

Capacity of *Candida* species to produce acetaldehyde at various concentrations of alcohol

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Abstract

Background: Along with tobacco use, alcohol consumption is one of the crucial factors for oral cancer. Acetaldehyde (ACH), a byproduct of alcohol, is reported as carcinogenic. One of the producers of ACH from alcohol is *Candida* species. The aim of the study was to quantify the ACH produced by *Candida* species at various concentrations of alcohol.

Materials and Methods: Clinical isolates of *Candida*, namely *Candida albicans*, *Candida krusei* and *Candida tropicalis* and *C. albicans* ATCC 18,804, were subjected to various concentrations of alcohol. Alcohol dehydrogenase and ACH were estimated using spectrophotometry and headspace gas chromatography, respectively.

Results: Out of all three clinical isolates, *C. tropicalis* produced more ACH (412.1 µM) at 10 mM alcohol concentration by 10⁵ colony-forming unit/ml followed by *C. albicans* (233 µM) and *C. krusei* (53.7 µM). *C. albicans* of clinical isolate and ATCC species (222 µM) did not show much difference.

Conclusion: The study results conclude that *Candida* species are capable of producing carcinogenic levels of ACH on exposure to various concentrations of alcohol.

Keywords: Acetaldehyde, alcohol, *Candida albicans*, *Candida* species, oral cancer

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INTRODUCTION

Alcohol consumption is the main risk factor for oral and pharyngeal cancers, together with tobacco use.^[1-4] Although alcohol (ethanol) has been classified by the International Agency for Research on Cancer as a human carcinogen, there is no clear evidence of its association.^[5,6] Ethanol-associated carcinogenicity is due to the major oxidized metabolite called acetaldehyde (ACH).^[7] Exposure to ACH over a period of time influences the cellular activity and triggers DNA modifications that may bring about mutations and cancer initiation.^[8,9]

Although it is thought that ethanol is mainly metabolized in the liver,^[10] several studies in the literature have demonstrated elevated levels of ACH in saliva than those found in the blood soon after alcohol intake.^[11,12] Metabolism of ethanol to ACH in the oral cavity is a direct function of oral microbiome, associated metabolism, salivary glands and compromised oral hygiene along with several other nutritional, psychosocial and environmental factors.^[13]

A direct correlation has been found between the levels of oral microbes (*Streptococcus salivarius*, hemolytic *Streptococcus*

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viridans var., *Corynebacterium* sp., *Stomatococcus* sp. and yeasts) and ACH levels in saliva. *Candida* species in particular have been associated with increased levels of ACH,^[14] and this is mediated through the microbial enzyme alcohol dehydrogenase (ADH).

Previous studies have demonstrated the ACH production in oral cavity after alcohol consumption or use of alcohol-based mouthwashes and correlated with the bacterial analysis.^[14-16] However, the detailed analysis of bacterial species with the quantification of ACH production was unclear. Limited studies have been published in particular with *Candida* species.^[17]

Yeasts are the high producers of ACH from alcohol. ACH binds to cellular proteins and DNA, interferes with DNA synthesis and repair and leads to replication errors or mutations causing development of tumor. With this background, the present study made an attempt to correlate the alcohol dose-dependent ACH production by *Candida* species.

MATERIALS AND METHODS

This study was conducted after the approval by the Ethics Committee, D.Y. Patil Vidyapeeth, Pimpri, Pune, No: DYPV/EC/49/2016. Three clinically isolated strains of *Candida*, namely *Candida albicans*, *Candida krusei* and *Candida tropicalis*, from oral saliva samples were used along with *C. albicans* ATCC 18,804 as standard culture strain. Identification of the clinically isolated cultures was on the basis of colony morphology, microscopy and growth on chromogenic culture media. Antibiotic susceptibility of all cultures was tested. Cell density for further experiments for *Candida* standard strain along with clinical isolates was estimated by suspending colonies from pure culture in phosphate-buffered saline and optical density at 500 nm was adjusted spectrophotometrically. Dilution plating was used to verify the microbial concentration. Absorbance of 0.4 at 500 nm corresponds to 1×10^5 colony-forming unit (CFU)/ml; absorbance of 0.34 at 500 nm corresponds to 1×10^4 CFU/ml; absorbance of 0.28 at 500 nm corresponds to 1×10^3 CFU/ml; absorbance of 0.19 at 500 nm corresponds to 1×10^2 CFU/ml. Crude enzyme extract from the cells was prepared by ultrasonication of cells and taking supernatant after centrifugation. For estimation of ADH, the continuous spectrophotometric rate determination (A340, light path = 1 cm) assay was used. ACH levels in the reactions were tested by headspace gas chromatography. All enzyme activity was expressed as total activity 1.0 μ mol of ethanol to ACH per min.

