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Interaction of Long-Chain Alcohol with Dry Yeast, Cholesterol, and Sea Firefly Luciferase

Kibo Nagasaki[1]; Shinya Nagasaki[2*], PhD

[1]*Oakville Christian School, Oakville, Ontario, Canada*
(Current Affiliation: Mentor College, Mississauga, Ontario, Canada)
[2]*Department of Engineering Physics, McMaster University, Hamilton, Ontario, Canada*

Abstract

Background: A hand sanitizer containing alcohol, usually ethanol or isopropanol, is typically used for disinfection, but given that cholesterol is one of the main components of virus envelopes, long-chain alcohol may be more effective. To better understand the potential disinfection activity of long-chain alcohols, we studied their interactions with dry yeast, cholesterol, and sea firefly luciferase.

Methods and Results: We measured, at 30 ºC and 39 ºC, the minimum inhibition concentration (MIC) of dry yeast fermentation and the stability of cholesterol and sea firefly luciferase with alcohols, diols, cetyltrimethylammonium chloride, and stearyltrimethylammonium chloride. The MIC decreased with the chain length at C≤12 for dry yeast and cholesterol with alcohol at 30 ºC. At C_{13} and higher, the cut-off region was observed. At 39 ºC, the cut-off region shifted to C_{15} and higher. The reduction of MIC was measured with the diol or sea firefly luciferase at C≤14.

Conclusion: The presence of the cut-off region is suggested to be related to whether the alcohol is in the liquid state. For the liquid alcohol, the longer the chain length, the lower the MIC. This suggests a potential disinfection activity of long-chain alcohol. **(International Journal of Biomedicine. 2021;11(4):460-466.)**

Key Words: cut-off region • liquid alcohol • minimum inhibition concentration

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Introduction

During the COVID-19 pandemic, people are recommended to wash their hands with soap or an alcohol-based hand sanitizer.[1] The viral envelope of the COVID-19 virus is lipophilic/hydrophobic due to its composition of lipids and cholesterol. However, a question arises as to why we use ethanol or isopropanol but not long-chain alcohols.

The effects of ethanol and isopropanol on disinfection have been widely studied, reviewed, and summarized.[2-8] However, only a few studies have investigated the effect of long-chain alcohols. Kubo et al.[9] investigated the antimicrobial activity of long-chain alcohols and reported only the trend and qualitative results from the standpoint of the alcohol structure. Using alcohols, Mukherjee et al.[10] investigated the antibacterial properties of long-chain fatty alcohols against mycobacteria. Mukherjee et al.[10] found that the best activity was shown by alcohol with a C_{10} chain length, but this was not consistent with the report by Kubo et al..[9] Fletcher et al.[11] studied the inhibitory activity of long-chain alcohols on the growth of *Mycoplasma gallisepticum* and *Mycoplasma pneumoniae* and found that the primary saturated alcohols were more effective than both the unsaturated and secondary alcohols. However, the reason for this difference was not clear. Ingram and Vreeland[12] compared the effects of ethanol with that of hexanol only on the inhibition of the growth of *Escherichia coli*. Gill and Ratledge,[13] Teh,[14] and Kato and Shibasaki[15] studied the inhibition of growth of various microorganisms by *n*-alcohols and found that the inhibition potency increased with the chain length. In sum, few studies have been conducted on

***Corresponding author**: Prof. Shinya Nagasaki, PhD. Department of Engineering Physics, McMaster University, Hamilton, Ontario, Canada. E-mail: nagasas@mcmaster.ca*

the activity of long-chain alcohols from the perspective of disinfection, and the effect is not fully understood.

Many previous studies delved into the impact of long-chain alcohol on the anesthetic effects (a local or general loss of sensation, including pain, achieved by action on the brain or peripheral nervous system to suppress responses to sensory stimulation)[16-18] and the growth of microorganisms[9,19] and reported the presence of a cut-off region (i.e., where alcohols or other compounds suddenly lose their activity and function). However, the reason for this cut-off phenomenon in anesthetic effects and microorganism growth is unknown although some explanations have been proposed.[18,20-22] Furthermore, to the best of our knowledge, no previous study has investigated whether the cut-off is observed in the case of disinfection with long-chain alcohol.

