Evaluating the influence of phenolic compounds to gut bacteria-host cell interaction via HPLC-MS/MS based targeted metabolic profiling

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Abstract

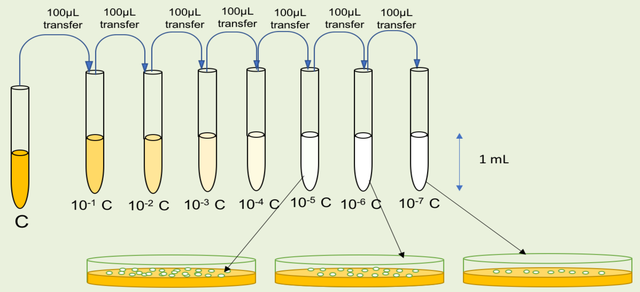
This study evaluated the influence of phenolic compounds and their influence to the interaction of human gut bacteria and host cells. Four bacterial stains were studied in this project. The minimum inhibitory concentration of phenolic compounds on these four strains were investigated. The effects of phenolic compounds on adhesion of bacteria to cultured Caco-2 cells were determined. Metabolite extractions of the cell cultures were conducted and samples were analyzed by HPLC MS/MS to investigate the metabolic difference of the host cells with or without addition of phenolic compounds.

Introduction

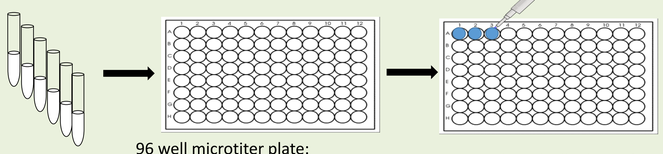
Polyphenols are a group of chemicals found in many fruits, vegetables, and other plants, such as berries, walnuts, olives, tea leaves, broccoli, grapes and even chocolate! They are classified as antioxidants, meaning that they can remove free radicals from the body. Some of these phenolic compounds were also known to have antimicrobial function, meaning they could inhibit microbe growth. The purpose of this study was to find out if polyphenols can influence the gut bacteria and host cells interaction. We investigated this topic via a combination of microbiology and metabolomics approaches.

Experimental Methods

Serial Dilution & colonies Count



Minimum inhibitory concentration (MIC)



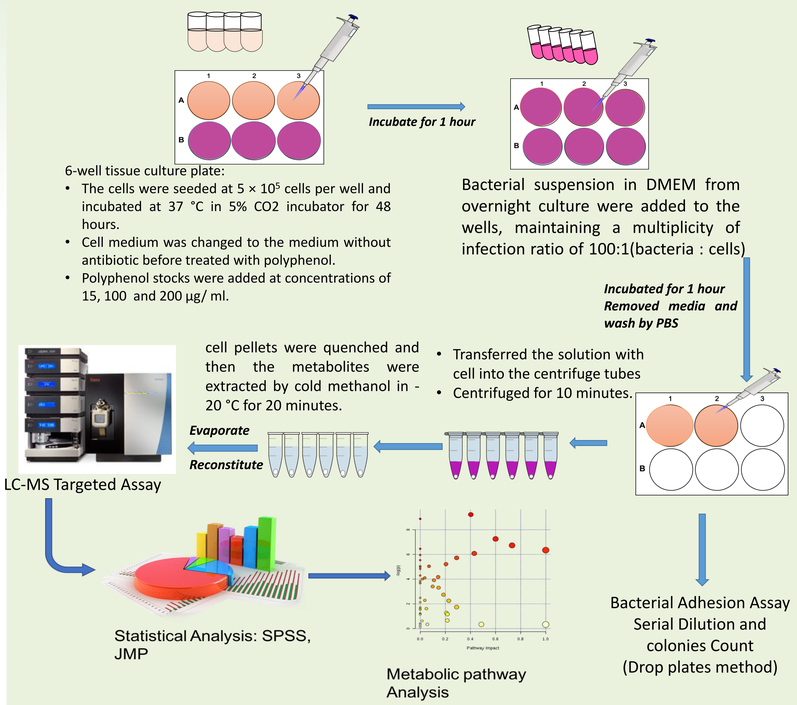
Bacterial suspension of 6 strains diluted to $5x10^4$ cfu/mL from overnight culture

96 well microtiter plate:

- Polyphenol solutions were prepared into 9 different concentrations included 3.5, 3, 2.5, 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL.
- 100µL of polyphenol solution was added to all wells in the plate.
- 100µL of diluted bacteria culture of each strain was added to all wells in the plate
- Every treatment include 3 replicates

Incubated all plates for 16 hours and added 10 µL of Resazurine (dye) and incubate for one hour.

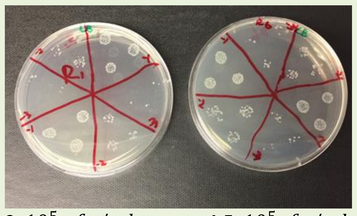
Experimental Methods



Results

Polyphenol	Minimum inhibitory concentration (µg/ml)			
	Escherichia coli: K12	Escherichia coli: O157:H7	Staphylococcus aureus: ATCC29213	Staphylococcus aureus: RN450
Caffeic acid	950	950	480	950
EGCG	120	120	60	60
Catechin	950	1428	>1428	>1428
Daidzein	950	>1428	>1428	>1428
Quercetin	950	>1428	1428	1660
Chlorogenic acid	950	>1428	>1428	>1428
Phloridzin	950	>1428	>1428	>1428

Table 1: Minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited the growth of the bacteria by polyphenols.



