

# The role of yeasts in oral cancer by means of endogenous nitrosation

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Krogh P. The role of yeasts in oral cancer by means of endogenous nitrosation. *Acta Odontol Scand* 1990;48:85–88. Oslo. ISSN 0001-6357.

Oral leukoplakias, particularly non-homogenous types, are often invaded by yeasts, with *Candida albicans* being the dominant species. The more advanced precancerous leukoplakia lesions yield more rarely occurring biotypes of *C. albicans*, suggesting a causal role for these biotypes in the malignant transformation. *N*-nitroso-benzylmethylamine (NBMA) is a compound able to induce carcinoma of the esophagus and the oral cavity in the rat. The catalytic potential of yeasts, isolated from leukoplakia lesions and from normal mucosa, to produce NBMA from the precursors *N*-benzyl-methylamine and nitrite was assessed at pH 6.8. The yeast strains differed in nitrosation potential, ranking from 0 to 1.2 µg NBMA/10<sup>6</sup> cells. *C. albicans* strains of the more rarely occurring biotypes showed the highest nitrosation potential, whereas *C. tropicalis*, *C. parapsilosis*, and *Torulopsis glabrata* were ranked lower. Strains with high nitrosation potential were generally isolated from lesions with more advanced precancerous changes. Thus, further evidence is provided supporting the hypothesis that yeasts play a causal role in oral cancer by means of endogenous nitrosamine production. □ *Biotypes; Candida albicans; N-nitrosobenzylmethylamine; oral leukoplakia*

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Oral leukoplakia occurs in 3–4% of the adult population (1), and, if untreated, 5–10% of the cases will develop into carcinoma (2). Non-homogenous types of oral leukoplakia have a higher probability than homogenous types for malignant transformation (3), and the fact that most non-homogenous leukoplakias have been found invaded by yeasts suggests a causal role of yeasts in malignant development of oral precancer (4–6). *Candida albicans* is by far the fungal species most commonly isolated from oral leukoplakia (7), and the biotypes of *C. albicans* associated with homogenous and non-homogenous leukoplakias differ from those isolated from normal oral mucosa (8). This observation suggests that malignant development of oral precancer may be elicited by particular biotypes of *C. albicans*. Microbial carcinogenesis may involve nitrosation in which microbial cells catalyze the formation of *N*-nitroso compounds from the precursors nitrite and from amines, amides, or other nitrosatable compounds.

## Yeast nitrosation

Several species of bacteria encompass strains capable of catalyzing nitrosation, in particular *Escherichia coli* (9). Yeasts may also include nitrosating organisms. In an etiologic study of esophageal carcinoma, the second most common cancer in China, the strain of *C. albicans* investigated was able to catalyze the formation of *N*-nitrosobenzylmethylamine (NBMA) from the precursors (10). As indicated above, certain biotypes of *C. albicans* seem to be associated with the malignant development of oral precancer. It would therefore be relevant to elucidate the distribution of the nitrosation potential among oral yeast species and biotypes of *C. albicans*, to identify an association between nitrosation potential and malignant development. No information is at present available on the prevalence of various nitrosamines and nitrosatable compounds in saliva, which might otherwise have been useful in suggesting the specific nitrosation process to be studied in the assessment of yeasts' nitro-

sation potential. As the nitrosation potential is likely to be dependent of the molecular structure of the nitrosatable compound, the relevant compounds to be studied might be identified by considering the nitrosamines known to induce mucosal cancer in experimental animals. NBMA has been found capable of inducing tumors in the esophagus, forestomach, and tongue of the rat (11) and is therefore a relevant compound for study. Altogether 26 strains of yeasts, obtained from 12 cases of oral precancerous lesions and from the normal mucosa of each patient, were studied, comprising 21 strains of *C. albicans* belonging to 15 biotypes, 2 strains of *C. tropicalis*, 1 strain of *C. parapsilosis*, and two strains of *Torulopsis glabrata* (12). The catalytic potential of the strains to