According to Veenstra et al.,[23] the long-chain alcohols showed low acute and repeat-dose toxicity with high-dose effects related to minimal liver toxicity. There was no evidence of toxicity to the reproductive system or the developing organism with these chemicals. The former was performed on male and female rats during premating, mating, and gestation. For up to a year, the latter was studied with rats and dogs. Alcohols with C_6–C_{11} chain lengths are generally considered irritants, whereas those with intermediate chain lengths (C_{12}–C_{16}) are considered mild irritants. These chemicals are broadly and safely used across the consumer products industry with the highest per person consumer exposures resulting from use in personal care products.

Therefore, to understand the activity of long-chain alcohol for disinfection, the dependence of the minimum inhibitory concentration (MIC) on the chain length of alcohol should be studied. Further, we should examine whether the cut-off region is present, and if it is, explain the reason for its presence.

In this work, we studied the MIC of alcohol for dry yeast fermentation and cholesterol stability, examined whether a cut-off region existed, and if yes, investigated the reason for the existence of the cut-off region. This study provides no direct evidence of the efficacy of long-chain alcohols as disinfectants. We believe, however, that it can present new and basic data for discussing a potential activity and possibility of these alcohols for disinfection.

In many studies on disinfection, real microorganisms, bacteria, and viruses were used. Some studies[24,25] have shown that dry yeast can be used to study disinfection, antimicrobial action, and infectious diseases. Moriyama[26] developed biological control materials and used mycovirus and dry yeast to prevent virus-induced rice diseases. The inhibitory effect of alcohols on mycoplasmas was investigated using cholesterol.[11] Furthermore, cholesterol is one of the main components of the envelope of viruses such as SARS-CoV-2. Therefore, we used dry yeast and cholesterol instead of real viruses. In this study, we also used dried sea firefly luciferase.

Materials and Methods

Chemicals

In this study, straight-chain alcohols and diols, cetyltrimethylammonium chloride, and stearyltrimethylammonium chloride were used. All chemicals listed in Table 1 (except 1,4-butanediol, 1,6-hexanediol, and 1,16-hexadecanediol), *N*, *N*-dimethylformamide (DFM), pH buffer solution (pH 6), cholesterol, and cholesterol fluorescence kit were purchased from Fisher Scientific Canada. MilliporeSigma supplied three diols for this experiment. Dried sea firefly was purchased in Monotaro, Japan. The Metro Supermarket supplied dry yeast (Instaferm by Lallemand Inc.) and sugar (granulated sugar by Lantic Sugar Ltd.) (Oakville, Ontario, Canada). Milli-Q Direct 8 equipment was used to create deionized water.

Solutions

The concentrations of ethanol, 1-butanol, 1,2-ethanediol, and 1,4-butanediol were adjusted by diluting the original solutions with deionized water. The long-chain alcohols, diols ($C_6 \leq$ chain length $\leq C_{16}$), cetyltrimethylammonium chloride, and stearyltrimethylammonium chloride were first dissolved in DFM and then their concentrations were adjusted. Since the fermentation of dry yeast is the most active at a pH of ~6, the pH buffer solution (pH=6) was added to all solutions. The final concentration of DFM was 1%. It was previously confirmed that the pH buffer solution and 1% DFM do not affect the fermentation of dry yeast or the stability of cholesterol and sea firefly luciferase.

Based on the preliminary tests, the concentrations of chemicals were decided as listed in Table 1 at first. Using the chemicals listed in Table 1, the lower limit of MIC was determined, below which dry yeast fermentation and the stability of cholesterol and sea firefly luciferase were observed, and the upper limit of MIC was determined, above which these characteristics were not observed. The chemicals were then prepared with concentrations that were slightly finely adjusted between the upper and lower limits of the MIC, and the experiment was repeated. The upper limit of the MIC decided with these slightly, finely adjusted concentrations was adopted as the final MIC value.

