to be one of the effective modules in integrated disease management (IDM) strategies and potential alternative approach in modern agriculture. Besides antagonists' fungi and bacteria species, soil is also habitat for yeast and the soil yeast can also be antagonist to soil borne plant pathogens. Studies on various aspects of soil yeast were done [1]. The yeasts are a phylogenetically diverse group of fungi characterized by unicellular growth, spherical to ovoid, lemon, pear or cylindrical shape and reproduce asexually by forming buds. There are about 100 genera and 700 species of yeasts [2]. Their population is usually relatively low as compared to bacteria and fungi in soil. The yeast population is also affected by soil depth where they occur in soil. They are most numerous in the upper soil layers of approximately 2 to 10 cm depth [3] and play their role in maintaining biological activities and properties of soil [4]. Soil yeasts may also play a role in soil aggregate formation and maintenance of soil structure and serving as a nutrient source for bacterial and protistan predators [5]. Yeasts are common in soils of widely different texture, chemical composition, humidity and pH value at various geographical locations and under diverse climatic conditions [6]. *Cryptococcus albidus* is the most common species consistently associated with soil [7]. In recent years, some private firms have formulated yeast product as biocontrol agent for control of postharvest diseases of fruits and vegetables. Among these products Aspire TNt biofungicide is one which comprises yeast *Candida oleophila* [8]. More recently, two additional commercial products have appeared in the market i.e. yield Plus of Anchor Yeast company Cape town, South Africa comprising yeast *Cryptococcus albidus* [9] and Shemer of Agro Green company of Israel comprising yeast *Metschnikowia fructicola* [10, 11, 12] for control of postharvest decays on several fruits including pome, grapes, stone and citrus fruit.

However, yeasts have been paid little attention as biocontrol agents of soil borne fungal plant pathogens as compared to bacterial, actinomycetes, and fungal antagonists. The ability of certain taxa of yeasts to multiply rapidly, to produce antibiotics and cell wall degrading enzymes, to produce plant growth regulators indicates the potential to exploit them as biocontrol agents and plant growth promoters. Yeasts appear to be promising biocontrol agents as it produces antibiotics [13]. The control of phytopathogenic fungi by yeasts has been studied with great potential usage, against the molds that cause fruit rotting in post-harvest period, because the yeasts are good competitor for nutrient and space [14, 15, 16]. Some examples of yeast isolates used as biological control agents are *Cryptococcus infirmo-miniatus* which control decay of sweet cherry [17] and *Pichia membranefaciens* which completely inhibit *Botrytis* storage rot in kiwifruit [18]. A new ascosporic yeast *Metschnikowia fructicola* isolated from grapes in central Israel has biocontrol activity against Botrytis rot of stored grapes [10]. Therefore, the possibilities of using soil yeast as biocontrol agent for damping off pathogens in nursery was considered important and need to be explored. Hence, it was decided to study the occurrence and diversity of yeast population present in different agro climatic zones of Maharashtra.

Material and Methods

Collection of soil samples

Soil samples were collected from the various locations of different districts from diverse geographic regions of Maharashtra state and were processed for the isolation of yeast cultures. A total of 150 rhizosphere soil samples (0-15 cm depth) were collected by using GPS technique as per the soil series prescribed [19] from different physiographic regions of Maharashtra viz., Western Konkan Coast, Western Ghats (Sahyadris) and North Deccan Plateau (Upper, Lower and Metamorphic).

Isolation of yeast cultures

A specialize medium was used for yeast isolation namely malt extract agar (g/L) consisting of malt extract 20 g, mycological peptone 5 g, agar 20 g, distilled water (1000 ml) and pH=7 and Glucose yeast extract peptone agar (g/L) containing glucose 20 g, bacteriological peptone 10 g, yeast extract 5 g, agar 20 g, distilled water (1000 ml) and pH=7. Serial dilution technique (10^{-4} cfu/ml) was used for the isolation of soil yeast. The isolated yeast colonies in the Petri plates were further purified by serial dilution technique and the single colonies thus obtained were maintained as pure culture of the yeast. These yeast cultures were designated as per their location and by giving a numerical number.