form NBMA from the precursors *N*-benzylmethylamine (BMA) and nitrite was assessed at pH 6.8, representing an average pH value for saliva (13), and included a 48-h nitrosation period at 37°C. The nitrosamine produced was identified and quantitated by high-performance liquid chromatography, confirmed by gas chromatography-mass spectroscopy, and related to the number of yeast cells involved, as nitrosation rate. One-way analysis of variance showed that the 26 yeast strains were different in terms of nitrosation potential, ranging from zero to 1.12 µg NBMA/10<sup>6</sup> cells (Table 1). The strains were ranked by nitrosation potential, and by use of the calculated least significant difference (0.40 µg NBMA/10<sup>6</sup> cells) sub-groups of strains with identical nitrosation

Table 1. Ranking of yeast strains isolated from the oral cavity of precancer cases, according to nitrosation rate (adapted from Ref. 12)

Yeast		Nitrosation rate (µg NBMA 10 <sup>6</sup> cells)	Ranking†						
Collection no.	Species*								
910	<i>C. albicans</i>	1.12			A				
901	<i>C. albicans</i>	0.74	B		A				
900	<i>C. albicans</i>	0.69	B		C				
944	<i>C. albicans</i>	0.63	B		C	D			
950	<i>C. albicans</i>	0.61	B		C	D			
911	<i>C. albicans</i>	0.48	B	E	C	D			
949	<i>C. albicans</i>	0.46	B	E	C	D			
908	<i>C. albicans</i>	0.42	F	B	E	C	D	G	
903	<i>C. albicans</i>	0.39	F	B	E	C	H	D	G
904	<i>C. albicans</i>	0.37	F	B	E	C	H	D	G
907	<i>T. glabrata</i>	0.37	F	B	E	C	H	D	G
906	<i>C. albicans</i>	0.34	F		E	C	H	D	G
909	<i>T. glabrata</i>	0.28	F		E	C	H	D	G
932	<i>C. albicans</i>	0.20	F		E	C	H	D	G
905	<i>C. albicans</i>	0.19	F		E	C	H	D	G
934	<i>C. albicans</i>	0.19	F		E	C	H	D	G
937	<i>C. tropicalis</i>	0.19	F		E	C	H	D	G
951	<i>C. albicans</i>	0.17	F		E	C	H	D	G
933	<i>C. albicans</i>	0.15	F		E	C	H	D	G
952	<i>C. albicans</i>	0.12	F		E	C	H	D	G
936	<i>C. tropicalis</i>	0.11	F		E	C	H	D	G
902	<i>C. albicans</i>	0.08	F		E	C	H	D	G
961	<i>C. albicans</i>	0.06					H	D	G
962	<i>C. albicans</i>	0.02					H	D	G
935	<i>C. albicans</i>	0					H	D	G
912	<i>C. parapsilosis</i>	0					H	D	G

One-way analysis of variance of nitrosation potential between yeast strains: SSD = 3.3326;  $f = 24$ ;  $s = 0.1389$ ;  $F = 4.06$  ( $P < 0.001$ ).

\* *C.* = *Candida*; *T.* = *Torulopsis*.

† Ranking of the yeast strains was done as an extension of the one-way analysis of variance; the least significant difference between yeast strains was calculated to be 0.40 µg NBMA/10<sup>6</sup> cells.

Table 2. Comparison of yeast nitrosation rate and characteristics of precancer patients (adapted from Ref. 12)