Minimum inhibitory concentration

Ishijima and Abe[25] demonstrated the use of the culture media, measurement of the growth inhibition circle, and assessment of the MIC for antibacterial, disinfection, and sterilization purposes. We first measured the MIC for dry yeast fermentation with ethanol using the Ishijima and Abe's method[25] and experimental procedure described below, and the MICs measured using both methods were identical. Therefore, in this study, we used the experimental procedure described below. To confirm the reproducibility of MIC, all measurements were performed 10 times in 10 independent and separate experiments.

Dry yeast fermentation

Five grams sugar and 1g dry yeast were added to a 50 mL centrifuge tube. After 10 mL alcohol solution was added to the tube, the tube was put in the incubator for 30 min. The temperature was controlled at 30 °C or 39 °C.

After 30 min of fermentation, the MIC was determined to be the lowest concentration that completely inhibited the fermentation of dry yeast.

The same procedure was applied with diols, cetyltrimethylammoniumchloride,orstearyltrimethylammonium chloride at 30 °C. The solubilities of cetyltrimethylammonium

chloride and stearyltrimethylammonium chloride are higher than those of alcohols with C_{16} and C_{18}.

Table 1.
Concentrations of chemicals

Chemicals	Target	Concentrations (M)
ethanol	dry yeast cholesterol	1×10^{-2}, 1×10^{-1}, 1, 1.5, 2, 7
1-butanol		1×10^{-2}, 5×10^{-2}, 1×10^{-1}, 5×10^{-1}, 8×10^{-1}, 1, 2
1-hexanol		1×10^{-3}, 1×10^{-2}, 3×10^{-2}, 5×10^{-2}, 1×10^{-1}
1-octanol		1×10^{-4}, 1×10^{-3}, 3×10^{-3}, 5×10^{-3}, 1×10^{-2}, 1×10^{-1}
1-decanol		1×10^{-5}, 5×10^{-5}, 8×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}
1-dodecanol		1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, 5×10^{-5}, 1×10^{-4}
1-tridecanol		1×10^{-7}, 1×10^{-6}, 3×10^{-6}, 5×10^{-6}, 1×10^{-5}
1-tetradecanol		1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 5×10^{-6}, 1×10^{-5}
1-pentadecanol		1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}
1-hexadecanol		1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}
1,2-ethanediol		1×10^{-2}, 1×10^{-1}, 1, 1.5, 2, 7
1,4-butanediol		1×10^{-2}, 5×10^{-2}, 1×10^{-1}, 5×10^{-1}, 8×10^{-1}, 1, 2
1,6-hexanediol		1×10^{-3}, 1×10^{-2}, 3×10^{-2}, 5×10^{-2}, 1×10^{-1}
1,8-octaediol		1×10^{-4}, 1×10^{-3}, 3×10^{-3}, 5×10^{-3}, 1×10^{-2}, 1×10^{-1}
1,10-decanediol		1×10^{-5}, 5×10^{-5}, 8×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}
1,12-dodecanediol		1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 2×10^{-5}, 5×10^{-5}, 1×10^{-4}
1,13-tridecanediol		1×10^{-7}, 1×10^{-6}, 3×10^{-6}, 5×10^{-6}, 1×10^{-5}
1,14-tetradecanediol		1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 2×10^{-6}, 3×10^{-6}, 5×10^{-6}, 1×10^{-5}
1,15-pentadecanediol		1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}
1,16-hexadecanediol		1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 1×10^{-4}
cetyltrimethyl-ammonium chloride		1×10^{-8}, 5×10^{-8}, 1×10^{-7}, 1×10^{-6}
stearyltrimethyl ammonium chloride		1×10^{-9}, 1×10^{-8}, 5×10^{-8}, 1×10^{-7}
ethanol	dry sea firefly luciferase	1×10^{-2}, 1×10^{-1}, 5×10^{-1}, 8×10^{-1}, 1, 2
1-butanol		1×10^{-3}, 5×10^{-3}, 1×10-2, 5×10^{-2}, 1×10^{-1}
1-hexanol		1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3}, 1×10^{-2}
1-octanol		1×10^{-5}, 1×10^{-4}, 5×10^{-4}, 1×10^{-3}, 5×10^{-3}
1-decanol		1×10^{-6}, 5×10^{-6}, 1×10^{-5}, 5×10^{-5}, 1×10^{-4}
1-dodecanol		1×10^{-7}, 1×10^{-6}, 1×10^{-5}, 5×10^{-5}, 1×10^{-4}
1-tridecanol		1×10^{-7}, 1×10^{-6}, 2×10^{-6}, 5×10^{-6}, 1×10^{-5}
1-tetradecanol		5×10^{-8}, 1×10^{-7}, 5×10^{-7}, 1×10^{-6}, 5×10^{-6}
1-pentadecanol		1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}
1-hexadecanol		1×10^{-8}, 1×10^{-7}, 1×10^{-6}, 1×10^{-5}