In vitro testing efficacy of antagonistic activity of yeast isolates

For testing efficacy of yeast isolate (s) as biocontrol agent against most important soil borne plant pathogen causing damping off of seedlings (*Pythium* sp.) in the nurseries were used as per methodology given [20]. The potential isolates with significant strains were designated as effective isolates.

Isolation of damping off pathogen from tomato seedlings

For isolation of damping off soil borne pathogen of tomato, the tomato seedling showing damping off symptoms in the nurseries were collected. The collected samples were cleaned with the help of running tap water gently and dried on the blotter paper to remove the soil particles and other material cling to the seedling. The infected radicle portion were sterilized with 0.1% $HgCl_2$ for 2 min and were washed subsequently thrice in distilled sterile water. These sterile radicles were cut vertically into 3-4 pieces and these were kept on the sterilized PDA in Petri plates. The inoculated plates were incubated at 30 ℃ for 3 days for observation of growth of *Pythium*.

Characterization of effective yeast isolates

The methods as described [21], in "The yeast: A taxonomic study, 5th edition", were used for morphological (Fig. 2), physiological and biochemical characterization of yeast isolates. Similarly, isolation and purification of DNA from effective isolates were performed [22]. The molecular identification of predominant isolate was performed by PCR ITS-rDNA and gel electrophoresis technique [23]. The purified amplicon of the isolates was sent for sequencing to microbial identification services of Gene Ombio Technologies Pvt. Ltd., Pune (India) for yeast identification using Internal Transcribed Spacer region gene sequence for confirmation of the yeast genus and species.

Results and discussion

Total 34 soil yeast cultures were yielded from collected 150 soil samples from diverse agro climatic zones comprising 26 districts of Maharashtra. These soil samples were then subjected for the isolation of soil yeast on a specialized media i.e. malt extract agar (MEA) and Glucose yeast extract peptone agar (GYEPA). Upon isolation on this specialized media only the yeast colonies were developed. The isolated yeast colonies were round to irregular in shape with either flat or raised elevation. The colour of these colonies were white creamy to light-dark brown (Fig.1). The colonies were developed within 48 h of incubation at the temperature of 27±2 ℃. The colony shape, colour and elevation were variable among the isolates from various agro climatic regions and the specific locations. The isolated and purified yeast colonies were confirmed microscopically (Fig. 1). The results (Table 1) indicated that in South Konkan coastal zone the soils of Sindhudurg district 12 soil samples from 12 villages of 5 tahsils were collected particularly of Tahsil Devgarh (Village-Pural) and Tahsil, Sawantwadi (Village-Amba) harboured the yeast. There was no correlation between the soil depth and the presence of yeast in the soil. Soil depth as low as 4 cm and as high as 165 cm were found to harbour the yeast. Out of 12 samples collected from 12 villages of 5 tahsils of Sindhudurg district only 2 samples i.e. from Devgarh and Sawantwadi tahsil harboured the yeast. Thus, in Sindhudurg district only 16.66% soil sample harboured the yeast. Similarly, in Ratnagiri district 9 soil samples from 9 villages of 5 tahsils were collected. Out of these 9 samples 5 samples yielded the yeast population. The yeast was present in the soils of villages. Ratnagiri and village Harchil of Ratnagiri tahsil; village-Nandgaon of Chiplun tahsil; village-Kenjule of Khed tahsil and village-Ojahar Budrukh of Sangameshwar tahsil. Thus, the presence of yeast was recorded in 55.10% soil samples collected from Ratnagiri district. Further, the soil sample of village-Gunde of Shahapur tahsil; village-Nane of Wada tahsil and village Dahisar of Palghar tahsil harboured the yeast population (Table 1). Thus, 37.5% samples from Thane district harboured the yeast population. In Raigad

