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Rapid separation of antimicrobial metabolites by microchip electrophoresis with UV linear imaging detection

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Abstract

This research examines microchip electrophoresis with linear imaging UV detection for the analysis of antimicrobial metabolites, monoacetylphloroglucinol (MAPG) and 2,4-diacetylphloroglucinol (2,4-DAPG) from *Pseudomonas fluorescens* F113. Initial results show the separation of MAPG, 2,4-DAPG and resorcinol in less than 20 s. This was achieved using a quartz microchip with a separation channel length of 25 mm. In order to quantitate the amount of MAPG and 2,4-DAPG in a microbial cultured supernatant sample, on-chip sample introduction in a methanol/buffer matrix was investigated. Sample introduction/injection parameters were optimized to improve sensitivity and thus decrease the limit of detection (LOD). The amount of antimicrobial metabolites present was quantitated with a separation time of 15 s. A previously developed capillary electrophoretic method was compared to the microchip method in relation to speed, efficiency, precision, linear range and limit of detection. This investigation shows the fastest separation so far of these antimicrobial metabolites with high efficiency.

Keywords: Monoacetylphloroglucinol (MAPG); 2,4-Diacetylphloroglucinol (2,4-DAPG); Antimicrobial metabolites; Microchip electrophoresis (MCE); *Pseudomonas fluorescens* F113

1. Introduction

The human genome project has left a legacy of interest in gene expression. This has resulted in intense research focus in areas such as genomics, proteomics and metabolomics. The metabolome includes hundreds of low-molecular-weight compounds that play vital roles in relation to the complex biochemical and metabolic processes occurring within cells and biological fluids. Metabolites are chemically diverse compounds that are released in a wide concentration range [1]. The term secondary metabolite [2] was first mentioned in the early 1960s to indicate microbial metabolites found as products of differentiation in restricted taxonomic groups and not necessary for metabolism.

Microchip electrophoresis (MCE) [3] is an emerging new technology that epitomises the current trend towards miniaturization of chemical systems. The main advantages include the capability of assaying complex multicomponent matrices in record time periods of a few seconds. Microchips have the potential to challenge more traditional columns and capillaries due to speed, size, versatility, portability, reduced solvent and sample consumption and lower cost. The ability of a microchip platform to deliver high-speed separations and thus high sample throughput has allowed this format to deliver some of the fastest separations reported to date [4]. Constant demands within the pharmaceutical, clinical and biotechnology sectors for rapid, cheaper, highly efficient separations have catalyzed the need for these miniaturized separation techniques and devices.

Phloroglucinols have many important roles to play in both medicine and agriculture. Release of antimicrobial agents by microorganisms, in particular phloroglucinols, is recognized as a significant biocontrol mechanism for inhibiting plant disease and death. The polyketide metabolite 2,4-diacetylphloroglucinol (2,4-DAPG) is made by many strains of fluorescent *Pseudomonas* spp. with a major function being biocontrol of soil-borne fungal plant pathogens [5]. While monoacetylphloroglucinol (MAPG) possesses antibacterial properties, 2,4-DAPG displays stronger potency [6].

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2,4-DAPG plays a major role in the biological control of numerous plant-destroying diseases. This phenolic compound has broad-spectrum antibacterial and antifungal properties [7]. As with many natural products, phloroglucinol (benzene-1,3,5-triol) is used in medicine as a muscle relaxant, as it shows no anticholinergic potency and appears to have lower toxicity than other antispasmodic agents [8]. To date, chromatographic methods have played an important role in the analysis of phloroglucinol compounds. Lartigue-Mattei et al. employed a method for determining phloroglucinol in human plasma by gas chromatography–mass spectrometry [9]. Similar studies by Kim et al. resulted in a sensitive and selective liquid chromatography–mass spectrometric (LC–MS) method for determining phloroglucinol in plasma samples [8]. Bonsall et al. have reported antibiotic isolation from both soil and broth cultures with further quantitation by reversed-phase high-performance liquid chromatography [10]. Shanahan et al. had previously published a reversed-phase liquid chromatographic analysis of 2,4-DAPG in culture and soil samples [11]. Later, Shanahan et al. employed a gradient LC assay for determining MAPG and 2,4-DAPG in growth culture media [12]. More recently, Picard and Bosco studied 2,4-DAPG production from different strains of *Pseudomonas* using [13], a method previously described by Keel et al. [14]. Kamei and Isnansetyo published findings of a novel antibacterial mode of action of 2,4-DAPG against methicillin-resistant *Staphylococcus aureus* (MRSA) [15]. Recently, Siddiqui and Shaukat revealed the importance of the bacterial metabolite 2,4-DAPG in the suppression of root-knot disease in tomatoes [16].

