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Identifying antibacterial targets of flavonoids by comparative genomics and molecular modeling

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ABSTRACT

Flavonoids are among most common natural products that exhibit a broad spectrum of antibacterial activity. In order to decipher their antibacterial mechanisms, we used comparative genomics method to identify the targets in E. coli for 19 antibacterial flavonoids, and then validated these targets by molecular docking. Five important enzymes, namely, fumarate reductase flavoprotein, dihydroorotate dehydrogenase, dihydrofolate reductase, NADH-dependent enoyl-ACP reductase, and the DNA gyrase subunit, were identified as potential targets of 19 flavonoids. Docking results also showed that the 3-O-galloyl or 3-O-glycosides side chain at flavonoid pyrane ring are important for inhibiting these enzymes. This study not only provides important clues to understanding antibacterial mechanisms of flavonoids, but also demonstrates that comparative genomics is useful in predicting natural product targets.

INTRODUCTION

Flavonoids are a major family of secondary metabolites, which are widely distributed in plants and are common constituents of human diet. To date, more than 9000 flavonoids have been found in plants [1]. In general, flavonoids consist of a flavan scaffold and various modifications, producing an extremely diverse range of derivatives. They can be classified into six classes according to their chemical structures, that is, flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins [2]. Flavonoids have a number of biological activities, including antiviral, antibacterial, antiprotozoal and anti-inflammatory, etc. [3].

Flavonoids have been recognized as potential natural sources of antimicrobial drugs. They can inhibit the growth and replication of a variety of pathogenic bacteria that cause human illness, such as Escherichia, Gingiva, Pseudomonas, Staphylococcus, and Canadida

genera [4]. Some flavonoids, such as flavone, panduratin A, and isobavachalcone, have been identified as novel types of effective antimicrobial compounds [5]. Therefore, flavonoids are useful not only for finding new antibacterial drugs but also for improving the shelf life and safety of foods [6].

Flavonoids can exert antibacterial activities through multiple mechanisms, such as disruption of cytoplasmic membrane, inhibition of nucleic acid synthesis, inhibition of energy metabolism, inhibition of cell wall synthesis, and inhibition of cell membrane synthesis [5]. Several reports suggest that flavonoids usually have multiple mechanisms of action [7-9]. However, the antibacterial targets remain largely unknown for most flavonoids. Our recent analysis about flavonoid targets in human genome revealed that many flavonoid targets have homologues in human gut bacterial metagenomes [10], suggesting that we can predict flavonoid targets in pathogenic bacteria by comparative genomics. Thus, in this paper, we first use comparative genomics approach to identify the potential antibacterial targets of flavonoids in *Escherichia coli* (E. coli). Then, these targets were validated by molecular docking. The results are helpful to understand antibacterial mechanisms of flavonoids.

MATERIALS AND METHODS

Data collection

Antibacterial flavonoids were collected from literatures. Then, we retrieved their targets in Homo sapiens from STITCH database (http://stitch.embl.de/), which integrates information about interactions from crystal structures, binding experiments and drug-target relationships. The successful antibacterial targets in E. coli were taken from TTD database (http://bidd.nus.edu.sg/group/ttd). The protein sequence files were downloaded from UniProt (http://www.uniprot.org/). And their three-dimensional structures were obtained from Protein Data Bank (http://www.rcsb.org).

Comparative genomics analysis

The homologues sequence searching using BLASTP was performed on all the successful antibacterial targets of E. coli against the flavonoid targets in Homo sapiens. An E-value of < 10-6 and a sequence coverage of >= 50% were used as criteria to identify the homologous sequences. This method has been successfully employed in previous studies to predict potential targets of flavonoids in gut bacteria genomes [10].

Protein-ligand docking

The antibacterial flavonoids were optimized using PRODRG server with full charges [11]. AutoDock 4.2 [12] was used to dock antibacterials with the predicted targets. The AutoDockTools was used to prepare, run, and analyze the docking simulations. The ligands were treated as flexible, and only torsional degrees of freedom were explored, holding bond angles and bond lengths constant. Atomic Gasteiger charges were used for the ligands, and Gollman atom charges were used for the receptor atoms.