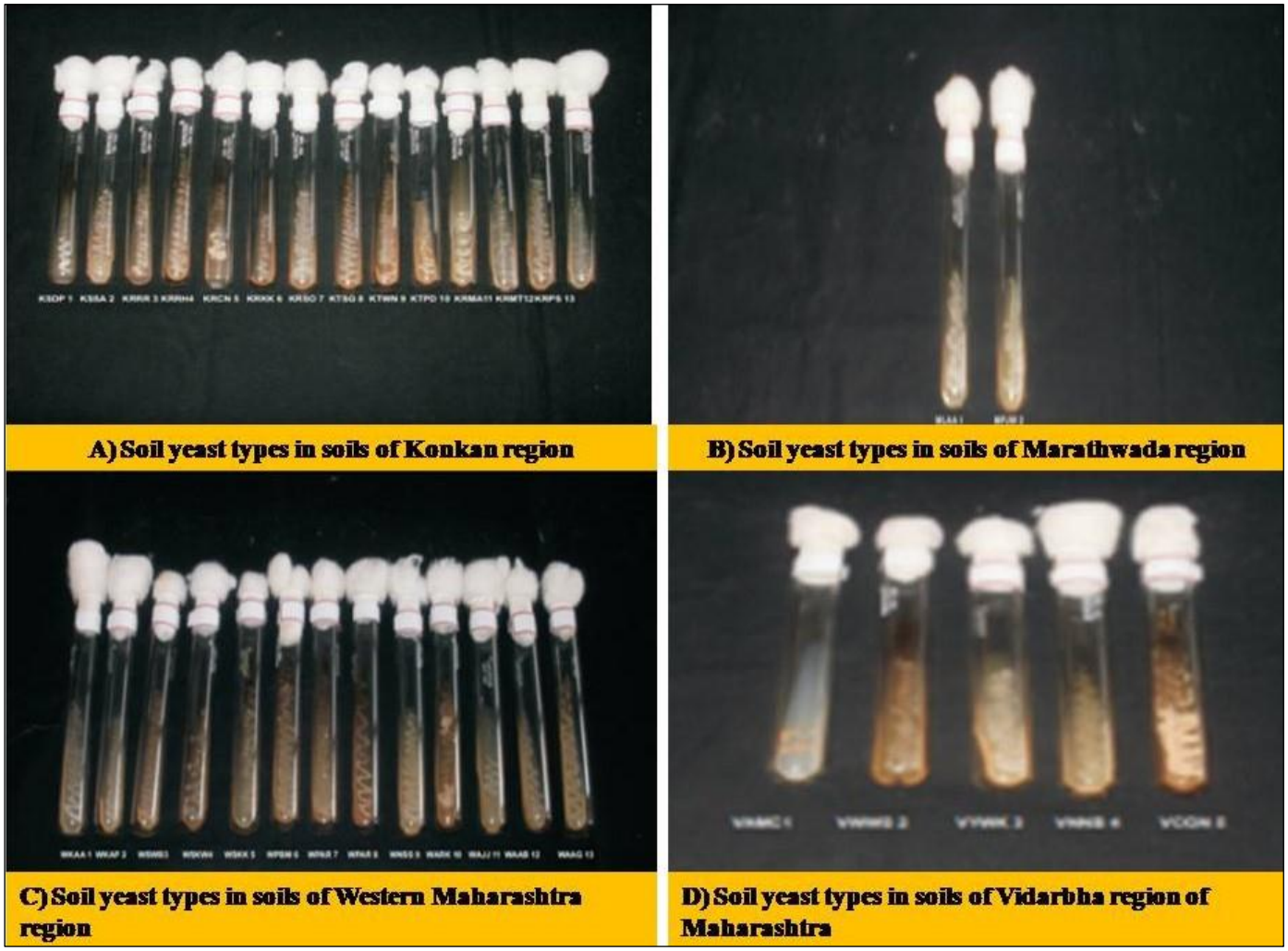
district, soil samples from 4 villages of 4 tahsils particularly Man, Mahad, Poladpur and Mangaon were used for isolation and these were found to harbour yeast population. Thus, in Raigad district all the soil samples harboured the yeast population. The soils of two villages i.e. village-Ambarde and Parpoli of Ajara tahsil yielded the yeast population out of soils of 11 villages from 6 tahsils of Kolhapur district. Hence, 18.1% soil samples from Kolhapur district harboured the yeast population. Furthermore, in Satara district the soil samples from 6 villages of 3 tahsils was analyzed for yeast population. The yeast population was present in the soils of village-Barsewadi of Wai tahsil and of village-Wathar of Koregaon tahsil. So, 33.3% soil samples in Satara district harboured the yeast population. Accordingly, in Sangli district the soil samples from 7 villages of 5 tahsils were analyzed for yeast population. The yeast population was present in the soil of village-Kawathemahankal of Kawathemahankal tahsil. Therefore, 14.28% soil samples in Sangli district yielded the yeast population. Likewise, the soils of 3 villages i.e. village-Mahakoshi of Bhor tahsil and village Rokadewadi and Ranjani of Ambegaon tahsil harboured the yeast population out of 8 villages from 4 tahsils of Pune district. Thus, 37.5% soil samples from Pune district harboured the yeast population. From Nashik district the soil samples of 15 villages of 7 tahsils were analyzed for yeast population. The yeast population was present in the soil of village-Sonmbre of tahsil Sinnar. Thus, 6.66% soil samples in Nashik district harboured the yeast population.