Stability of cholesterol

One gram of cholesterol was dissolved in 1% DFM. After diluting, 1 mM cholesterol solution was prepared. Then, 100μL cholesterol solution was mixed with 10 mL alcohol or diol in the 15mL centrifuge tube. After 30 min at 30°C or 39°C (incubator), 100 μL of the solution was taken from the tube and fed to the cholesterol fluorescence kit. At McMaster University (Ontario, Canada), the MIC was calculated using a fluorescence spectrometer (excitation: 560 nm, fluorescence: 590 nm). When cholesterol reacts with alcohol, it undergoes esterification and no fluorescence occurs. The MIC represents the concentrations of alcohol and other chemicals below which fluorescence is not detected.

Dried sea firefly luciferase

The bioluminescence of sea firefly (460 nm) is induced by a reaction in which luciferin is oxidized by the O_2 dissolved in the water under the catalytic action of the enzyme luciferase. The bioluminescence emission experiment was conducted according to the experimental procedure by Toya and Ito.[27]

First, 0.1 g of dried sea firefly was pulverized thoroughly in a mortar. Immediately after mixing the pulverized, dried sea firefly with a 1mL alcohol at 30°C, the bioluminescence was measured with the light detector.[28] The light detector converts light intensity to sound intensity. The light detector has a high sensitivity to blue light (460 nm) and was calibrated with a fluorescence spectrometer. It detected bioluminescence from 0.1g of ground dried sea firefly mixed with 1L of distilled water. The MIC was set to be the alcohol concentration at which the sound intensity became the background noise level.

Considering the good reproducibility of MIC measured in 10 experiments, the variation in the concentrations of luciferin and enzyme luciferase between the experiments was considered negligible.

Results

No difference was observed in the MIC values measured in the 10 experiments in this study. This suggests that the measurement data were highly reproducible. Therefore, error bars are not plotted in Figures 1–4 shown below.

Minimum inhibition concentration (MIC) by alcohol for dry yeast fermentation and cholesterol stability at 30°C and 39°C

The extent to which the MIC for the dry yeast fermentation and the stability of cholesterol depends on the chain length of alcohol at 30°C and 39°C are shown in Figures 1 and 2, respectively.

The MIC for dry yeast fermentation decreased with chain length up to C_{12} at 30°C. This is consistent with the lipophilicity/hydrophobicity. Furthermore, the cut-off region was observed at C_{13} and C_{14}. The anesthetic cut-off is a well-known phenomenon at C≥13.[16] This mechanism has yet to be solved. Figure 1 depicts a similar cut-off phenomenon in the inhibition of dry yeast fermentation.