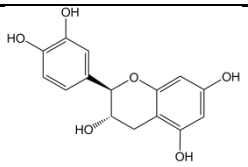
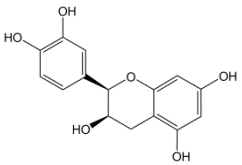
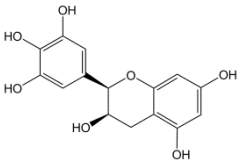
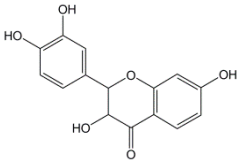
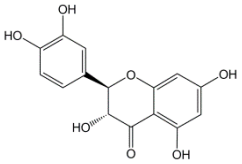
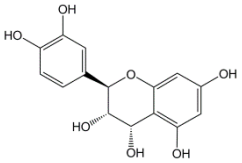
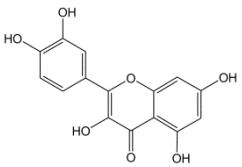
The center of the binding site was chosen based on information from the literature. The grid box was defined around the substrate binding site. A grid spacing of 0.375 Å were used for the energetic map calculations. The Lamarckian genetic algorithm (LGA) implemented in Autodock was used for docking experiments. For each simulation, the compounds were subjected to 200 runs of the Autodock search using 250000 steps of energy evaluation and default values for the other parameters. Further, cluster analysis was performed by sorting binding modes based on the binding energy within the specified RMSD threshold, which was set to 1.5 Å. The optimal binding models of the ligands were those with the lowest binding energies picked up from the cluster, which had maximum number of similar conformations. The interactions mediating ligand-protein complex were analyzed and visualized using Poseview (http://poseview.zbh.uni-hamburg.de) and Pymol (http://www.pymol.org), respectively. The cavity volumes of the ligand-binding sites were calculated by CASTp [13] using a 1.4 Å radius probe.

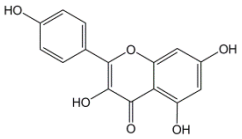
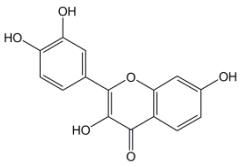
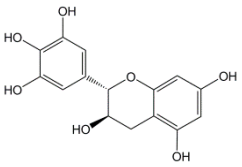
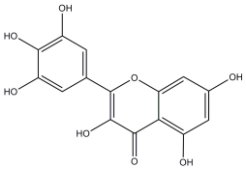
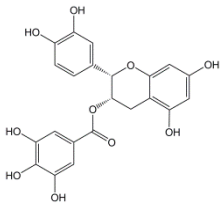
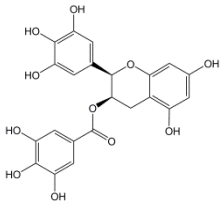
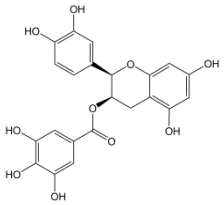
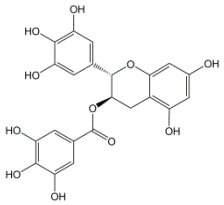
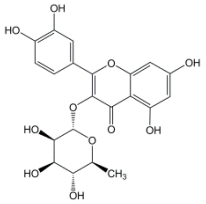
RESULTS AND DISCUSSION

Nineteen antibacterial flavonoids were collected from literatures (Table 1) [2, 5, 14-16]. These flavonoids have shown activities against E. coli. Through searching the STITCH database, we

identified 1336 human targets for these flavonoids. In order to identify the potential targets of these flavonoids in E. coli, we performed a BLASTP comparison of the successful antibacterial targets with the targets from the STITCH database. Totally, 19 successful antibacterial targets of E. coli derived from TTD database were used for the comparison (Table 2). Five of them were identified as homologues of flavonoid targets in human genome, with the E-values ranging from 3.00e–118 to 9.00e–74 (Table 3). These proteins are known to be essential enzymes for biosynthesis and cell growth. Four of them are related to oxidoreductase activity. The fumarate reductase flavoprotein (FrdA) of E. coli is a membrane-bound flavoprotein which catalyzes the interconversion of fumarate and succinate, and is essential for anaerobic growth on non-fermentable substrates [17]. The dihydroorotate dehydrogenase (PyrD) catalyzes the oxidation of dihydroorotate to orotate. The dihydrofolate reductase (DYR) catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate. The NADH-dependent enoyl-ACP reductase (FabI) plays a determinant role in completing cycles of elongation in type II fatty acid synthase systems. The DNA gyrase subunit (GyrB) catalyzes ATP-dependent negative supercoiling of DNA and is involved in DNA replication, recombination, and transcription. These potential targets were further evaluated by docking analysis.

Table 1. Antibacterial flavonoids studied in this work.