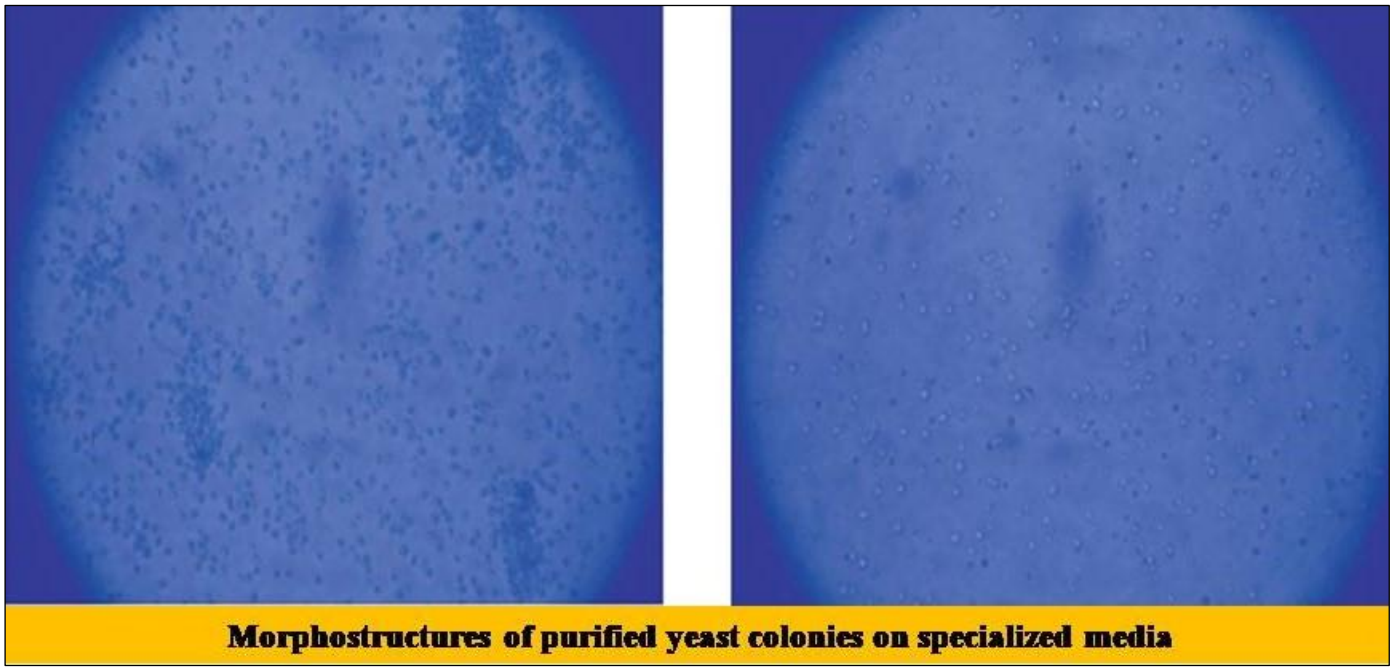
In transition zone-II, 15 soil samples were collected from Ahmednagar, Dhule, Nandurbar and Jalgaon districts. The soil samples from 9 villages of 5 tahsils of Ahmednagar district were subjected to yeast isolation. Out of these, soils of 4 villages i.e. village-Jhikri of Jamkhed tahsil, village Kolyachiwadi of Rahuri tahsil and village Bhoirepathar and Ghospuri of Ahmednagar tahsil yielded the yeast population. Consequently, 44.44% soil sample from Ahmednagar district harboured the yeast population. Similarly, in Marathwada region, Latur district the soil samples of 4 villages of 3 tahsils were analyzed for yeast population. The yeast population was present in the soil of village-Ambulga of Ahmadpur tahsil. Therefore, 25% soil samples in Latur district harboured the yeast population. Parbhani district soil samples from 2 villages of 2 tahsils were analyzed for yeast population. The yeast population was present in the soils of village-Wadachiwadi of tahsil Jintur. Hence, 50% soil samples in Parbhani district harboured the yeast population.

