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APPLICATION NOTE No. 397

Fast Screening Method for Natural Anti-Biofilm Compounds with the ep*Motion*® 96xl

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Abstract

The screening for active compounds with antibacterial or anti-biofilm effects on microorganisms are often conducted in microtiter plate-based assays. Many samples can be tested with a sufficient number of replicates in a short time. In this application note, the screening of the naturally occurring antioxidative flavanonol taxifolin, found in several plant species, for its potential anti-biofilm and antimicrobial activities is described. For the pipetting steps in the biofilm assay, the ep*Motion* 96xl was used to reduce the time demand and increase the reproducibility.

Taxifolin concentrations of 500, 600 and 700 µg/mL showed a significant reduction of *Escherichia coli* cell adhesion. In addition, the effect on the growth rate of planktonic *E. coli* cells was measured; here, a maximal reduction of the growth rate was observed at 700 µg/mL, but without statistical significance. These experiments with taxifolin make it a promising reagent to be tested further on different microorganisms and to analyze its mode of action. The use of the ep*Motion* 96xl decreased the time and improved the reproducibility of the data. Therefore, it is highly recommendable for screening purposes.

Introduction

Biofilms are sessile life forms of various microorganisms, mainly bacteria and fungi. During the formation of the biofilm the microorganisms produce a three-dimensional matrix consisting of extracellular polymeric substances [1]. In this sessile state, the microorganisms are more resistant to environmental impacts and antibiotic treatment. Formation of a biofilm can cause physical damage in industrial systems, but also several diseases are linked to biofilms [2]. To avoid biofilm formation, new and less toxic substances than known anti-biofilm compounds are needed. For medical applications, these substances should be non-toxic for humans to avoid side effects. Natural compounds like flavonoids found in traditional medicinal plants are often non-toxic and might present anti-biofilm activities [3]. For testing these substances, fast and high-throughput applications are useful. In this work, the flavonoid taxifolin was analyzed for its anti-biofilm activity with the ep*Motion* 96xl. Flavonoids are polyphenolic compounds produced in plants to color flower petals, but also act as antioxidants and reduce light stress. Taxifolin can be found in plants like the Siberian larch [4], but also in many other species in lower concentrations. There is evidence of inhibitory activity of taxifolin on quorum sensing in *Pseudomonas aeruginosa* [5], which indicates an inhibiting effect on the formation of biofilms. It is also approved for human consumption as a food additive, as it has no toxic properties [6].

This makes taxifolin an interesting candidate for further analysis of its biofilm-inhibiting activities. *Escherichia coli* was chosen as a model organism for gram-negative bacteria to test for a general ability to inhibit the attachment of bacteria to surfaces, the first step of biofilm formation.

Microtiter plates with 96 wells allow the handling of a high number of samples with sufficient replicates on one plate. The tests require many pipetting steps, which are usually carried out by hand. A semi-automated pipetting system can reduce the time and improve the accuracy and comparability of the test results.

Materials and Methods

Cells of *Escherichia coli* ATCC 25404 were grown in Luria-Bertani (LB) medium at 37°C over night with constant shaking. The next day, 2 mL of the culture were centrifuged for 6 min at 7,788 g and dissolved in 2 mL fresh LB medium. The cell density was calculated using a hemocytometer. A concentration of 108 cells/mL was prepared. Taxifolin standard (Sigma Aldrich®, Taufkirchen, Germany) was diluted to 1.25, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 and 17.0 mg/mL with ethanol. Samples were prepared in sterile reaction tubes with 3 % *E. coli cells* (108 cells/mL) and 4 % taxifolin standard or ethanol as control. In this way, the taxifolin concentrations in the samples were 50, 100, 200, 300, 400, 500, 600 and 700 µg/mL. From each sample, 200 µL were filled in one well of a black 96 well microtiter plate (96F nontreated, Thermo Fisher Scientific®, Dreieich, Germany) with six replicates per sample. Three plates were prepared in parallel and incubated for 24 h at 37°C. In addition, one clear 96 well plate (Microtest plate 96 well R, Sarstedt®, Nümbrecht, Germany) was filled in the same way, sealed with a foil (Breathe-easy, Diversified Biotech®, Dedham, USA) and placed in a microplate reader (Synergy™ Mx, BioTek, Winooski, USA), which measured the OD600 for 24 h every 20 min. After incubation, the liquid in the black microtiter plates was removed with the ep*Motion* 96xl. Tip height was set to around 2 mm above the well bottom, so the adhered cells were not removed. Remaining liquid in the edges was carefully removed with a pipette. The plates were dried at 40°C for 30 min and then the adhered cells were stained with 150 µL 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, Sigma Aldrich) for 20 min. The staining solution was removed and 200 µL phosphate buffered saline (PBS) were added and removed again to wash excessive staining twice. These steps were conducted with the ep*Motion* 96xl and 1,000 µL epT.I.P.S.® motion reloads. After the last removal of liquid, the plate was dried and the fluorescence was measured at 355/460 nm.