Species of yeast from lesion*	Nitrosation rate ( $\mu\text{gNBMA}/10^6$ cells)	Site of lesion	Clinical characteristics of lesion	Patient characteristics	
				Epithelial†	
				Hyperplasia	Dysplasia
<i>C. albicans</i> <sup>a</sup>	1.12	Commissural	Nodular	+	—
<i>C. albicans</i>	0.69	Retrocommissural	Erythroleukoplakia	+	—
<i>C. albicans</i>	0.63	Buccal	Homogenous	NI	NI
<i>C. albicans</i>	0.46	Bottom of mouth	Nodular	+	Moderate
<i>C. albicans</i> <sup>b</sup>	0.42	Retrocommissural	Erythroleukoplakia	+	—
<i>C. albicans</i> <sup>a</sup>	0.37	Commissural	Nodular	+	—
<i>T. glabrata</i> <sup>b</sup>	0.28	Retrocommissural	Erythroleukoplakia	+	—
<i>C. albicans</i>	0.20	Retrocommissural	Homogenous	+	Slight
<i>C. albicans</i>	0.19	Retrocommissural	Homogenous	NI	NI
<i>C. albicans</i>	0.17	Labial groove	Verrucous	+	Slight
<i>C. tropicalis</i>	0.11	Retrocommissural	Erythroleukoplakia	+	—
<i>C. albicans</i>	0.08	Retrocommissural	Erythroleukoplakia	+	—
<i>C. albicans</i>	0.06	Floor of mouth	Homogenous	+	Moderate
<i>C. parapsilosis</i>	0	Floor of mouth	Erythroleukoplakia	+	Carcinoma in situ

\* *C.* = *Candida*; *T.* = *Torulopsis*. <sup>a,b</sup> Two yeast strains isolated from the pathologic site of one patient.

† NI = no histologic investigation conducted.

potential could be identified. The species *C. albicans* encompassed the strains with the highest nitrosation potential, but there were also *C. albicans* strains with low or zero potential.

Although there was no apparent association between yeast nitrosation potential and advanced premalignant histologic changes, lesions characterized clinically as nodular, which constitute a type of leukoplakia with a high probability for malignant transformation (6), were exclusively associated with high-yielding yeasts (Table 2). The location of the examined leukoplakias was almost entirely confined to the retrocommissural area and the bottom of the mouth. It is noteworthy that the leukoplakias in the bottom of the mouth, which is a drainage area for saliva presumably containing the precursors nitrite and amines, showed the most advanced precancerous changes, moderate dysplasia and carcinoma in situ.

## Precursor supply

### Nitrite

Although some *Candida* species, like *C.*

*nitrophila* and *C. utilis*, are able to reduce nitrate to nitrite (14), the yeast species occurring in the oral cavity cannot assimilate nitrate and do not synthesize the nitrate reductase enzyme. However, there is plenty of nitrite in saliva, mainly of bacterial origin. Thus average values of 6–10  $\mu\text{g}$  nitrite/ml saliva have been reported (15, 16), with peak values up to hundreds of micrograms per milliliter of saliva, depending on the nitrate intake (17). As only a few micrograms of nitrite per 1 ml are required for maximal yeast nitrosation, there is obviously surplus supply of nitrite in the oral cavity.

### Nitrosatable compounds

No estimate has yet been made of human exposure to nitrosatable compounds. BMA has been found in vegetables (carrots, green salad), herring oil, and freeze-dried coffee in a concentration range of 0.2–20  $\mu\text{g}/\text{kg}$  food (18). The requirement for maximal yeast production of NBMA is 1–2  $\mu\text{g}$  BMA/ml substrate. Thus, human exposure to this amine may well be sufficient for maximal yeast nitrosation. In this connection it is interesting to note that NBMA was detected

in the gastric juice of 11% of people from an area at high risk for esophageal cancer in China (19); whether the nitrosamine was of exogenous or endogenous origin was not established.

## Conclusion

Yeasts isolated from oral leukoplakias were able to catalyze the formation of the nitrosamine NBMA, provided the precursors were available. The highest nitrosation potentials were encountered in the species *C. albicans*, but pronounced differences in the potential were observed between *C. albicans* biotypes. It is noteworthy that more advanced precancerous leukoplakias generally yielded yeast strains with high nitrosation potential. Nitrite is always available in the oral cavity in surplus. The human exposure to the amine (BMA) has never been assessed, but considering its presence in various foods, it is likely that there is a surplus supply of this precursor. Thus, available evidence suggests the existence in the oral cavity of yeast-catalyzed endogenous nitrosation, implying a causal role in the development of oral cancer. However, several factors affecting endogenous nitrosation are known, including enhancing factors such as thiocyanate and preventing factors such as vitamin C, and these factors may be operating in the oral cavity as well. Work is in progress to assess the influence of these factors on yeast-induced nitrosation.

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