The soil of village-Chandhai from Mangrulpir tahsil of Akola district harboured the yeast population (Table 1). From Vidarbha region, Wardha district soil samples from 5 villages of 3 tahsils were analyzed for yeast population. The yeast population was present in the soil of village-Sewagram of Wardha tahsil. Thus, 20.00% soil samples in Wardha district yielded the yeast population. Whereas, Nagpur district soil samples from 13 villages of 6 tahsils were analyzed for yeast population. Out of these soils, the soils of village-Bahadura of Nagpur tahsil harboured the yeast population. So, 7.69% soil samples in Nagpur district harboured the yeast population. Yavatmal district the soil samples from 3 villages of 3 tahsils were analyzed for yeast population. Out of these soils, the soil of village Koregaon of Wani tahsil harboured the yeast population. Hence, in Yavatmal district 33.33% soil samples harboured the yeast population. In eastern Vidarbha zone, the soil samples were collected from Chandrapur, Gadchiroli and Bhandara district. The soil samples from 4 villages of 2 tahsils of Chandrapur district were analyzed for yeast population. The soil sample of village-Nandgaon of Gondpipri tahsil harboured the yeast population. Thus, 25% soil samples from Chandrapur district harboured the yeast population. However, in Konkan region approximately 42.42% of locations harbour the soil yeast. But, in Western Maharashtra region 18.05% of locations harbour the soil yeast, whereas in Marathwada region 16.16% of locations harbours the soil yeast. In Vidarbha region 15.15% of locations harbour the soil yeast. The distribution of soil yeast in the zone or the places within the zone was random because although the soil of one village in a particular tahsil harbour the yeast the soil of another village in the same tahsil did not harbour the yeast. Further, there was no correlation between soil depth and presence of yeast in the soil. Soil depth as low as 4 cm and as high as 165 cm were found to harbour the yeast population. The isolated yeast isolates were variable in their colony colour which was not specific to the locations of isolation. On the basis of morphological, physiological and biochemical characteristics, the yeast isolates were identified as *Trichomonascus petatosporus* [24, 21], *Candida labiduridarum* [25], *Candida shehatae* [26], *Eremothecium ashbyi* [27] and *Eremothecium cymbalariae* [28] and *Moniliella megachiliensis* [29]. A yeast culture identified as *Eremothecium cymbalariae* on the basis of morphological and biochemical characteristics [28] was further confirmed by molecular studies. The results indicated that some of the yeast isolates inhibited the growth of *Pythium* sp. and did not allow the *Pythium* sp. to multiply in the presence of these yeast isolates. The yeast isolates *viz*. WAJJ-11, WAAB-12, MLAA-1, and VCGN-5 were most effective in inhibiting the growth of *Pythium* sp. under *in vitro* condition whereas the yeast isolates like KSSA-2, KRSO-7 and WKAP-2 were ineffective in inhibiting the growth of *Pythium* sp. and the fungus grew in control broth. The yeast isolates which were found effective against the *Pythium* sp. were KTPD-10, WSWB-3, WNSS-9, WAJJ-11, WAAB-12, MLAA-1 and VCGN-5 (Table 2).

