Production of antiviral lignin from sugarcane bagasse by microwave glycerolysis
（マイクロ波グリセロリシスによるサトウキビバガスからの抗ウイルスリグニンの生産）

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Infectious viral diseases threaten human and animal lives and cause huge economic crises. A rapid increase in global transportation and expansion of human and domestic animal populations has magnified the risk of infectious diseases. In addition, recent changes in human behavior, such as excess use of fossil fuels, have intensified global warming as well as the interaction among humans, livestock, and wildlife, thereby increasing the risk of spillover of potential pathogens. In this context, there is increased demand for safe and clean living environments for both humans and animals, in addition to the development of antiviral drugs. If lignocelluloses, the plant cell wall components, could be converted into antiviral agents, the production of antivirals from renewable bioresources would meet the demand for supplying economical antiviral agents, which would be of great help in preventing the spread of infectious viral diseases. At the same time, the development of lignocellulose-derived antivirals adds value to the biomass and thus promotes societal implementation of lignocellulosic biorefinery, which contributes to the goal of a low-carbon society.

In this doctoral dissertation, strategic screening of the chemical reactions that produce antiviral substances from sugarcane bagasse was conducted. Sugarcane bagasse is one of the most abundant lignocellulosic agricultural residues produced from sugar factories. More than 100 million dry tons of sugarcane bagasse are produced annually, and this material is mostly used as fuel for the sugar factories. However, a large amount of bagasse is still unutilized, presenting an opportunity to develop new applications of the residual biomass. In this study, the author studied the production of antiviral substances from sugarcane bagasse and found that antiviral lignin can be produced from the biomass through microwave solvolysis. The detailed chemical structure and antiviral mechanism of the lignin were analyzed. Furthermore, antiviral cellulose-lignin assembly was prepared by coating the lignin on cotton cellulose fibers. Based on the results, it appears that an antiviral material that is totally composed of lignocellulosic biomass was successfully developed in this study.

In Chapter 2, the screening of the reactions that produced the antiviral compounds is described. Sugarcane bagasse was decomposed by microwave solvolysis using Brønsted–Lowry acid, Lewis acid, and alkali. The products were subjected to antiviral activity assay against encephalomyocarditis virus (EMCV), a non-enveloped RNA virus belonging to the family *Picornaviridae*. As a result, it was found that the product reacted through acidic glycerolysis at 200 °C strongly inhibited the viral infection without cytotoxicity. Chemical and spectroscopic analysis revealed that the antiviral substance was structure-altered lignin, designated FR_{200}. To analyze the relationship between antiviral activity and chemical structure, a series of lignins with

different antiviral activities was prepared through bagasse reactions between 140 and 200 °C. When the bagasse was decomposed at lower temperatures, the products retained native lignin structure but exhibited much lower antiviral activity, indicating that the structural alteration of lignin is an important trigger for inducing the inhibitory activity. The innate immune system of the host cell, L929, was not activated by the FR_{200} treatment, and no antiviral activity was found when L929 was pretreated with the lignin before viral infection. Thus, it was found that the antiviral lignin inactivated EMCV through direct contact between the lignin and virions.

In Chapter 3, the characteristics of the antiviral lignin, FR_{200}, in particular the antiviral mechanism and spectrum against enveloped and non-enveloped viruses, is discussed. The lignin inhibited the plaque formation of non-enveloped viruses, EMCV and Theiler's murine encephalomyelitis virus (TMEV), and enveloped viruses, vesicular stomatitis virus (VSV), Sindbis virus (SINV), and Newcastle disease virus (NDV), showing that its antiviral activity emerged regardless of the presence of the envelope. Preparation of a series of lignins with different antiviral activities revealed that higher hydrophilicity induced higher affinity to the EMCV virion and stronger anti-EMCV activity; however, no difference in zeta potential was observed among the lignin preparations. In the acidic microwave glycerolysis, glycerol was incorporated into the lignin, thereby increasing the hydrophilicity of FR_{200}. In addition, FR_{200} had the most abundant carboxylic groups among the series of lignin preparations. However, lignosulfonate, a water-soluble lignin that has high hydrophilicity, exhibited no antiviral activity against EMCV. Thus, it can be concluded that the changes in the hydrophobicity and hydrophilicity balance, along with structural alteration, is essential for the antiviral activity to emerge. Analysis of the antiviral mechanism showed that the lignin inhibited the viral attachment to host cells. Transfection and electrophoresis experiments indicated that the EMCV RNA was intact after the treatment with FR_{200}. Therefore, the lignin functions as entry inhibitor by interfering with the viral attachment to host cells through the interactions with the capsid surface.

In Chapter 4, the antiviral assay involving feline calicivirus (FCV) as a target virus is described. It was found that FCV was strongly inhibited by the lignin through direct contact. Given that FCV is used as a surrogate of human norovirus, the results of the assay demonstrate the potential of the lignin as a disinfectant against human norovirus. The lignin was immobilized on cotton with the use of simple soaking methods. Even coating of the lignin onto cellulose fibers was confirmed through fluorescence microscopy and ATR FT-IR analysis. Although the solid lignin exhibited no anti-FCV activity, the lignin cellulose assembly reduced the FCV titer by filtration of a FCV solution. In addition, the FCV RNA in the filtrate was decreased, supporting that the lignin-coated fibers captured the virion. The development of antiviral fibers that are totally composed of plant biomass will contribute to the safeguarding of daily life environments against viral threats.