Compound Name	PubChem ID	Structure
(+)-Catechin	9064	
(-)-Epicatechin	72276	
(-)-Epigallocatechin	72277	
Fustin	246330	
Taxifolin	439533	
leucocyanidin	440833	
Quercetin	5280343	

kaempferol	5280863	
Fisetin	5281614	
(-)-Gallocatechin	9882981	
Myricetin	5281672	
Epicatechin-3-gallate	65056	
(-)-Epigallocatechin gallate	65064	
(-)-Epicatechin gallate	107905	
(-)-Gallocatechin gallate	199472	
quercitrin	5280459	

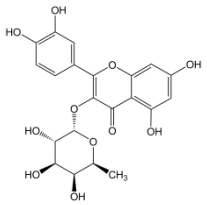
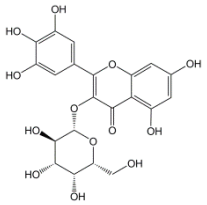
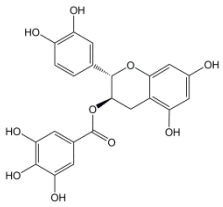
Quercetin 3-rhamnoside	5359430	
Myricetin 3-galactoside	5491408	
(-)-Catechin gallate	6419835	

Table 2. Successful antibacterial targets in *E. coli*.

Uniprot identification	**Protein name**
AMPC_ECOLI	Beta-lactamase
DCM_ECO57	DNA-cytosine methyltransferase
DDLA_ECOLI	D-alanine--D-alanine ligase A
DHPS_ECOLI	Dihydropteroate synthase
DYR_ECOLI	Dihydrofolate reductase
EFG_ECO57	Elongation factor G
FABI_ECOLI	NADH-dependent enoyl-ACP reductase
FRDA_ECOLI	Fumarate reductase flavoprotein subunit
FTSI_ECO57	Peptidoglycan synthase FtsI
GYRA_ECOLI	DNA gyrase subunit A
GYRB_ECOLI	DNA gyrase subunit B
IMDH_ECO57	Inosine-5'-monophosphate dehydrogenase
MANA_ECOLI	Mannose-6-phosphate isomerase
MURA_ECOLI	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
PARC_ECOLI	DNA topoisomerase 4 subunit A
PBP2_ECO57	Penicillin-binding protein 2
PYRD_ECOLI	Dihydroorotate dehydrogenase (quinone)
RPOB_ECOLI	DNA-directed RNA polymerase subunit beta
SYI_ECOLI	Isoleucine--tRNA ligase

Molecular docking of 19 flavonoids into 5 potential targets were performed by Autodock program. As shown in Table 4, the binding free energies of the flavonoids range from –6.68 kcal/mol to –13.75 kcal/mol. The binding free energies of a set of successful inhibitors for the 5 targets were also calculated with the Autodock program, which vary in a range of –6.32 ~ –10.68 kcal/mol (Table S1). It can be seen that the flavonoids have comparative binding affinities to the successful drugs, providing further evidence to support that the five E. coli proteins are antibacterial targets of the flavonoids, which has significant implications for interpreting their antibacterial mechanisms. The previous genetic studies have revealed the phenotypic changes associated with the mutation of these targets. For instance, FabI mutant can cause membrane perturbations including leakage of cytoplasmic material [18]; GyrB mutant inhibit the nucleic acid synthesis and cell division [19]; FrdA mutant represses the anaerobic respiration metabolism and cellular energy synthesis [20]. These genotype-phenotype associations for the potential flavonoid targets imply that the inhibition of these targets will disrupt the cytoplasmic membrane function, nucleic acid synthesis and energy metabolism, which agree well with the reported antibacterial mechanisms of flavonoids [5].

Table 3. *E. coli* homologues of flavonoid targets in human genome.

Human targets* (protein name)	**BLASTP hits**** (protein name)	**Identity (%)**	**E-value**
DHSA_HUMAN (Succinate dehydrogenase flavoprotein subunit)	FRDA_ECOLI (Fumarate reductase flavoprotein subunit)	38.28	3.00e–118
PYRD_HUMAN (Dihydroorotate dehydrogenase)	PYRD_ECOLI (Dihydroorotate dehydrogenase)	43.50	9.00e–74
DYR_HUMAN (Dihydrofolate reductase)	DYR_ECOLI (Dihydrofolate reductase)	27.87	3.00e–13
DECR_HUMAN (2,4-dienoyl-CoA reductase)	FABI_ECOLI (NADH-dependent enoyl-ACP reductase)	28.57	5.00e–15
TOP2A_HUMAN (DNA topoisomerase 2-alpha)	GYRB_ECOLI (DNA gyrase subunit B)	25.95	6.00e–32

* Flavonoids targets in *Homo sapiens*.

** Successful antibacterial targets in *E.coli*

Table 4. Docking results of 19 flavonoids into 5 targets.