$2x10^5\ cfu/ml$ $4.5x10^5 cfu/ml$

Figure 1: Adhesion of Escherichia coli O157:H7 culture to Caco-2 cells after 1 hour of pretreatment with (left) and without (right) EGCG.

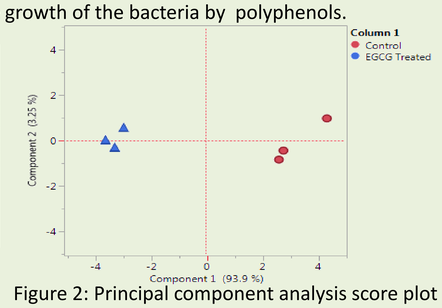


Figure 2: Principal component analysis score plot showing the metabolic profiles different of Caco-2 cells to *Escherichia coli* O157:H7 culture (right group, red) and Caco-2 cells to Escherichia coli O157:H7 with EGCG treated culture (left group, blue). The plot shows 14 significantly different metabolites to the separation of the two testing culture groups.

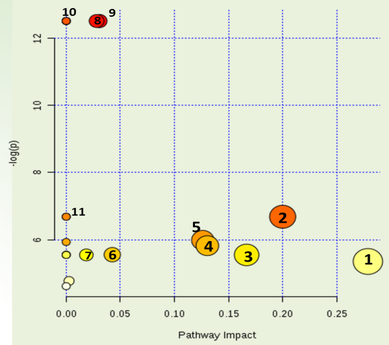


Figure 3: A metabolome view showing all impacted metabolic pathways in this study analyzed using MetaboAnalyst (3.0). Major pathways that were impacted by EGCG:

1. Glycine, serine and threonine metabolism
2. Tryptophan metabolism
3. Methane metabolism
4. Aminoacyl-tRNA biosynthesis
5. Nicotinate and nicotinamide metabolism
6. Citrate cycle (TCA cycle)
7. Cysteine and methionine metabolism
8. Starch and sucrose metabolism
9. Galactose metabolism
10. Streptomycin biosynthesis
11. Nitrogen metabolism

Metabolites	Fold Change	T-test
D-glucose	6.10E-04	2.75E+00
Isocitric acid	2.34E-03	5.14E-01
Fructose 1,6-biphosphate	2.45E-03	3.63E-01
Threonine	8.14E-03	4.76E-01
tryptophan	8.50E-03	6.57E-01
NAD	9.28E-03	5.02E-01
proline	1.20E-02	5.46E-01
Glutamic acid	1.60E-02	4.62E-01
D-(-) 3 Phosphoglyceric-Acid	2.12E-02	1.40E+00
D-orinthine	2.30E-02	7.59E-01
2'-deoxycytdine 5'-monophosphate	2.87E-02	5.20E-01
serine	3.07E-02	5.56E-01
cytosine	3.22E-02	7.56E-01
Keto-glutaric acid	3.62E-02	4.40E-01

Table 2: Metabolites with p-value lass than 0.05 in comparison of Escherichia coli O157:H7 culture treated with EGCG. Three replicates were tested in each group.

Discussion and Future Directions

This project is still in progress, we have only the results of bacteria adhesion assay of Escherichia coli O157:H7 to Caco-2 cells treated with EGCG. The result shows that the bacteria colony number decrease from $4.5x10^5$ to $2x10^5$ cfu/ml after pretreatment with 15 µg/ml EGCG. 14 of 52 metabolites detected from cells shows significant different between control and experiment group.

The project may reveal more about influence of phenolic compounds to gut bacteria-host cell interaction. In the future we will focus on other bacteria strains (Escherichia coli K12, Staphylococcus aureus ATCC 29213 and Staphylococcus aureus RN450) to Caco-2 cells treat with polyphenols (EGCG, Caffeic acid and Quercetin). And apply the HPLC-MS targeted assay, statistic analysis, and pathway analysis. The biomarker will be selected and the influence of phenolic compounds to gut bacteria-host cell interaction will be determined.

References

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3. (a) Zhu, J.; Djukovic, D.; Deng, L.; Gu, H.; Himmati, F.; Abu Zaid, M.; Chiorean, E.; Raftery, D., Targeted serum metabolite profiling and sequential metabolite ratio analysis for colorectal cancer progression monitoring. Anal Bioanal Chem 2015, 407 (26), 7857-7863; (b) Zhu, J.; Djukovic, D.; Deng, L.; Gu, H.; Himmati, F.; Chiorean, E. G.; Raftery, D., Colorectal Cancer Detection Using Targeted Serum Metabolic Profiling. Journal of Proteome Research 2014, 13 (9), 4120-4130.
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Acknowledgements

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