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Development of Biomass Conversion System with Hydrophilic Ionic Liquid and Enzyme from Extremophile

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Research aims

Ionic liquids (ILs) are composed of a bulky asymmetrical cation and a small anion, both of which are easily replaced; therefore, numerous compositions of ILs are possible. Unlike conventional organic solvents, ILs are able to dissolve many compounds. Thus, ILs are useful as reaction media[1].

Using a hydrophobic IL as reaction solvent, we developed a convenient procedure for enzymatic synthesis of 3-cyclohexylpropyl caffeate, which exhibits a strong antiproliferative activity on human tumor cells[2]. However, we found difficulties in using hydrophilic ILs as reaction solvents in the enzyme reaction[3]. It is suggested that hydrophilic ILs can cause changes in enzyme conformation and thereby enzyme denaturation, probably through their effect of reducing water activity[4]. Thus, it would be desirable to develop enzymes that are stable and functional in hydrophilic ILs.

The purpose of this research was to elucidate the mechanism of resistance to hydrophilic ILs in hydrophilic IL-tolerant microorganisms, and to identify enzymes that are stable in hydrophilic ILs.

Methods

Using various food samples as bacterial sources, we obtained a number of bacterial isolates that could grow in broth media containing a hydrophilic or hydrophobic IL. The hydrophilic ILs used were 1-ethyl-3-methylimidazolium trifluoromethanesulfonate ([EMIM][CF_3SO_3]), 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([BMIM][CF_3SO_3]), and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]), and the hydrophobic ILs were 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]) and 1-butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide ([BMIM][NTf_2]). The bacterial isolates were identified by 16S rRNA gene sequence analysis. Using LC-MS (Waters, MA), the [BMIM] cation (*m*/*z* 139.1 as molecular ion, *m*/*z* 83.1 as fragment ion) was quantified. Enzyme activities in the culture supernatant containing the hydrophilic IL were detected with the APIZYM system (bioMérieux, France).

Results

(1) Isolation of bacteria that could grow in the presence of hydrophilic ILs

Mixed bacterial floras from kusaya gravy and Korean pickled cabbage were inoculated into a broth medium containing [BMIM][PF_6] or [EMIM][CF_3SO_3]. From the bacterial communities grown in the media, we obtained bacterial isolates that could grow in broth media containing hydrophilic or hydrophobic IL. The 16S rRNA gene sequences of all isolates had the highest similarity to those of bacteria belonging to the genus *Staphylococcus*.

(2) Enzyme activities detected in culture containing hydrophilic IL

A mixed bacterial flora from kusaya gravy was inoculated into a broth medium containing hydrophilic IL (10% [EMIM][CF_3SO_3]). Good growth was observed with the culture. In the supernatant of the bacterial culture thus ob-

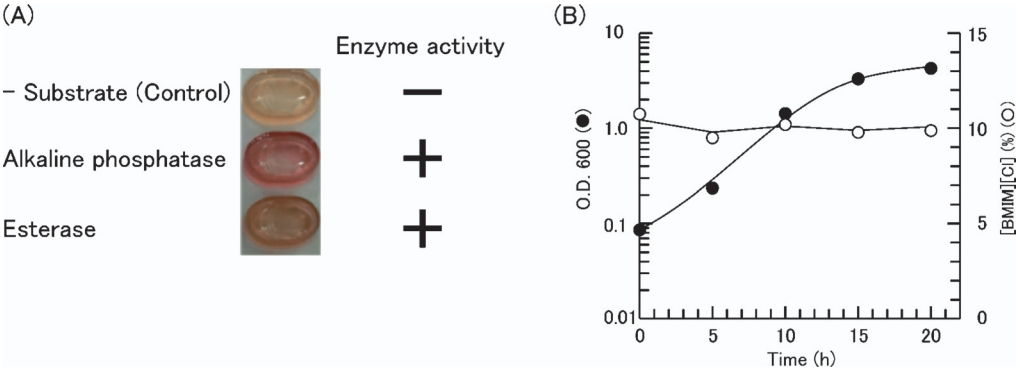

Fig. 1. Enzyme activities detected in the supernatant of culture broth containing hydrophilic IL ([EMIM][CF_3SO_3]), in which a mixed bacterial flora from kusaya gravy was grown (A) and growth of *Staphylococcus* sp. SM-1-W in the presence of the hydrophilic IL (B)

tained, alkaline phosphatase activity and esterase activity were detected with the APIZYM system (Fig. 1A).

(3) Characterization of an IL-tolerant *Staphylococcus* sp. strain

The bacterial isolate *Staphylococcus* sp. SW-1-M was found to be capable of growing in broth containing 10% [EMIM][CF_3SO_3], 10% [BMIM][Cl], 10% [BMIM][PF_6], or 10% NaCl, but not in broth containing 10% [BMIM][NTf_2] or 10% [BMIM][CF_3SO_3]. LC-MS analysis of the culture broth samples suggested that [BMIM] cation was not degraded during cultivation. Thus, it was indicated that *Staphylococcus* sp. SW-1-M is a hydrophilic IL ([BMIM][Cl])-tolerant bacterium (doubling time 102.2 min) (Fig. 1B).

Conclusion

Bacterial isolates obtained from food samples were examined for growth in broth containing hydrophilic or hydrophobic ILs. One isolate, *Staphylococcus* sp. SW-1-M, grew adequately in the broth containing 10% [BMIM][Cl]. When a mixed bacteria population from kusaya gravy was grown in culture medium containing 10% [EMIM][CF_3SO_3], alkaline phosphatase activity and esterase activity were detected in the culture supernatant. It was reported that cellulose is soluble in certain hydrophilic ILs, namely, [BMIM][Cl] and *N*-ethyl-*N′*-methylimidazolium methylphosphonate[5,6]. We have already obtained bacterial communities capable of growing adequately in the presence of carboxymethyl cellulose or [BMIM][Cl]. Thus, using those IL-tolerant bacteria and hydrophilic ILs, a novel system for efficient conversion of cellulose could be constructed.

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