Results and Discussion

The flavanonol taxifolin was tested in different concentrations on the adhesion ability of *E. coli* to hydrophobic surfaces of 96 well microtiter plates. After static growth for 24 h, the attached cells on the bottom of the microtiter wells were stained with a fluorescent dye (DAPI) and the signal was measured. Concentrations of 500, 600 and 700 µg/mL taxifolin showed a significant reduction in the fluorescence signal of the attached cells (Fig. 1) with the highest reduction of 71.6% at 700 µg/mL (Fig. 2). Lower concentrations of 50 to 400 µg/mL showed no significant reduction. The same taxifolin dilutions were analyzed for their influence on the general growth rate of *E. coli*. The optical density of cells growing at 37°C for 24 h was recorded in clear microtiter plates. Here, the results showed a reduced growth rate, but with no statistical significance (Fig. 3).

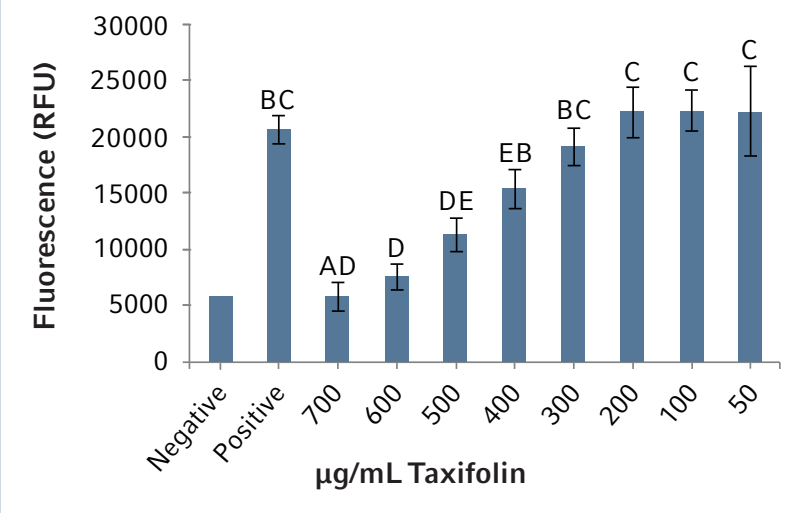

Figure 1: Fluorescence signal in relative fluorescent units (RFU) of *E. coli* cell adhesion after 24 h. Mean of three individual plates with six replicates (wells) per sample are shown. Different letters indicate a statistically significant difference with $p<0.05$ calculated with a one-way ANOVA followed by Tukey's test.

