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Allyl Alcohol Found in Heated Garlic is a Potent Selective Inhibitor of Yeasts

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Abstract Allyl alcohol (2-propen-1-ol), found in considerable amounts in heated garlic, was able to discriminate yeasts from bacteria and was approximately three orders of magnitude more inhibitory towards yeasts than bacteria. The average minimum inhibitory concentration (MIC) of allyl alcohol for bacteria and yeasts was 5.0% and 0.0056%, respectively. The unsaturated primary alcohols, including allyl alcohol and 2-buten-1-ol, seemed to work differently from all the other saturated alcohols and unsaturated secondary alcohols in inhibiting various yeasts. An alcohol dehydrogenase-negative (ADH^-) strain of *Saccharomyces cerevisiae* was as resistant to allyl alcohol as various bacteria, exhibiting an MIC of 5.0%. The unsaturated primary alcohols were apparently oxidized into the corresponding unsaturated aldehydes before they inhibited the yeasts.

Key words: Allyl alcohol, 2-buten-1-ol, garlic, alcohol dehydrogenase, acrolein

Allyl alcohol was recently found to be formed in quite considerable quantities when garlic or alliin is heated at cooking temperatures, plus it also exhibits an extremely potent anti-yeast activity [3], and its minimum inhibitory concentration (MIC) is as low as 0.002% for *Candida utilis*. However, allyl alcohol is not as potent for bacteria, the MICs being only equal to or more than 4.0%. Allyl alcohol is already known to be a contributing factor to the flavor of heated garlic [7, 24, 25]. A mechanism for the formation of allyl alcohol has previously been proposed by Block *et al.* [2]. When garlic is heated at 120°C for 45 min or longer, the amount of allyl alcohol formed ranges from 0.080 to 0.162%, depending on the content of alliin in the garlic [11]. Allyl alcohol is the only nonsulfur antimicrobial compound derived from alliin in garlic [3].

Several alcohols, including ethyl alcohol, isopropyl alcohol (propan-2-ol), and *n*-propanol, are widely used as effective skin antiseptics or hard-surface disinfectants [15]. Alcohols exhibit a broad spectrum of antimicrobial activity against all kinds of vegetative microorganisms, including viruses. Generally, the antimicrobial activity of alcohols is significantly lower at concentrations lower than 50%, and is optimal at 60 to 90%. The most effective concentration of ethanol is known to be about 76% (v/v) [9]. The concentrations of ethanol used in food preservation are much lower than those used in antiseptics and disinfectants, yet sufficient to inhibit the proliferation of various microorganisms, although not kill them [9].

Not much is known about the specific modes of the antimicrobial action of alcohols; however, they are generally believed to cause membrane damage and the denaturation of proteins [5], with subsequent interference with the metabolism and cell lysis [10, 14].

Alcohols, including methyl, ethyl, propyl, isopropyl, butyl, amyl, isoamyl, and hexyl alcohols, have all been identified in various foods [8]. Methyl alcohol is formed from the spontaneous enzymatic destruction of pectic substances in over-ripe fruit and wines. Considerable amounts of ethyl alcohol are formed by the anaerobic fermentation of sugars by *Saccharomyces cerevisiae*. Other aliphatic alcohols, including propyl, isopropyl, butyl, amyl, isoamyl, and hexyl alcohols, are found as small-quantity byproducts in products where alcoholic fermentation has taken place.

Allyl alcohol has not been found in foods until recently [3]. Yet, since allyl alcohol is an important industrial starting material for making such products as resins and plasticizers [1], the effects of allyl alcohol have already been extensively investigated [17]. Allyl alcohol is cytotoxic [17] and antimicrobial [3, 12, 16, 18, 22], and the specific potent inhibition of yeasts by allyl alcohol has been attributed to its metabolite formed inside yeast cells. Allyl alcohol is oxidized to a toxic aldehyde, acrolein (acryl aldehyde, 2-propen-1-al), by alcohol dehydrogenase (ADH) [18, 20]. More recently, Lemar *et al.* [12] proposed a cell death mechanism for *Candida albicans* by allyl alcohol, where the yeast cells die from programmed cell death induced by

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oxidative stress, mediated by the generation of reactive oxygen species or alternatively by the depletion of cellular thiols.

Accordingly, the objective of this investigation was to show the selective potent inhibitory activity of allyl alcohol against yeasts in comparison with other commercially available alcohols.

MATERIALS AND METHODS

Materials

The allyl alcohol was purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). The methyl alcohol, propyl alcohol, and isopropyl alcohol were purchased from J. T. Baker (Phillipsburg, NJ, U.S.A.), Mallinckrodt Chem. (Phillipsburg, NJ, U.S.A.), and Janssen Chimica (Beerse, Belgium), respectively. The ethyl and butyl alcohols were purchased from Duksan Pure Chem. Co. (Ansan, Korea). The amyl and isoamyl alcohols were purchased from Wako Pure Chem. Ind. (Osaka, Japan). The hexyl and butenyl alcohols were purchased from Across Organics (Geel, Belgium).

