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GC-MS analysis of metabolites in tritrophic interaction of yeast (*Pichia Kudriavzevii)* against soft rot pathogen of carrot (*Daucus carota*)

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Carrot (*Daucus carota* L.) is an important root vegetable cultivated worldwide, valued for their high levels of alpha and beta carotene, essential vitamins A, K, and B_6, as well as minerals that enhance eye health. They are widely used in various culinary dishes (Kitinoja *et al.* 2018). However, carrots are susceptible to postharvest diseases during transportation, handling, and storage. Yeasts have been utilized to control these diseases, supported by successful reports from various studies. Yeasts are beneficial microorganisms known for their strong antagonistic properties, environmental friendliness, and safety for human consumption (Zhang *et al.* 2020). These single-celled fungi thrive in diverse environments, do not produce harmful metabolites, and can be easily cultured in large quantities. They can colonize fruit surfaces for extended periods and resist chemical pesticides commonly used in postharvest disease management (Spadaro and Droby 2016). Liu *et al.* (2020) demonstrated that the yeast strain *Pichia kudriavzevii* effectively inhibited the growth of *Botrytis cinerea, Alternaria alternata*, and *Rhizopus stolonifer* in cherry tomatoes. In carrots, the primary diseases are soft rot caused by *Pectobacterium carotovorum* subsp. *carotovorum* and sour rot caused by *Geotrichum candidum*. This study focused on the metabolites extracted from soft rot pathogen, antagonist, and their interaction, which were analyzed through GC-MS. The metabolites from the effective yeast isolate were found to play a role in managing postharvest diseases in carrots.

A study was carried out during 2022–23 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Bacterial strain *P. carotovorum* subsp. *carotovorum,* isolated from carrot soft rot symptoms, and yeast strain *P. kudriavzevii,* isolated from the carrot surface, were cultivated separately in YEPDA broth. Both isolates were then co-inoculated in YEPDA broth and incubated for 48 h with continuous agitation. For metabolite extraction, the actively growing cultures were centrifuged at 12,000 rpm for 15 min. The supernatant was collected and filtered through Whatman No. 1 filter paper. The filtrate was mixed with an equal volume of ethyl acetate and shaken overnight. The ethyl acetate layer was separated using a separating funnel and collected in a separate container. This layer was then evaporated using a vacuum flask evaporator at 60°C and 80 rpm, resulting in a concentrated residue. The residue was dissolved in HPLC-grade methanol for analysis with Gas Chromatography-Mass Spectrometry (GC-MS) (Sasidharan *et al.* 2012).

Effect of crude metabolites by effective yeast isolates against P. carotovorum *subsp* carotovorum: The antagonistic activity of *P. kudriavzevii* against *P. carotovorum* subsp. *carotovorum* was tested using the agar well diffusion method. A petri dish containing Nutrient Agar (NA) medium was prepared, and a bacterial lawn was established by evenly spreading a bacterial suspension with an L-shaped rod and allowing it to air-dry. Four wells, each 1 cm from the edge of the plate, were made using a cork borer. The extracted metabolite was added to the wells in volumes of 50, 100, 150, and 200 µL, while sterile distilled water served as a control. An inhibition zone was observed around the wells containing the metabolite (Chiranjeevi *et al.* 2021).

Detection of crude metabolites through GC-MS analysis: The extracted crude metabolites were analysed using GC-MS. An Elite-5MS column (30 mm × 0.25 mm, 0.25 µm thickness) was used, with helium as the carrier gas at a flow rate of 1 ml/min. The column temperature was initially set at 40°C for 2 min, then increased to 160°C at 6°C/min, and finally raised to 260°C at 10°C/min, where it was held for 4 min. The ion source temperature was 230°C, with electron energy of 70 eV, and the mass range scanned from 40–450 Da. Compound identification was performed by comparing the spectra with the NIST MS Data library using computer-based sources (Mulatu *et al.* 2022).

Statistical analysis: The experiments were analysed through completely randomized design (CRD). The mean differences of the treatment were compared using Duncan's Multiple Range-Test (DMRT) at 5% significance level

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(Gomez and Gomez 1984). All the data were analysed statistically using SPSS software (version 16).

Effect of crude metabolites of P. kudriavzevii *YKP 12 against* P. carotovorum *subsp.* carotovorum: The study investigated the impact of crude metabolites from *Pichia kudriavzevii* YKP 12 on *P. carotovorum* sub sp. *carotovorum* using an agar well diffusion assay. The metabolites exhibited varying levels of inhibition, with the highest inhibition zone of 18.33 mm observed at 100 µL and the lowest of 5.66 mm at 50 µL against *P. carotovorum* subsp. *carotovorum* (Table 1, Fig. 1). Previous studies, such as those by Sheeba *et al.* (2019), also utilized the agar well diffusion method to assess similar antibacterial activity.