This work investigates the rapid separation of resorcinol along with metabolites MAPG and 2,4-DAPG on a quartz microchip with UV linear imaging detection. UV detection eliminates the need for sample derivatization and is more convenient than the commonly used laser induced fluorescence detection systems [17]. Typical separation parameters varied during optimization, included voltage, buffer concentration and pH, and sample introduction and separation times. A sub-study of different methods of on-chip sample introduction was also undertaken. A previously developed CE method [18] is compared with the microchip method in relation to speed, efficiency, precision, and limit of detection (LOD). A microbial cultured supernatant sample from *Pseudomonas fluorescens* F113 was for the first time analyzed by MCE, and its metabolite content determined in 15 s.

2. Experimental

2.1. Instrumentation

All electrophoretic separations were performed using a Shimadzu microchip electrophoresis (MCE) 2010 system (Shimadzu GmbH, Duisburg, Germany). The detection system was a UV linear imaging system (190–370 nm) under normal polarity settings (towards the cathode). The operating system was Windows 2000 and the software was MCE-2010. The system utilised a 0–1800 V high-voltage supply. The Shimadzu quartz microchip employed for all separations have dimensions of 30 μm width × 30 μm depth with a total separation/analytical channel length of 25 mm. There were four platinum electrodes on the chip to apply voltages between the sample introduction and separation reservoirs, the chip were encased in a polypropylene frame. The quartz microchip was rinsed between separations using an inbuilt automatic buffer wash. If blockage was suspected, the microchip was rinsed with 0.1 M NaOH using a hand-held plastic syringe for 15 min. At the end of each day, an inbuilt automatic water-flushing programme was switched on so that the microchip would not become dry. At all times, the microchip was stored in the MCE instrument. On applying voltages between the microchip reservoirs, SI and SO refer to sample inlet and outlet positions, while BI and BO refer to the buffer inlet and outlet.

2.2. Reagents

Sodium tetraborate (borax), phloroglucinol (anhydrous) and boron trifluoride diethyl etherate complex (for the synthesis of 2,4-DAPG) were purchased from Sigma–Aldrich (UK). HPLC grade methanol was purchased from Labscan Ltd. (Dublin, Ireland). HC1 and NaOH were purchased from E. Merck (Darmstadt, Germany). Tetrahydrofuran (THF) was purchased from Labscan Ltd. (Dublin, Ireland). Resorcinol (99%) was purchased from Sigma–Aldrich (Dublin, Ireland). 2,4-DAPG was synthesized in our laboratories according to the Dean and Robertson procedure [19]. MAPG (98%) was purchased from Sigma–Aldrich (Dublin, Ireland) under the trade name 2,4,6-trihydroxyacetophenone monohydrate. Dual filter membranes (0.45 μm) were purchased from Millipore Ltd. (Cork, Ireland). Disposable plastic syringes (2 mL) were purchased from Lennox Laboratory Supplies (Dublin, Ireland). A plastic 1 mL syringe unit with an inbuilt filter for manually rinsing the microchip was supplied with the microchip courtesy of Shimadzu (Duisburg, Germany).

2.3. MAPG and 2,4-DAPG standard preparation

Standard solutions in the range of 0.5–400 mg/L MAPG and 2,4-DAPG were prepared in 50% sodium tetraborate (25 mM, pH 9.3) and 50% methanol for calibration and method development studies. These standard solutions had a final concentration of 12.5 mM sodium tetraborate. In addition, a stock solution of MAPG, 2,4-DAPG and resorcinol (500 mg/L) was prepared in 100% sodium tetraborate (50 mM, pH 9.3), for analysis by pinched sample introduction. All solutions, buffers and samples were filtered through dual filter membranes (0.45 μm), and stored in a fridge.