RESULTS

Three clinical isolates of *Candida* species, i.e., *C. albicans*, *C. krusei*, *C. tropicalis* and *C. albicans* ATCC 18,804, were assessed for their capability of converting various levels of alcohol to ACH at different time intervals. Clinical isolate of *C. albicans* produced more ACH when compared to *C. albicans* (ATCC). Out of three clinical isolates, *C. tropicalis* produced the highest ACH. At a particular concentration of alcohol, the concentration of ACH was increasing as the CFU/ml of the species was increasing by all the *Candida* species. Similarly, at particular CFU/ml of species, the ACH concentration was decreasing as the concentration of the alcohol increases for all the species. As the concentration of the alcohol increases, the ability of the *C. albicans* to produce ACH reduces. Beyond the level of 1000 mM, the organisms of the level 10^2 – 10^4 cannot produce ACH [Table 1]. *C. albicans* (clinical isolates) are able to produce ACH even with increasing concentration of alcohol, but the amount reduces significantly [Table 2]. *C. krusei* did not produce ACH from alcohol of concentration beyond 1000 mM [Table 3]. *C. tropicalis* was able to produce ACH irrespective of the alcohol concentration, but the amount of ACH reduced as the concentration increased and also as the concentration of the organism reduced. Production of ACH was inversely proportional to the alcohol concentration [Table 4].

DISCUSSION

The progression of cancer involves a series of steps, all of which are characterized by accumulation of genetic alterations followed by clonal evolution.^[18] The well-recognized risk factors implicated in the pathogenesis of oral cancer are smoking, tobacco chewing as well as alcohol consumption.^[19] Presumable role of microbes such as human papillomavirus, Epstein–Barr virus and *C. albicans* has also been substantiated.^[20] Alcohol consumption has been regarded as an independent risk factor for cancer development irrespective of the type of beverage, pattern of drinking or tobacco use.^[21] The exposure to alcohol in terms of both intensity and duration is associated with the risk of oral cancer development.

Alcohol is not the direct carcinogen, ACH which is its primary metabolite is known to be mutagenic. Apart from hepatic metabolism of alcohol to ACH, there is a good evidence of extrahepatic metabolism of alcohol to ACH in particular to occur in the oral cavity.^[22] ACH is synthesized by mucosal aldehyde dehydrogenase 2; greater amounts are acquired from oral microflora-mediated microbial oxidation of ethanol through ADH-mediated carbohydrate

Table 1: Acetaldehyde production (μM) at various concentrations of alcohol (mM) at different colony-forming unit/ml by *Candida albicans* (ATCC)

Alcohol Conc. (mM)	CFU/ml							
	10^5		10^4		10^3		10^2	
	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity
100	222	2.83 \pm 0.50	168	2.23 \pm 0.08	132.2	1.77 \pm 0.13	87.22	1.06 \pm 0.06
500	219.7	2.72 \pm 0.22	122	1.26 \pm 0.04	98	1.02 \pm 0.12	54.54	0.77 \pm 0.12
1000	135.12	1.6 \pm 0.20	86.6	0.93 \pm 0.07	76.12	0.82 \pm 0.08	16	0.21 \pm 0.04
2000	58	0.16 \pm 0.02	-	-	-	-	-	-
2500	53.7	0.13 \pm 0.02	-	-	-	-	-	-

CFU: Colony-forming unit, ACH: Acetaldehyde

Table 2: Acetaldehyde production (μM) at various concentrations of alcohol (mM) at different colony-forming unit/ml by *Candida albicans* (clinical isolate)

Alcohol Conc. (mM)	CFU/ml							
	10^5		10^4		10^3		10^2	
	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity
100	233	2.68 \pm 0.08	202.0	2.46 \pm 0.12	182.6	2.22 \pm 0.08	161.0	1.83 \pm 0.11
500	220	2.65 \pm 0.16	175.2	1.90 \pm 0.19	158.3	1.67 \pm 0.15	109.2	1.06 \pm 0.19
1000	202.1	2.24 \pm 0.13	144.6	1.46 \pm 0.08	102.2	1.30 \pm 0.03	69	0.92 \pm 0.07
2000	148.9	1.21 \pm 0.04	98.5	0.96 \pm 0.04	79.2	0.88 \pm 0.06	32.3	0.46 \pm 0.13
2500	78	0.63 \pm 0.05	56.4	0.58 \pm 0.07	32	0.44 \pm 0.13	-	-

CFU: Colony-forming unit, ACH: Acetaldehyde

Table 3: Acetaldehyde production (μM) at various concentrations of alcohol (mM) at different colony-forming unit/ml by *Candida krusei* (clinical isolate)

Alcohol Conc. (mM)	CFU/ml							
	10^5		10^4		10^3		10^2	
	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity
100	53.7	0.96 \pm 0.07	40.2	0.76 \pm 0.12	22.8	0.71 \pm 0.04	8.2	0.46 \pm 0.11
500	46.3	0.55 \pm 0.02	16.6	0.32 \pm 0.06	12	0.23 \pm 0.07	-	0.09 \pm 0.04
1000	11.2	0.12 \pm 0.05	-	-	-	-	-	-
2000	-	-	-	-	-	-	-	-
2500	-	-	-	-	-	-	-	-

CFU: Colony-forming unit, ACH: Acetaldehyde

Table 4: Acetaldehyde production (μM) at various concentrations of alcohol (mM) at different colony-forming unit/ml by *Candida tropicalis* (clinical isolate)