Table 1 Effect of crude metabolites of *P. kudriavzevii* YKP 12 against *P. carotovorum* sub sp *carotovorum* by agar well diffusion assay

Yeast isolate	Concentrations (µl)	Inhibition zone (mm)
YKP12	25	5.66[d]
	50	12.67[c]
	75	15.66[b]
	100	18.33[a]
	Control	0.00[e]

Mean followed by a common letter as superscript does not differ significantly at 5% level by DMRT. Values in parentheses are arc sine transformed values.

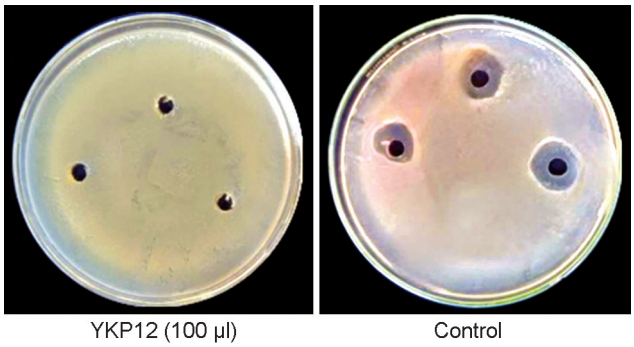

Fig. 1 Effect of crude metabolites of *P. kudriavzevii* YKP 12 against *P. carotovorum* sub sp *carotovorum.*

Detection of crude metabolites through GC-MS analysis:

GCMS analysis of metabolites extracted from P. carotovorum *subsp.* carotovorum: GC-MS analysis of bacterial isolates identified three major compounds: Carbamic acid phenyl ester, 1-Hexanol 2-ethyl, and Hexadecanoic acid methyl ester. Among these, 1-Hexanol 2-ethyl had the highest peak area at 13.56% with a retention time of 4.8 min, while 1-Octanol 2, 7-dimethyl had the lowest peak area at 0.5% with a retention time of 5 min.

Similar findings by Meziani *et al.* (2015) linked compounds like hexadecenoic acid trimethylsilyl ester to pathogenesis in potato tubers infected with *P. atrosepticum*. Marquez-Villavicencio *et al.* (2021) noted that *P. carotovorum* subsp. *carotovorum* strain PCC21 from Chinese cabbage exhibited butanoate metabolism, contributing to a rotting odor.

GCMS analysis of metabolites extracted from yeast YKP12 isolate: GC-MS analysis of *Pichia kudriavzevii* identified three major compounds: 2,4-bis (1,1-dimethylethyl) phenol, 1,2-benzenedicarboxylic acid, and hexamethyl cyclotrisiloxane. The most prominent compound was 1, 2-benzenedicarboxylic acid, which accounted for 13.09% of the peak area and had a retention time of 16.130 min. The compound with the smallest peak area, Cyclotrisiloxane (hexamethyl), was recorded at 0.67% with a retention time of 15.643 minutes (Fig. 2). Research by Zhao *et al.* (2023) on the aroma-producing yeast *Wickerhamomyces anomalus* identified metabolites such as phenylethyl alcohol, isobutyl alcohol, isothiocyanate esters, myrcene, and dimethyl disulfide, highlighting the best ether compound found in an ethyl acetate mixture. Additionally, *Hannaella sinensis* has demonstrated