Moreover, the MIC was measured at C_{13} and C_{14} at 39 °C, but not at C_{15} and C_{16}. That is, the cut-off region was shifted to a longer chain length range of alcohol at 39°C.

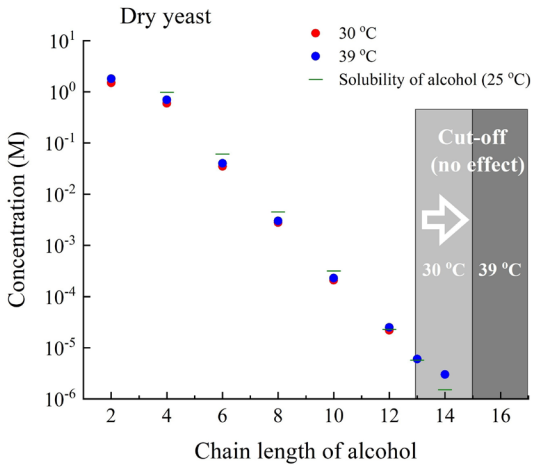

Fig. 1. *Dependence of the MIC for dry yeast fermentation on the chain length of alcohol and solubility of alcohol in water*[32]

The MIC for the stability of cholesterol was slightly smaller than that for dry yeast fermentation, but the trend of the former was the same as that of the latter. That is, the MIC decreased with the chain length of alcohol. There was a cut-off region at a long-chain range, and the threshold of the cut-off region shifted to a longer chain length at 39 °C.

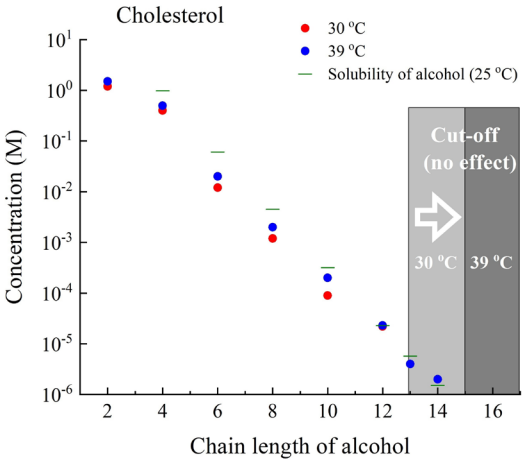

Fig. 2. *Dependence of the MIC for cholesterol stability on the chain length of alcohol and solubility of alcohol in water.*[32]

In the experiment with cholesterol, alcohol directly reacted with cholesterol in the solution. Further, to inhibit the dry yeast fermentation, alcohol had to interact with mannoprotein, β-glucan, chitin, and envelope.[29] This may be why the MIC for cholesterol was slightly smaller than that for dry yeast.

Minimum inhibition concentration (MIC) by diols and cetyltrimethylammonium chloride and stearyltrimethylammonium chloride for dry yeast fermentation and cholesterol stability at 30 °C

Figure 3 depicts the MIC by diol at 30 °C. The MIC decreased as the diol chain length increased. For both dry yeast fermentation and cholesterol stability, the MIC of diol was nearly equal to that of alcohol. The cut-off region was observed at C_{15} and higher at 30 °C.

Furthermore, it was also found that cetyltrimethylammonium chloride (C_{16}) and stearyltrimethylammonium chloride (C_{18}) inhibited the dry yeast fermentation and cholesterol stability at 30 °C.