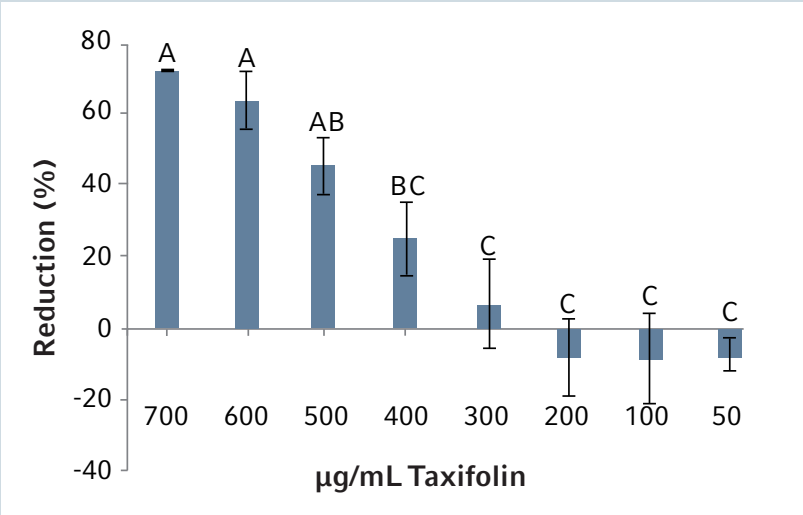

Figure 2: Reduction of *E. coli* cell adhesion after 24 h of growth compared to the positive control in %. Mean of three individual plates with six replicates (wells) per sample are shown. Different letters indicate a statistically significant difference with $p<0.05$ calculated with a one-way ANOVA followed by Tukey's test.

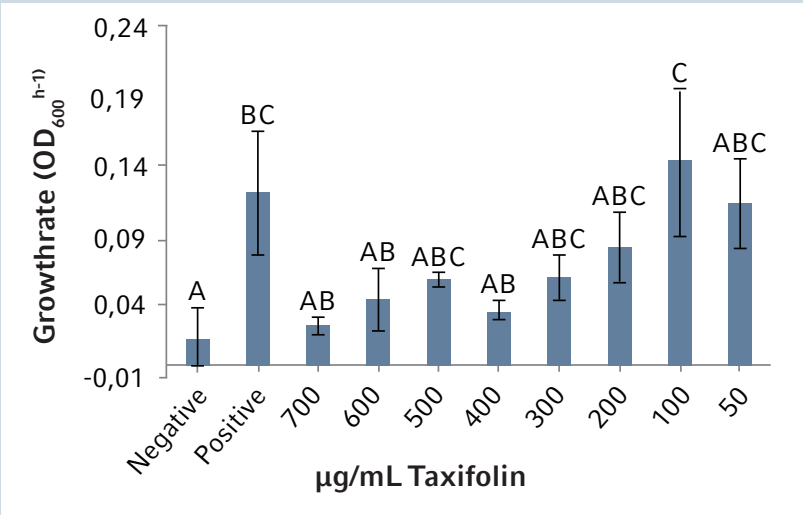

Figure 3: Maximal growth rate of *E. coli* cells grown for 24 h at 37°C. Mean of three individual plates with six replicates (wells) per sample are shown. Different letters indicate a statistically significant difference with $p<0.05$ calculated with a one-way ANOVA followed by Tukey's test.

The reduction of *E. coli* cell adhesion to the microtiter plates shows the potential of taxifolin as a biofilm-inhibiting compound. At the same time, high concentrations of taxifolin have an antimicrobial effect, as they reduce the growth rate of *E. coli*. Considering these results, taxifolin can be seen as a flavanonol with both activity against biofilm formation and planktonic cell growth. Compared to manual pipetting, the invested time for plate staining was reduced by one third, from three to two hours. In addition, it is possible to analyze three or more plates in parallel, which is not convenient with manual pipetting. The fast dispensing into and aspiration of liquids from the plate also leads to a more even treatment of the 96 wells, as there are no delays. In further experiments by using the ep*Motion* 96xl, the effect of taxifolin on microorganisms more relevant for technical and clinical applications could be evaluated.

Conclusion

In this application note, the ep*Motion* 96xl semi-automated pipetting system was successfully applied for a biofilm screening assay with *E. coli*. The naturally occurring flavanonol taxifolin was found to exhibit anti-biofilm and antimicrobial activity. This fast method of metabolite screening can be further applied on more detailed experiments on taxifolin or other promising compounds.

Literature

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Ordering information

Description	Order no. international	Order no. North America
ep*Motion*® 96xl, with 2-position slider, semi-automated electronic pipette for parallel 96 channel microplate processing (without iPod® controller), 100 – 240 V ±10 %/50 – 60 Hz ±5 %, 5 – 1000 µL	5069 000.314	5069000306
epT.I.P.S.® motion as Reload System, with filter PCR clean, 1000 µL, 2,304 tips (24 trays x 96 tips)	0030 014.510	0030014510

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