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Foliar penetration enhanced by biosurfactant rhamnolipid

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ABSTRACT

With recent environmental and health concerns, biosurfactants have obtained increasing interest in replacing conventional surfactants for diverse applications. In agriculture, the use of surfactant in stimulating foliar uptake is mainly for wetting leaf surface, resisting deposition/evaporation, enhancing penetration across cuticular membrane (CM) and translocation. This paper aimed to address the improved foliar uptake by rhamnolipid (RL) in comparison with the currently used alkyl polyglucoside (APG). As found, compared with APG at 900 mg/L (1 × critical micellar concentration, CMC), RL at a much lower concentration of 50 mg/L (1 × CMC) showed much better wettability and surface activity, indicative of its high effectiveness as surfactants. Its performance on resistance to deposition and evaporation was at least as same as APG. Moreover, RL could significantly improve the penetration of herbicide glyphosate and other two small water-soluble molecules (phenol red and Fe^{2+}) across CM at an equivalent efficiency as APG at 1 × CMC. Finally, the greatly enhanced herbicidal actitivity of glyphosate on greenhouse plants confirmed that RL and APG could both enhance the foliar uptake including translocation. Overall, RL should be more applicable than APG in agriculture due to its more promising properties on health/environmental friendliness.

1. Introduction

Surfactants are usually amphiphilic organic compounds containing both hydrophobic head and hydrophilic head. As surfactants can lower the surface tension of liquids, over 12 million metric tons per year are consumed in divers industries and daily life [1]. Likewise, modern agriculture also needs huge quantities of surfactants in either controlling pests or promoting plant growth, aiming to meet the growing global demand for food productions. In agriculture, surfactants are not only used as emulsifiers and dispersing/wetting agents in stabilizing fertilizers and pesticides [1], but also function as spreaders, stickers and penetrants for enhancing the biological activities. Particularly, surfactants are critical important in foliar spraying agents to stimulate the uptake of the soluble active ingredients by plant leaf [2]. Otherwise, the hydrophilic molecules are difficult to cross the cuticular membrane (CM) which is covered with insoluble wax components [3].

Nevertheless, most surface-active compounds are chemically synthesized from petrochemical sources and thus can adversely affect the environment due to low biodegradability. Similarly, the surfactants annually consumed in agriculture at a volume of at least 0.23 million metric tons [4] have been frequently reported for its environmental toxicity [5,6]. Unlike the application in industrial field, the surfactants as agrochemicals are generally released into the environment without prior waste treatment and then accumulate in the river, sea or soil [7], causing potent organic pollutions on soil and aquatic environment [8]. Hence, the feature of biodegradability will be critically important for surfactant at their use in agriculture.

The biologic safety is another major concern at selecting surfactant for agriculture use because they can damage cells by disrupting cell membrane at low concentrations. Hence, such agricultural adjuvants have been reported of adverse effect on bacteria [9], animals [10] and plants [11], particularly being worse on the aquatic animals/micro-ecosystems like tadpoles, frogs and fish [12,13]. Moreover, such adverse effect are much severe than those of pesticide alone [14]. For an example, the surfactants in commercial glyphosate products of Roundup largely exacerbated the toxic sensitivity of glyphosate by reducing a half of toxic dose on rats [15]. The surfactants in glyphosate formula caused much severer cell necrosis than the active ingredient alone on human cells [16]. Even worse, some chemical surfactants like phenyl phenols showed adverse allergic effects, endocrine disruptions [17] and gene toxicity [9] on human/environment biological systems.

Abbreviations: RL, rhamnolipid; RLs, rhamnolipids; CMC, critical micellar concentration; APG, alkyl polyglucoside; CM, cuticular membrane.

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Considering the environmental and health safety, green surfactants have being encouraged for use in enhancing crop yield without any harmful side effects. Biosurfactants produced by microbials are the most desired "green" surfactants for such use in view of their biological production process and friendly end-use for both environment and health [18,19]. Moreover, biosurfactants could also improve the quality of agriculture soil via fascinating biodegradation of hydrocarbonates or removal of heavy metals[6], making their uses intensively attractive in agricultural area.

However, the potential application of biosurfactant on enhancing the foliar penetration of soluble molecules has never been scientifically investigated. This study will address the possible function of rhamnolipid (RL) in enhancing the foliar penetration of soluble molecules in comparison to alkyl polyglucoside (APG) [4], an extensively used chemical surfactant in agriculture. As well accepted, RL secreted by microbials for solubilization of hydrocarbons as well as modification of surface properties [20], is the most intensively focused biosurfactants for their superior environment/health friendliness as well as functions on stimulating bioremediation of soil or water [21,22]. Also, RL has been produced at commercial scale and might sustain a cost-efficiency as chemical surfactants in near future [23]. These features could provide excellent opportunities for the use of RLin agriculture.

2. Materials and methods

2.1. Materials

RL with purity over 95% were obtained from Huzhou Gemking Biotechnology Co., Ltd. (Huzhou, China) and its major components are di-rhamnolipid ($C_{32}H_{58}O_{13}$) and mono-rhamnolipid ($C_{26}H_{48}O_9$) at a mass ratio of 2.6 [18]. The aqueous glyphosate solution at a concentration of 41% (wt) (isopropyl amine salt) was obtained from Hangzhou Wynca Corp (Hangzhou, China). *Epipremnum aureum* and *Iris pseudacorusand*s at seedlings were both purchased from local market and incubated in green house. The remaining chemicals were supplied by a local supplier and all are of reagent grade.