The plant growth promotion activity (PGP) was identified in yeast *Saccharomyces cerevisiae*, a biocontrol agent for *Fusarium oxysporum*, but did not find the accelerated PGP activity [30]. Eighty-seven yeast strains from the rhizosphere of potato, maize, vegetable marrow and cabbage plants were isolated [33]. Similarly, [32] isolated one hundred and eleven yeast stains from 60 agricultural soils in Slovakia. Likewise, [31] isolated 4 genera and 5 species of yeast from cultivated soil and 12 genera and 16 species from garden soil of Pakistan. On the basis of morphological and biochemical characteristics as described [21], the seven effective soil yeast isolates were characterized upto Genus and Species level. Out of seven isolate two yeast isolates belonged to the genus *Eremothecium* with species *cymbalariae* (yeast isolate no. WAAB-12) and species ashbyi (yeast isolate no. WNSS-9). Both these yeast isolates were present in the oils of western Maharashtra region but were found in different districts. *Eremothecium cymbalariae* was present in the soils of Ahmednagar district whereas *Eremothecium ashbyi* was present in the soils of Nashik district. Two yeast isolates i.e. WSWB-3 and MLAA-1 belonged to the genus *Candida*. Isolate no. WSWB-3 was *Candida labiduridarum* whereas isolate no. MLAA-1 was *Candida shehatae. Candida labiduridarum* was found in the soils of western Maharashtra region particularly in the soils of Satara district whereas

Candida shehatae was found in Marathwada region particularly in the soils of Latur district. Further, two identified isolates i.e. isolate no. VCGN-5 and isolate no. KTPD-10 belonged to the genus *Trichomonascus* and species *petasosporus* was present in the soils of Vidarbha and Konkan region. As Vidarbha and Konkan region has got high rainfall, it is presumed that these yeast genera is more prevalent in the high rainfall zone. A yeast isolate WAJJ-11 belonged to the genus *Moniliella* and species *megachiliensis* found in western Maharashtra region in the soils of Ahmednagar district. Thus, these seven yeast isolates belonged to three yeast genus and their distribution and prevalence was specific in the soils of particular region. The confirmation of the genus *Eremothecium* and species *cymbalariae* of the yeast isolate was carried out on the basis of internal transcribed spacer (ITS) region gene sequencing. On the basis of ITS region gene sequencing technique, the yeast culture was confirmed as *Eremothecium cymbalariae.* The yeast *Eremothecium cymbalariae* thrives on dead organic material and decaying fruits however, it is not reported as a soil yeast. The biocontrol activity of this yeast is reported in the control of fruit infections but not for soil borne plant pathogens. Thus, to the best of our knowledge, this is the first report that the soil yeast *Eremothecium cymbalariae* from Maharashtra soil has reported the biocontrol activity against the *Pythium* damping off pathogen. The ecology of *Eremothecium cymbalariae* is described as a plant pathogen associated with plant feeding insect, but because an only a single strain is known the ecology remain speculative. This species was reported as a pathogen of flax and other plants and was redescribed [34] and [35]. Our results added that the species was prevalent in the soils of western Maharashtra region in Ahmednagar district and has potential to act as biocontrol agent against damping off pathogen *Pythium. Eremothecium ashbyi* is reported to be a pathogen of cotton and citrus and occurs in warmer northern and southern parts of hemisphere where these crops are normally grown [27]. This species causes cotton boll rot in various species of *Gossypium* and induces cankers on citrus fruit. Transmission of this yeast pathogen causes infection to various genera of hemipterous insects [27, 36]. However, our results indicated that this species is present in the soils of Nashik district in the western Maharashtra region and has got the potential as biocontrol agent against damping off pathogen *Pythium* sp. Similarly, [37] reported the biocontrol activity of *Candida* species whereas the biocontrol activity of *Eremothecium, Trichomonascus, Moniliella* is not reported in the literature and therefore these three genera of yeast are reported for the first time to have the potential of biocontrol agents. Similarly, these yeasts are not reported previously from India, thus it is the first report of these yeast from the soils of India.