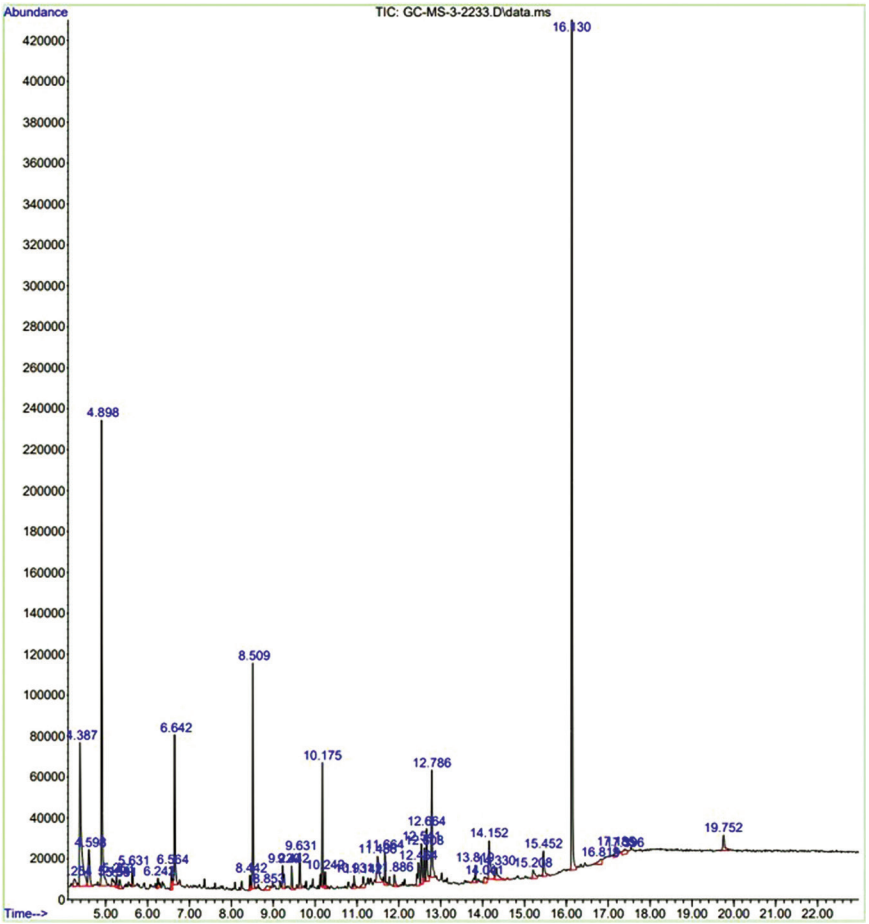

Fig. 2 GCMS analysis of metabolites extracted from Pichia kudriavzevii YKP 12 isolate.

Table 2 GCMS analysis of metabolites extracted from interaction of *P. kudriavzevii* YKP 12 isolate and *P. carotovorum* sub sp. *carotovorum*

Compound	RT	Area (%)	Molecular weight	Molecular formula	Structure	Biological activity	Reference
1-Hexanol, 2-ethyl-	4.898	13.89	130.2279	$C_8H_{18}O$		Antifungal, Nematicidal	(Hewavitharana *et al.* 2014)
Heptocosane	5.154	0.60	380.7	$C_{27}H_{56}$		Antimicrobial Antioxidant	(Marrufo *et al.* 2013)
Tricosanoic acid, methyl ester	13.819	0.77	368.6367	$C_{24}H_{48}O_2$		Antimicrobial	(Valiei *et al.* 2011)
1,2-Bis (trimethylsiyl) benzene	15.208	0.80	222.47	$C_{12}H_{22}Si_2$		Antioxidant Antimicrobial	(Momin and Thomas 2020)
Cyclobarbital	14.041	0.65	236.27	$C_{12}H_{16}N_2O_3$		Antioxidant Antimicrobial	(Joshi *et al.* 2020)
Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenyl methyl)-	12.664	2.34	244.29	$C_{10}H_{16}N_2O_2$		Antioxidant	(Manimaran and Kannabiran 2017)
Compound	RT	Area (%)	Molecular weight	Molecular formula	Structure	Biological activity	Reference
Cyclopentane tridecanoic acid	13.819	0.22	268.50	$C_{18}H_{32}O_2$		Antibacterial	(Kawahara *et al.* 1996)
Hexadecane	12.430	0.17	226.44	$C_{16}H_{34}$		Antibacterial, Antioxidant	(Yogeswari *et al.* 2012)

effectiveness in managing post-harvest diseases in apples by degrading patulin (PAT) through intracellular enzymes (Ma *et al.* 2023).

GCMS analysis of metabolites extracted from the interaction of Pichia kudriavzevii YKP12 isolate and P. carotovorum *subsp* carotovorum: In the bipartite interaction analysis, four bioactive compounds were identified: 1-Hexanol, 2-ethyl, Heptacosane, and Pyrrolo [1,2-a] pyrazine-1,4-dione, as well as 1,2-Bis(trimethylsilyl) benzene. Among these, 1-Hexanol, 2-ethyl exhibited the highest peak area at 13.89% with a retention time of 4.89 min. Pyrrolo [1,2-a] pyrazine-1,4-dione showed a peak area of 2.34% and a retention time of 12.66 min, while Heptacosane had the lowest peak area at 0.60% (Table 2).

SUMMARY

Carrot a fleshy edible root vegetable, abundant in bioactive compounds such as carotenoids and dietary fibres. The market value of carrots is impacted by post-harvest illnesses, which have emerged as a serious production constraint. A promising advancement in the battle against post-harvest illnes is yeast-based biocontrol, because of its colonization on dry surfaces for longer periods and low nutrient utilization and can multiply easily. A study was carried out during 2022–23 at Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu to evaluate the GC-MS analysis of metabolites in tritrophic interaction of yeast (*Pichia Kudriavzevii)* against soft rot pathogen of carrot. The tritrophic interaction of soft rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum* and yeast isolate YKP 12 *Pichia kudriavzevii* was analyzed through GCMS analysis and the metabolite 1-Hexanol, 2-ethyl showed highest peak area of 13.89% with the retention time of 4.89 min. The tri-trophic interaction results revealed that the compound 1, 2-Benzenedicarboxylic acid monoester had both antibacterial and antifungal activity.

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