ABSTRACT

Propolis, commonly referred to as “bee glue,” has been well documented in literature as a remedy to fight infections including the viruses that cause herpes and HIV-1. Propolis is a resinsous substance gathered by bees to line and reinforce their hives, and the antimalarial properties of propolis have been shown to prevent microbial infection of bee larvae, honey stores, and combs. In our experiments, we further investigated the antiviral properties of propolis by inhibiting the replication of Ty1, an active long terminal repeat (LTR) retrotransposon in the budding yeast, Saccharomyces cerevisiae. Ty1 retrotransposition replication shares many common steps with retroviruses that infect humans, including HIV, and therefore compounds that inhibit Ty1 have the potential to also inhibit retroviruses. Antiviral effects of ethanol-extracted propolis were tested on the Ty1 retrotransposon. Ty1 replication was measured in their presence of different concentrations and sources of propolis using a Ty1 retrotransposition assay. The Ty1 gene was tagged with a sequence designed to produce its own histidine. Ty1 retrotransposition occurred uninterrupted, the yeast would be able to thrive on a His-plate. The retrotransposition frequency was calculated using the standard formula for calculating mobile elements transposition. Our hypothesis stated that propolis would decrease the retrotransposition frequency of Ty1 by interfering with the reverse transcriptase activities of Ty1 and thus lower the frequency of transposition. While the majority of the samples had little effect on reducing the frequency of retrotransposition, as hypothesized, there was evidence of a reverse effect, i.e., an increase in the retrotransposition frequency of Ty1 in a few cases. While it is unclear why propolis did not impact the frequency of transposition as expected, it is clear that further studies, such as the mechanism of entry of propolis into the yeast cells and effect of propolis on the cells’ reproductive growth period, would be needed to better understand this phenomenon.

INTRODUCTION

Propolis is a resinous exudate of the flower bloss of poplar and other trees and is primarily produced by bees. Propolis origination from various geographic regions vary in both chemical content and amount generated (Schnitzler). Typically, raw propolis is composed of 50% plant resin, 30% waxes, 10% essential and insecticidal oils, 5% polens and 3% other organic substances, including flavonoids. Aside from adding some wax to the substance, the bees largely leave the chemical makeup of the propolis unchanged. Propolis has a variety of uses outside of the hive. It has been used medicinally since 300 BC as an antiviral, antimicrobial, anti-inflammatory agent. Many studies also support its antibacterial, antifungal and antitumor properties. In our studies, we investigated the potential antiproliferative properties of propolis using a standard Ty1 retrotransposition assay as a model system. The cell cycle interactions between the reproductive cycles of Ty1 and HIV are what make Ty1 an ideal model for such an inquiry. HIV is a retrovirus and a large portion of HIV’s reproductive cycle is directly modeled by Ty1 retrotransposition. Ty1 has a gene sequence that naturally exists in Saccharomyces cerevisiae, budding yeast, which was used in our experiments to host cells. The genome of such yeasts have multiple copies of the retrotransposable Ty1, and together with the fact that HIV and Ty1 elements are evolutionarily similar in their genomic structure, makes the yeast an ideal model system to study retrotransposition of the retrotransposable elements such as Ty1. We used a strain of yeast that was engineered with a reporter gene one of the Ty1 copies. Retrotransposition frequency of Ty1 can be measured by determining the percentage of the cells asymptotic that can grow in the absence of histidine. We tested the hypothesis that propolis would inhibit the replication of the Ty1 retrotransposase in Saccharomyces cerevisiae.

RESULTS

Every sample of propolis resulted in an increase in retrotransposition frequency when compared to the wild type (based on a 95% C.I.). Most of these increases were not significant. However one of the samples showed a significant increase at three different concentrations, 0.02%, 0.2%, and 2%. Another sample also showed a significant increase at a concentration of 0.2%. These results do not support our hypothesis, which claimed that the retrotransposition frequency would decrease in the presence of propolis. Propolis solution demonstrated faring characteristics and not of a homogeneous mixture as intended in our research.

PROCEDURE

Ethanol extracts of propolis were made by grinding propolis and dissolving it into 95% ethanol in sealed jars, which were wrapped in aluminum and placed in the dark for two weeks with intermittent shaking.

A dose response study was initially conducted on the yeast strain (Y20112) to test the propolis sample, for their antifungal properties, allowing determination of concentrations to be used on the rest of the assays without causing cell death. The optimal concentration of propolis was measured over a growth of 24 hours at 30°C using a spectrophotometer. Based on this initial study, four concentrations (2%, 0.2%, 0.02%, 0.002%) were determined to be ideal model for such an inquiry. HIV retrotransposition activity occurred uninterrupted, the yeast would be able to thrive on a His-plate. The retrotransposition frequency was calculated using the standard formula for calculating mobile elements transposition. Our hypothesis stated that propolis would decrease the retrotransposition frequency of Ty1 by interfering with the reverse transcriptase activities of Ty1 and thus lower the frequency of transposition. While the majority of the samples had little effect on reducing the frequency of retrotransposition, as hypothesized, there was evidence of a reverse effect, i.e., an increase in the retrotransposition frequency of Ty1 in a few cases. While it is unclear why propolis did not impact the frequency of transposition as expected, it is clear that further studies, such as the mechanism of entry of propolis into the yeast cells and effect of propolis on the cells’ reproductive growth period, would be needed to better understand this phenomenon.

CONCLUSION AND DISCUSSION

In conclusion, the Ty1 retrotransposition frequency of Ty1, it also showed a different result from the one expected in the hypothesis.

An increase in the frequency of retrotransposition when introduced to propolis was also observed in certain cases where normal activity decreased.

The majority of the retrotransposition activity occurred using the 2% propolis solution, the highest concentration used in our experiments. This implied that the propolis at a higher concentration had a greater impact and in many cases it appeared to increase the retrotransposition frequency. These findings contradicted our original hypothesis.

One possible explanation for our results would be that perhaps the propolis was unable to enter the cell and therefore could not interrupt the reverse transcriptase of the Ty1. In addition, the introduction of propolis to this hypervirulently-propionate Ty1 yeast could have encouraged yeast replication either by disturbing the cells’ equilibrium or encouraging reproductive growth, which would cause the retrotransposition frequency to increase. This thought stems from observations made by researchers when they found increased levels of Ty1 RNA and retrotransposition to occur when yeast were infected with various DNA damaging agents, such as UV light, drug, EtO-inhibiting DNA synthesis, (Khorasan). Future study would be valuable to note in the latter cases if propolis is able to enter the yeast cells, but also if propolis increased retrotransposition by encouraging reproductive growth.

Lastly, in adding the propolis to the YPD media, the propolis proved to be lacky solubility in the media. Review of research indicated that propolis is a hydrophilic substance. The 20% extract that was made initially became a thick, foaming substance when exposed to the media, which may have prevented propolis from dissolving completely. Considering propolis is one of the many reasons why propolis was unable to enter the cells might have had, in this case, a hydrophilic substance.

Further studies investigating whether propolis can enter the yeast cells in the first place without damaging them and this was able to cause an unintentioned increase in retrotransposition events would provide valuable insight into this issue.

REFERENCES


Lee B-L, B, Lai, Garrett, D, Balsi AM. Nucleoside Excipient Repair/ThP3: Helicase Rald and 52 Sibid Inhibi1g Sequence Reproduction and Ty1 Retrotransposition By Similar Mechanism. Molecular and Celluar Biology, 2000; 20: 2436-2445.


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