Morphostructures of purified yeast colonies on specialized media

Table 1: Status of soil yeast in different agro climatic zones of Maharashtra

Region	Agro climatic zone	District of Yeast isolation	Location of Yeast isolation	Soil type	Soil depth (cm)	Soil colour	Status of yeast	Yeast colony colour (on glucose yeast extract peptone agar medium)
Konkan region	South Konkan Coastal Zone	Sindhudurg	Village: Pural Tahsil: Devgarh	Loamy, extremely shallow	4	Yellowish brown	+	White creamy
		Sindhudurg	Village: Amba Tahsil: Sawantwadi	Fine-loamy, deep	125	Reddish brown	+	White creamy
		Ratnagiri	Village: Ratnagiri Tahsil: Ratnagiri	Loamy, extremely shallow	8	Reddish brown	+	Light brown
		Ratnagiri	Village: Harchil Tahsil: Ratnagiri	Loamy, very shallow	18	Yellowish red	+	Light brown
		Ratnagiri	Village: Nandgaon Tahsil: Chiplun	Clayey, moderately shallow	56	Dark reddish brown	+	Light brown
		Ratnagiri	Village: Kenjule Tahsil: Khed	Loamy, moderately deep	76	Dark red	+	White creamy
		Ratnagiri	Village: Ojahar Budrukh Tahsil: Sangameshwar	Loamy, shallow	40	Dark reddish brown	+	White creamy
	North Konkan Coastal Zone	Thane	Village: Gunde Tahsil: Shahapur	Loamy, very shallow	23	Dark brown	+	Light brown
		Thane	Village: Nane Tahsil: Wada	Fine, moderately shallow	56	Dark brown	+	Light brown
		Thane	Village: Dahisar Tahsil: Palghar	Clayey, very shallow	13	Black gravely clay	+	Light brown
		Raigad	Village: Ambet Tahsil: Man	Clay, moderately shallow	70	Brown	+	Light brown
Konkan region	North Konkan Coastal Zone	Raigad	Village: Tamhane Tahsil: Mahad	Clay, moderately shallow	70	Yellowish red	+	White creamy
		Raigad	Village: Sakhar Tahsil: Poladpur	Fine, moderately deep	90	Reddish brown	+	Light brown
		Raigad	Village: Bhorghar Tahsil: Mangaon	Clay, moderately shallow	56	Dark reddish brown	+	White creamy
Western Maharashtra Region	Western ghat zone	Kolhapur	Village: Ambarde Tahsil: Ajra	Fine, deep	125	Dark reddish brown	+	White creamy
		Kolhapur	Village: Parpoli Tahsil: Ajra	Fine, moderately deep	80	Brown & reddish brown	+	White creamy
		Satara	Village: Barsewadi Tahsil: Wai	Clayey, very shallow	12	Very dark grayish brown	+	Light brown
		Satara	Village: Wathar Tahsil: Koregaon	Fine, deep,	150	Very dark grayish brown	+	White creamy
		Sangli	Village: Kavthemahankal	Loamy, extremely shallow	5	Reddish brown	+	White creamy