Microbial Strains and Culture Condition

The *Escherichia coli* B34, *Staphylococcus aureus* B33, *Salmonella typhimurium* B38, *Bacillus subtilis* B96, *Saccharomyces cerevisiae* ATCC 4126, *Candida utilis* ATCC42416, and *Pichia membranefaciens* Y20 were all provided by Dr. Henry P. Fleming (Food Fermentation Laboratories, USDA/ARS, North Carolina State University, Raleigh, NC, U.S.A.). The *Lactobacillus plantarum* ATCC14917 *and Leuconostoc mesenteroides* ATCC8293 were provided by the Microbiology Laboratory of Korea University (Seoul, Korea). The *Enterobacter aerogenes* KCTC2190 and *Candida albicans* KCTC7121 were purchased from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea). The *Zygosaccharomyces rouxii* KCCM50523 was purchased from the Korea Culture Collection of Microorganisms (KCCM; Seoul, Korea). The alcohol dehydrogenase-negative mutant strain (ADH^-) of *Saccharomyces cerevisiae* ATCC 64456 was purchased from the American Type Culture Collection (ATCC; MD, U.S.A.).

The bacterial and yeast cultures were stored at −64°C in basal media containing 16% glycerol. The basal media were an MRS broth (Difco Laboratories, Detroit, MI, U.S.A.) for the lactic acid bacteria, tryptic soy broth (TSB; Difco Laboratories) for the non-lactic acid bacteria (non-LAB), and YMPG broth (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose) for the yeasts [4, 21].

Determination of Minimum Inhibitory Concentration

The alcohols were individually dissolved in the appropriate growth medium and filter-sterilized (0.45 μm pore size, 25 mm diameter, Millipore, Billerica, MA, U.S.A.) to make stock solutions that were diluted with a heat-sterilized culture broth to give the desired final concentrations. These media with the desired test concentrations of alcohol were then inoculated with microorganisms to give an initial number of approximately 10^4 CFU/ml and incubated at 30°C for 24 h for the bacteria and 48 h for the yeasts. The sensitivity of the microorganisms was expressed as the minimum inhibitory concentration (MIC). The experiments were all performed in duplicate, and the higher values recorded as the MIC. A complete absence of growth based on a viable count (Spiral Autoplate System, Spiral Biotech Inc., Norwood, MA, U.S.A.) after the incubation period was regarded as no growth.

RESULTS AND DISCUSSION

All the alcohols with 5 or more carbons, including the amyl, isoamyl, and hexyl alcohols, showed essentially the same antimicrobial activity toward both the bacteria and the yeasts, with an MIC within a range of 0.1 and 1.0%. Branching did not affect the antimicrobial activity of the alcohols, as the MICs for the propanol and amyl alcohols were essentially at the same level as the MICs for the isopropanol and isoamyl alcohols, respectively (Table 1).

Antimicrobial Activity of Saturated Alcohols

As the number of carbons in the saturated aliphatic alcohols increased, the antimicrobial potency increased (Table 1), as previously known [19]. The sensitivity towards the saturated aliphatic alcohols did not differ significantly between the yeasts and the bacteria.

Antimicrobial Activity of Unsaturated Alcohols

The anti-yeast activity of the unsaturated aliphatic alcohols showed a completely opposite trend compared with that of their saturated counterparts based on the limited regime of unsaturated alcohols. As the number of carbons in the unsaturated alkenyl alcohols decreased, the anti-yeast potency increased (Table 1). This differential inhibitory pattern was only found in the unsaturated primary alcohols. The anti-yeast activity of 3-buten-2-ol, an unsaturated secondary alcohol, was not as potent as that of its primary counterpart, 2-buten-1-ol (Table 1).

All the alcohols tested showed essentially the same growth inhibitory activity against the bacteria and yeasts, except for allyl alcohol and 2-buten-1-ol, which were approximately three and one orders of magnitude more potent toward the yeasts than the bacteria, respectively. The average MIC of allyl alcohol for the bacteria and yeasts was 5.0% and 0.0056%, respectively. However, the growth inhibitory activity of allyl alcohol toward the bacteria was essentially the same as that of propyl alcohol, a saturated counterpart of allyl alcohol, suggesting that

Table 1. Minimum inhibitory concentrations of various alcohols against selected bacteria[a] and yeasts[b].