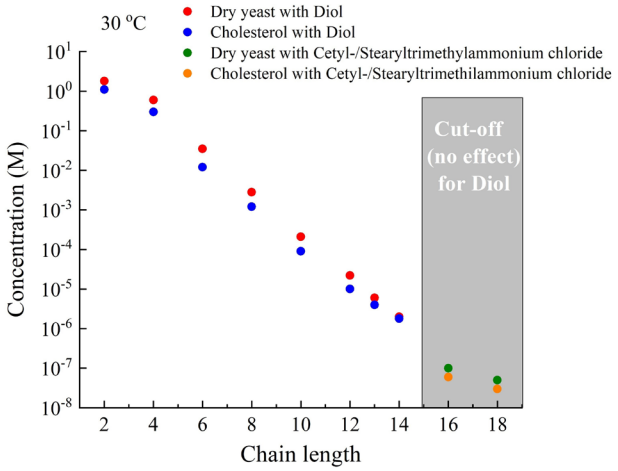

Fig. 3. *Dependence of the MIC for dry yeast fermentation and cholesterol stability on the chain length of the diol. The MIC values for cetyltrimethylammonium chloride and stearyltrimethylammonium chloride are included.*

Minimum inhibition concentration (MIC) by alcohol for dried sea firefly at 30 °C

The dependence of MIC for the bioluminescence of dried sea fireflies on the chain length of alcohol at 30 °C is shown in Figure 4. The MIC of alcohol decreased with chain length up to C_{14}, with the cut-off region observed at C_{15} and C_{16}. The MIC for bioluminescence was lower than the MIC for dry yeast fermentation and alcohol-induced cholesterol stability.

As Oba et al.[30] and Suzuki et al.[31] used firefly luciferase, the sea firefly luciferase is considered to have high sensitivity for alcohol. Hence, the MIC value for bioluminescence was smaller than that for dry yeast fermentation and cholesterol stability.

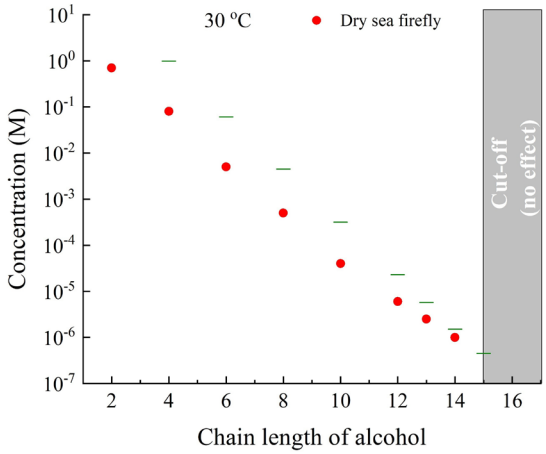

Fig. 4. *Dependence of the MIC for the bioluminescence of dried sea firefly on the chain length of alcohol at 30 °C and solubility of alcohol in water.*[32]

Discussion

Possible reasons for the origin of the cut-off region

Our study found (a) that the MIC decreased with the chain length of alcohols and diols in all our experiments and (b) that the cut-off region was clearly observed for longer chain alcohols and diols under our experimental conditions.

Figures 1 and 2 also show the plots of the solubility of alcohol in water at 25 °C.[32] The solubility of alcohol at 30 °C and 39 °C is unlikely to be lower than the solubility at 25 °C. The fermentation activity of dry yeast is hindered at high temperatures. In daily life, we do not use disinfectants at high temperatures such as 50 °C. The melting points of 1-tridecanol and 1-tetradecanol are 32.5 °C and 37.7 °C, respectively, whereas those of 1-pentadecanol and 1-hexadecanole are 45.5 °C and 49.3 °C, respectively. The solubility of solid alcohol increases upon melting. The melting point of alcohols with C≤12 is lower than 25 °C. Therefore, a temperature of 39 °C was selected in this study.

From Figures 1 and 2, the MIC values at C≤10 at 30°C and 39 °C were found to be smaller than the solubility of alcohol at 25°C; the MIC values at C_{12} at 30 °C and 39 °C were almost equal to the solubility at 25 °C. According to Bell,[32] the logarithm of the solubility of *n*-alcohol in water at 25 °C changes almost linearly with the chain length of alcohol up to C_{16}. When the temperature is raised to 30 °C and 39 °C, this linearity is unlikely to change. Assuming that both the logarithm of solubility at 30°C and the logarithm of MIC at 30 °C change linearly with the length of the alcohol chain, two linear plots intersect between C_{12} and C_{13}. Because solubility increases at 39 °C, the linear plot of solubility at 39 °C between C_{14} and C_{15} may intersect the MIC at 39 °C.