			Tahsil: Kavthemahankal					
	Transition Zone -1	Pune	Village: Mahakoshi Tahsil: Bhor	Loamy, very shallow	13	Brown	+	Light brown
		Pune	Village: Rokadewadi Tahsil: Ambegon	Loamy, very shallow	9-21	Dark yellowish brown	+	Light brown
		Pune	Village: Ranjani Tahsil: Ambegon	Loamy, very shallow	13-23	Brown & dark brown	+	Brown
Western Maharashtra Region	Transition Zone -1	Nashik	Village: Sonmbre Tahsil: Sinnar	Loamy, very shallow	18	Brown	+	White creamy
	Transition Zone -2	Ahmednagar	Village: Kolyachiwadi Tahsil: Rahuri	Fine, moderately deep	83	Brown & dark brown	+	Brown
		Ahmednagar	Village: Jhikri Tahsil: Jamkhed	Clayey, very shallow to shallow	12-36	Very dark grayish brown	+	White creamy
		Ahmednagar	Village: Bhoirepathar Tahsil: Ahmednagar	Fine, moderately shallow	60-75	Dark brown, very dark brown	+	White creamy
		Ahmednagar	Village: Ghospuri Tahsil: Ahmednagar	Clayey, shallow	28-45	Very dark grayish brown	+	White creamy
Marathwada Region Vidarbha region	Assured rainfall zone	Latur	Village: Ambulga Tahsil: Ahmadpur	Clayey, shallow	42	Very dark grayish brown	+	White creamy
		Parbhani	Village: Wadachiwadi Tahsil: Jintur	Fine, deep	100-150	Dark grayish brown & dark brown	+	White creamy
	Moderate rainfall zone	Akola	Village: Chandhai Tahsil: Mangrulpir	Clayey, shallow	27-36	Very dark grayish brown	+	Dark brown
		Wardha	Village: Sewagram Tahsil: Wardha	Loamy, extremely shallow	8	Yellowish brown	+	White creamy
		Yeotmal	Village: Koregaon Tahsil: Wani	Clayey, shallow	25	Very dark grayish brown	+	White creamy
		Nagpur	Village: Bahadura Tahsil: Nagpur	Fine, moderately deep	90	Dark brown	+	White creamy
	Eastern Vidarbha zone	Chandrapur	Village: Nandgaon Tahsil: Gondpipri	Fine, loamy, shallow	40	Brown & dark brown	+	Brown

Table 2: Characterization of effective yeast isolates into genus and species based on morphological and biochemical characterization (Kurtzman *et al*., 2011)

Sr. No.	Yeast isolate No.	Region of habitat	Morphological character					Biochemical character (Utilization of sugar source)						Yeast	Reference
			Colour	Pseudo mycelium	Septate hypha	Balisto conidia	Ascocarp	D-galactose	D-glucosamine	L-arabinose	Maltose	Starch	Erythritol		
1.	KTPD-10	R-Kokan V-Dahisar D-Thane	Light brown	-	-	-	-	+	+	+	+	+	-	*Trichomonascus petasosporus*	Kurtzman, 2004
2.	WSWB-3	R-Western MS V-Barsewadi D-Satara	Light brown	-	-	-	-	+	+	+	+	-	-	*Candida labiduridarum*	Suh *et al*., 2008
3.	WNSS-9	R-Western MS V-Sonambre D-Nasik	White creamy	-	-	-	-	-	-	-	+	-	-	*Eremothecium ashbyi*	Batra, 1973
4.	WAJJ-11	R-Western MS V-Jhikri D-Ahmednagar	White creamy	-	-	-	-	-	+	-	+	-	+	*Moniliella megachiliensis*	Stolk and Dakin, 1966
5.	WAAB-12	R-Western MS V-Bhoire Pathar D-Ahmednagar	White creamy	-	-	-	-	+	+	-	+	-	+	*Eremothecium cymbalariae*	Borzi, 1888
6.	MLAA-1	R-Marathwada V-Ambulga D-Latur	White creamy	-	-	-	-	+	+	-	+	-	-	*Candida shehatae*	Buckley and Van uden, 1967
7.	VCGN-5	R-Vidarbha V-Nandgaon D-Chandrapur	Brown	-	-	-	-	+	+	+	+	+	-	*Trichomonascus petasosporus*	Kurtzman, 2004

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