Microorganisms	MIC (%)										
	Saturated alcohols								Unsaturated alcohols		
	M	E	P	IP	B	A	IA	H	Allyl	Butenyl	
										PR	S
Escherichia coli B34	10.0	10.0	6.0	8.0	2.0	0.4	0.6	0.4	6.0	1.0<*	1.0<
Ent. aerogenes KCTC2190	10.0	6.0	4.0	6.0	2.0	0.4	0.6	0.4	4.0	1.0<	1.0
Staphylococcus aureus B33	10.0	6.0	4.0	4.0	2.0	0.6	0.4	0.4	6.0	1.0<	1.0<
Salmonella typhimurium B38	10.0	6.0	4.0	4.0	2.0	0.4	0.6	0.4	4.0	1.0<	1.0
Bacillus subtilis B96	10.0	6.0	4.0	6.0	2.0	0.4	0.8	0.4	4.0	0.4	1.0<
Lac. plantarum ATCC14917	10.0	8.0	4.0	6.0	3.0	1.0	0.8	0.6	6.0	1.0<	1.0<
Leu. mesenteroides ATCC8293	8.0	8.0	4.0	6.0	2.0	0.6	0.4	0.4	5.0	1.0<	1.0<
Sac. cerevisiae ATCC4126	10.0	10.0	4.0	8.0	1.5	0.4	0.4	0.4	0.004	0.08	1.0<
Sac. cerevisiae ATCC64456[c]	10.0	10.0	4.0	8.0	2.0	0.4	0.4	0.4	5.0	1.0<	1.0<
Candida albicans KCTC7121	8.0	6.0	4.0	4.0	2.0	0.4	0.6	0.1	0.007	0.06	1.0<
Candida utilis ATCC42416	6.0	7.0	2.5	4.0	1.4	0.4	0.4	0.1	0.002	0.04	1.0<
Zygo. rouxii KCCM50523	10.0	8.0	4.0	4.0	2.0	0.4	0.4	0.4	0.014	0.05	1.0<
Pichia membranefaciens Y20	4.0	4.0	2.0	2.0	1.0	0.2	0.2	0.2	0.001	0.02	1.0<

[a]MIC after 24 h of incubation, [b]MIC after 48 h of incubation, [c]ADH-negative strain.
* MICs were higher than maximum solubility (1.0%) of both butenyl alcohols.
Ent., *Enterobacter*; *Lac.*, *Lactobacillus*; *Leu.*, *Leuconostoc*; *Sac.*, *Saccharomyces*; *Zygo.*, *Zygosaccharomyces*.
M, Methyl; E, Ethyl; P, Propyl; IP, Isopropyl; B, Butyl; A, Amyl; IA, Isoamyl; H, Hexyl; PR, Primary (2-Buten-1-ol); S, Secondary (3-Buten-2-ol).

their inhibitory mechanisms against bacteria were no different from each other.

One explanation for the extraordinarily potent anti-yeast activity of allyl alcohol and 2-buten-1-ol is that the unsaturated alcohols were oxidized by the cellular ADH of the yeasts into corresponding aldehydes [22], where the keto groups are conjugated with double bonds, which in turn are known to be potent protein alkylating agents [16]. Rando [16] reported that yeast ADH oxidizes allyl alcohol into the highly cytotoxic agent acrolein. Secondary alcohols, including 3-buten-2-ol (Table 1), would not be expected to exhibit a potent anti-yeast activity, since they are metabolized into ketones, which are excreted without doing much harm to the cellular proteins [6].

Nonetheless, an ADH^- strain of the species *S. cerevisiae* ATCC64456 was not as sensitive, as the MIC was 5%, which was equal to the average MIC value of allyl alcohol for all the bacteria tested (Table 1). One explanation is that allyl alcohol itself only inhibits yeasts at higher levels, whereas acrolein, the oxidized product of allyl alcohol, inhibits yeasts at a much lower level. Therefore, the potent selective growth inhibitory activity of allyl alcohol is likely involved with the catalyzing activity of yeast ADH. As such, the permeability barrier hypothesis of Lamar *et al.* [12] does not seem to hold. In addition, the MICs of the three-carbon alcohols (propyl, isopropyl, and allyl alcohols) were essentially the same for the bacteria and yeasts (Table 1), with the exception of allyl alcohol for the yeasts, thereby challenging the permeability barrier hypothesis [12] again.

The three different ADH isozymes of microorganisms function differently depending on the type [22]. ADH1 is responsible for the reduction of acetaldehyde to ethanol, as in yeast alcohol fermentation. The function of the ADH type-2 isozyme is to catalyze the oxidation of ethanol to its corresponding aldehyde. Thus, mutations within the *ADH2* structural gene could shift the equilibrium concentrations of allyl alcohol and acrolein in the cells away from the poisonous aldehyde, thereby protecting the microorganisms from acrolein toxicity [18]. There is still another ADH (type 3) whose metabolic function has not yet been elucidated. The susceptibility of *Escherichia coli* to allyl alcohol has already been reported to correlate with the expression of NAD-specific ADH under anaerobic conditions [13]. Meanwhile, ADH-defective mutants of *E. coli* have been found to be resistant to allyl alcohol when they were grown aerobically and anaerobically [23].

Accordingly, the potent anti-yeast activity of allyl alcohol was apparently related to the oxidation of allyl alcohol by cellular ADH into acrolein, a potent protein alkylating agent. In addition under the present test conditions, it was also deduced that a very efficient NAD(P)-dependent ADH was expressed in the yeasts, but not in the bacteria.

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