In Figure 3, we used chemicals with higher solubility than alcohol. The MIC values for diol at 30 °C were almost identical to those for alcohol at 30 °C. We could not find the quantitative value of solubility of diol up to C_{16} in water at 25 °C or 30 °C in any research papers. A straight-chain diol, however, has a higher solubility than straight-chain alcohol. Therefore, if the logarithm of diol solubility changes linearly with chain length, we can assume that the diol solubility linear plot intersects with the MIC for dry yeast fermentation and the stability of cholesterol between C_{14} and C_{15}. For cetyltrimethylammonium chloride and stearyltrimethylammonium chloride, which have much higher water solubility than alcohols and diols, the MIC was discovered even at C_{16} and C_{18}.

We also used the sea firefly luciferase, which is highly sensitive to alcohol (Figure 4). Then, we found that the MIC was less than that for dry yeast or cholesterol. The MIC was smaller than the solubility of alcohol up to C_{14}. We considered that the linear plot of solubility of alcohol at 30 °C intersects the linear plot of the MIC between C_{14} and C_{15}.

To summarize, for alcohol to inhibit the fermentation of dry yeast and the stability of cholesterol and sea firefly luciferase, it must be in a liquid state. In a liquid state, the longer the chain, the lower the MIC. When the solubility is smaller than the MIC, the cut-off phenomenon occurs.

Potential activity of long-chain alcohols

In this study, we investigated the interaction of alcohol with the dry yeast, cholesterol, and sea firefly luciferase, not with the real viruses. However, dry yeast was used to study disinfection, antimicrobial action, and infectious diseases. Further, cholesterol is one of the main components of the viral envelope. As a result, the findings of this study suggest that long-chain alcohols may be suitable for disinfection and that the longer the chain of the alcohol, the lower the concentration required for disinfection. Of course, ethanol and isopropanol are useful for disinfection. However, if we need to disinfect wide surfaces such as walls, floors, and ceilings of rooms, long-chain alcohols are convenient, effective, and useful because their costs are lower, and roughening of hands by alcohol can be prevented because of the very low concentration required.

The mechanisms of disinfection with alcohol against viruses and bacterias are not completely elucidated. However, possible mechanisms are as follows:[33-35] (a) alcohol passes through the viral envelope/bacteria membrane and penetrates the cell, increasing the pressure inside the cell. As a result, the cells are destroyed, and the virus is eliminated. (b) The cholesterol reacts with alcohol, converting to ester. Therefore, the envelope is punctured, allowing the contents of the cell to leak out, killing the virus. Alcohol must be in liquid form to work in this manner. Solid alcohol cannot enter the cell or interact with cholesterol. In terms of phenomenology, this is consistent with the possible reasons for the origin of cut-off regions discussed above.

The real viruses were not used to prove the activity of long-chain alcohols and the presence of a cut-off region. However, we consider that the possibility of the potential activity of long-chain alcohols as disinfectants were qualitatively demonstrated. Therefore, further study with real viruses is desired.

Conclusion

The MIC for the dry yeast fermentation and for the stability of cholesterol and sea firefly luciferase decreased with the chain length of the alcohol used. However, the cut-off phenomenon was observed at longer chain lengths. One possible explanation for the cut-off phenomenon is that alcohol is either liquid or solid. Only liquid alcohol can inhibit dry yeast fermentation and maintain the stability of cholesterol and sea firefly luciferase. The results obtained in this study suggest that long-chain alcohols may have a potential activity as a disinfectant.

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Disclaimers

The views expressed in the submitted article are our own and not an official position of the institution.

Conflict of Interests

The authors declare that they have no conflicts of interest.

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