2.2. Surface tension and critical micellar concentration (CMC)

Surfactants (RL and APG) were dissolved in distilled water as a stock solution at the concentration of 10 g/L and 180 g/L, separately. The solution was then diluted before use for detecting the surface tension. The surface tension of the solutions was measured at 25 °C using a surface tensiometer (JYW-200A, Chengde, China) following the Du Nouy Ring method [24].

2.3. Detection of contact angle on the surface of plant leaves

Iris pseudacorusands and *Epipremnum aureum* were grown in green house where sunshine was sufficient and the temperature ranged from 20 to 26 °C. The leaves of each plant were cut into 2 cm pieces and fixed onto a glass slide with topside facing upward using double-sided adhesive tape. Droplets of the tested solutions at 1 μL were dropped onto one test specimen. Contact angles of the droplets were measured using a contact angle/surface tensionmeter (JC2000C1, Shanghai Zhongchen Digital Technology Equipment Co., LTD, Shanghai, China). During the measurement, photo of tested droplet was meanwhile taken.

2.4. Penetration of water-soluble molecules across isolated CM

Epidermal discs at a diameter of about 15 mm were cut from *Iris pseudacorusands* without the visual defects. Following a widely-used procedure [25], discs were incubated in citric acid buffer (20 mM, pH 3.8) containing 2% (w/v) pectinase (containing hemicellulase, cellulase, pectinesterase and xylanase with specific activity over 500 AJDU/mg, BBI) at 25 °C. The enzyme solutions were refreshed every 2 days until the CM was separated from the digested tissue. Subsequently, CM were rinsed with deionized water before dried on Teflon discs and stored at room temperature until use. All CMs used in this study were originated from the same collection site at the same harvest date.

The penetration of soluble molecules across cuticles is determined in infinite diffusion experiments [26]. Phenol red and ferrous ions (Fe^{2+}) were used for presenting for small water-soluble molecules while glyphosate represented for hydrophilic pesticide. Cuticles were mounted in filter paper holders sealed by epoxy resin and a rubber band [25], whereas the morphological outer side of the CM faced the donor cell. All CMs were checked for leakages/integrity by observing the penetration of PBS buffer solution which had been filled into the donor cells at a volume of 700 μL. The CM which could maintain constant hydrostatic pressure for at least 2 h was used in the subsequent experiments. In diffusion experiments, 150 μL of the soluble molecule solution containing phenol red (0.4 mmol/L) with/without presence of surfactants replaced the PBS buffer in the donor cell while 700 μL of PBS buffer was filled into the receiving cell. Samples were taken from the receiver at 24 h for determining the phenol red concentration using micro plate reader (Bio Rad 680, China) at 492 nm. The penetrations of Fe^{2+} and glyphosate were similarly investigated using 0.013 mol/L of Fe^{2+} solution or 0.071 mol/L of glyphosate solution instead of phenol red solution. The concentration of Fe^{2+} was detected following the previous assay procedure [27]. After derivatized with *p*-toluenesulfonyl chloride under alkaline conditions as previously described [28], the concentration of glyphosate was detected at 240 nm using Varian Prostar 210 Series HPLC (Walnut, creek, CA) with a C18 column, whereas 0.2 M phosphate buffer (pH 2.30)—acetonitrile (85:15, v/v) was used as a mobile phase.

2.5. Spread and evaporation of glyphosate droplet on leaf surfaces

Tested leaf samples and glyphosate solutions were respectively prepared as same as in the above-mentioned contact angle and penetration measuring experiment. A leaf segment of plant blade was fixed onto a glass slide with double-sided adhesive tape. For each plant, 1 droplet of deionized water, glyphosate, glyphosate with APG or RL was dropped onto 1 leaf segment separately with a volume pipette (Eppendorf, Germany). The droplet volume was 2 μL. Time for complete evaporation of the droplet was recorded with a stopwatch and the picture of each treated leaf was taken using a camera (Panasonic DMC ZS10, Japan). This procedure was conducted under the condition of temperature at 20 °C and relative humidity of 60–65%. The spread area was determined by tracing the marked outline of the droplet spread on the leaf surface using the free select tool of GIMP software (version 2.8). An integrated index (λ) was defined as the product of the spread area and the evaporation time of a droplet on a leaf surface [29].

2.6. Deposition of glyphosate on leaf surfaces

One 2 μL-droplet of the prepared glyphosate (isopropyl amine salt, 0.071 mol/L) solutions with or without presence of surfactants was dropped onto the topside of the *Iris pseudacorusands* with a volumetric pipette. The water was similarly dropped as a negative control. About 1 cm-long leaf piece after 6 h of treatment was cut from the plants after the droplet completely evaporated and then was fixed onto a specimen holder (NISSHIN EM. Co., Ltd. Tokyo, Japan). The deposited glyphosate on leaf surface was immediately observed under a scanning electron microscope (HITACHI