Alcohol Conc. (mM)	CFU/ml							
	10^5		10^4		10^3		10^2	
	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity	ACH	Enzyme activity
100	412.1	3.56 \pm 0.36	356.0	3.25 \pm 0.11	344.4	3.11 \pm 0.14	267.1	2.42 \pm 0.07
500	366.5	3.44 \pm 0.25	302.2	3.02 \pm 0.11	292.1	2.87 \pm 0.16	165.2	1.88 \pm 0.07
1000	301.6	2.86 \pm 0.16	223.6	2.52 \pm 0.14	203.6	2.45 \pm 0.13	104.0	1.12 \pm 0.13
2000	165.4	0.98 \pm 0.11	126.0	0.84 \pm 0.09	121.0	0.81 \pm 0.11	56.11	0.61 \pm 0.09
2500	111.0	0.77 \pm 0.09	94.6	0.66 \pm 0.15	82	0.57 \pm 0.13	-	0.13 \pm 0.08

CFU: Colony-forming unit, ACH: Acetaldehyde

fermentation.^[14,22-25] Yeasts are common oral resident flora with ADH activity.^[26] Therefore, they may constitute a significant part of ethanol-derived ACH.^[27] Furthermore, earlier studies have shown ACH accumulation in oral tissues enhanced by differential expression of enzymes in the oral cavity.^[22]

In the present study, at a particular CFU, the *C. albicans* both the clinical isolate and ATCC showed a maximum concentration of enzyme activity and ACH concentration at the lowest concentration of the alcohol. However,

as the concentration of alcohol increases, the enzyme activity and ACH concentration decreases. Similar findings were observed with other *Candida* species found in the clinical isolates, i.e., *C. krusei* and *C. tropicalis*. This could be attributed firstly the enzyme activity of *Candida* species to convert alcohol to ACH which could be limited to a particular concentration of alcohol. As the concentration of alcohol increases, the enzyme released by *Candida* species may not be sufficient to convert alcohol to ACH. Secondly, the *Candida* species may not sustain in an environment of higher alcohol concentration. Isolated

yeast, when incubated with 22 mM of ethanol *in vitro* for 60 min at 37°C, was capable of producing ACH and its production capacity was in proportion to the duration of incubation, the amount of yeast and the ethanol concentration (except a higher ethanol concentration, i.e., 2500 mM). A decrease in the production of ACH with increased ethanol concentrations was probably as a result of the death of viable yeasts. The difference in the production of ACH between species was about 180 fold, ranging from 1.3 nmol ach/10⁶ CFU (*C. parapsilosis* IH 112765) to 236.4 nmol ach/10⁶ CFUs (*C. albicans* IH 112344), with *C. albicans* accounting for 88% of all oral isolates (87% among the high and 90% among the low-producing saliva). Furthermore, three strains of *Candida glabrata* produced ACH of 13.6,14.1,15.4 nmol/106CFU and one strain of *C. tropicalis* was present with ACH production of 45.1 nmol/106CFU.^[27] Our study demonstrated *tropicalis* as the highest ACH producer from oral isolates, which was in line with the study conducted by Nieminen *et al.* where they studied thirty non-*C. albicans* isolates and observed that *C. tropicalis* produced the highest (252.3 µM) amount of ACH followed by *C. parapsilosis* (243.3 µM) compared to *C. albicans* (235.1 µM) that was studied earlier and *C. krusei* on the other hand produced the lowest (54.6 µM).^[17]

On incubation with ethanol (11 mM), all *Candida* species including *Candida kefyri* which was not earlier reported showed a significant amount of ACH production (>100 µM). *C. kefyri* produced the highest amount of ACH (299.3 ± 80.9 µM) from ethanol among all *Candida* species in contrast to the earlier reports which demonstrated *C. tropicalis* (248.9 ± 49.9 µM) to produce greater quantities of ACH.^[17] Higher levels of ACH production were found in the isolates from patients who reported regular alcohol intake than those from nondrinkers. The amount of ACH produced by the strains isolated from oral squamous cell carcinoma patients during 30 min of incubation was greater (mean 716.6 IM) than that of the strains obtained from control group (mean of 654.0 IM).^[28]

The ACH thus released accumulates in the oral mucosal cells and brings about changes in cellular and nuclear metabolisms leading to cytotoxicity. Many *Candida* species have been known to produce a mutagenic amount of ACH in the presence of ethanol.^[17] The effects of ACH on the oral cavity can be local, and oral hygiene may be linked to the joint effect of smoking and alcohol intake on the incidence of oropharyngeal to local production of ACH by oral microflora and yeast infection which could partly explain cancers.^[27] The presence of yeasts in the oral cavity has also been linked to epithelial dysplasia and oral carcinogenesis.^[20,29]

CONCLUSION

Based on the results of the study, *Candida* species can produce ACH which can be carcinogenic and can affect cellular activity. Exposure to ACH for a longer period of time could cause DNA mutations even at physiological concentrations. Maintaining oral hygiene is of at most importance to reduce the bacterial/fungal load and also prevent alcohol consumption as alcohol can alone be a factor for cancer initiation.

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Conflicts of interest

There are no conflicts of interest.

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