Compounds name	**Lowest Binding Affinity (kcal/mol)**				
	FrdA	**PyrD**	**FabI**	**DYR**	**GyrB**
(+)-Catechin	–10.46	–9.36	–9.11	–8.04	–7.44
(-)-Epicatechin	–10.02	–9.33	–8.54	–9.09	–7.61
(-)-Epigallocatechin	–10.25	–9.26	–9.11	–9.43	–7.42
Fustin	–9.30	–9.27	–8.85	–7.72	–8.52
Taxifolin	–10.11	–9.73	–8.7	–7.83	–8.45
leucocyanidin	–8.0	–8.8	–9.26	–7.15	–7.27
Quercetin	–10.22	–9.12	–8.55	–7.95	–8.5
kaempferol	–10.15	–8.14	–7.82	–7.79	–7.29
Fisetin	–9.84	–9.08	–8.54	–8.02	–8.47
(-)-Gallocatechin	–10.46	–8.74	–9.41	–7.75	–6.97
Myricetin	-8.26	-7.25	-7.58	-6.70	-7.0
Epicatechin-3-gallate	–8.22	–10.23	–11.35	–10.35	–7.62
(-)-Epigallocatechin gallate	–8.66	–11.3	–11.51	–9.46	–7.15
(-)-Epicatechin gallate	–8.43	–11.18	–11.22	–9.57	–6.99
(-)-Gallocatechin gallate	–8.95	–13.75	–11.19	–9.45	–8.05
Quercitrin	–8.01	–11.75	–10.79	–10.66	–8.86
Quercetin 3-rhamnoside	–7.52	–12.52	–10.18	–9.49	–8.47
Myricetin 3-galactoside	–7.84	–11.15	–10.41	–8.03	–6.68
(-)-Catechin gallate	–8.53	–12.88	–11.47	–9.45	–8.09

In the 19 flavonoids, (-)-epigallocatechin gallate (EGCG) and quercitrin indeed have been found as inhibitors of FabI reductases and GyrB, respectively [16]. The present results reveal that EGCG has the strongest binding with FabI in comparison with other flavonoids, and quercitrin is also the strongest inhibitor of GyrB in the 19 flavonoids. Previous studies also revealed that the presence of a galloyl side chain at position 3 of flavonoid pyrane ring have stronger antibacterial activities [21]. The present results indicate that flavonoids having galloyl moieties, such as EGCG, (-)-catechin gallate, (-)-epicatechin gallate, and (-)-

gallocatechin gallate, exhibit higher binding affinities to PyrD, FabI, and DYR than their cognates lacking the galloyl group, i.e., (-)-epigallocatechin, (+)-catechin, (-)-epicatechin, and (-)-gallocatechin, respectively (Table S2). A similar phenomenon is observed for flavonoids with glycosides. That is, quercetin 3-rhamnoside and myricetin 3-galactoside are more potent inhibitors to PyrD, FabI, and DYR than quercetin and myricetin, respectively (Table S3). To explore the underlying reasons, three types of interactions responsible for mediating the binding between agents and protein cavity are defined: i) hydrogen bond, assigned when the optimal distance between donor and acceptor is within 1.9 Å with a tolerance of 0.5 Å, and the acceptor-hydrogen-donor angle must not fall below 120º; ii) hydrophobic contact, considered formed if the distance between the hydrophobic atoms less than the sum of their van der Waals radii + 0.8 Å, and at least three hydrophobic ligand atoms lie in the range of their contact receptor residue; iii) π interactions including π-π or π-cation interactions, defined as the attractive interaction that distance between two parallel aromatic systems' centroids or an aromatic system's centroid and a cation is less than 5 Å or 4.5 Å. As shown in Table S4, flavonoids with galloyl or glycosides form more interactions with the three targets, PyrD, FabI, and DYR. Since galloyl or glycosides are hydrogen-bond donors and acceptors, and galloyl can form π interactions with aromatic or cation residues, they make positive contributions to the interactions between flavonoids and their targets. However, as the binding cavity in FrdA (498 Å3) is smaller than that in PyrD, FabI, and DYR (1143 Å3, 1197 Å3 and 1035 Å3, respectively), the binding of flavonoids with galloyl or glycosides to FrdA are hampered by steric hindrance. This is the reason why the FrdA affinities of flavonoids with galloyl or glycosides are weaker than those of the parents.

CONCLUSION

In this work, we identified 5 antibacterial targets for 19 flavonoids by comparative genomics analysis. These targets were further validated by molecular docking. Docking results also reveal that substitution of galloyl or glycosides at position 3 of heterocyclic pyrane ring in flavonoids enhances the binding affinity to three targets, i.e., FrdA, PyrD and FabI. The biological functions of the 5 targets are consistent with the reported antibacterial mechanisms of flavonoids. Therefore, comparative genomics method is very useful in identifying antibacterial targets and is expected to find important use in pharmacology and drug discovery.

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