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BBSdb, an open resource for bacterial biofilm-associated proteins

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Bacterial biofilms are organized heterogeneous assemblages of microbial cells encased within a self-produced matrix of exopolysaccharides, extracellular DNA and proteins. Over the last decade, more and more biofilm-associated proteins have been discovered and investigated. Furthermore, omics techniques such as transcriptomes, proteomes also play important roles in identifying new biofilm-associated genes or proteins. However, those important data have been uploaded separately to various databases, which creates obstacles for biofilm researchers to have a comprehensive access to these data. In this work, we constructed BBSdb, a state-of-the-art open resource of bacterial biofilm-associated protein. It includes 48 different bacteria species, 105 transcriptome datasets, 21 proteome datasets, 1205 experimental samples, 57,823 differentially expressed genes (DEGs), 13,605 differentially expressed proteins (DEPs), 1,930 'Top 5% differentially expressed genes', 444 'Threshold-based DEGs' and a predictor for prediction of biofilm-associated protein. In addition, 1,781 biofilm-associated proteins, including annotation and sequences, were extracted from 942 articles and public databases via text-mining analysis. We used *E. coli* as an example to represent how to explore potential biofilm-associated proteins in bacteria. We believe that this study will be of broad interest to researchers in field of bacteria, especially biofilms, which are involved in bacterial growth, pathogenicity, and drug resistance.

Availability and implementation: The BBSdb is freely available at http://124.222.145.44/#!/.

KEYWORDS

biofilm-associated protein, prediction, database, bacteria, machine learning

1 Introduction

Bacterial biofilms are adhesion structure formed by single or multiple bacteria and their metabolites. In clinical practice, biofilms can greatly improve the ability of pathogenic bacteria to resist antibiotics, thus increasing the risk of infection (Jamal et al., 2018; Schwarzer et al., 2020). Biofilm-associated proteins are defined as a type of protein molecules closely related to bacterial biofilm formation, which include constitutive proteins located downstream of the biofilm regulatory network and upstream transcriptional regulators. The understanding and discovery of biofilm-associated genes and proteins can help us to better understand the molecular mechanisms of bacterial biofilm formation. Over the last decade, more and more biofilm-associated genes and proteins have been discovered and investigated with the development of omics techniques including transcriptomes and proteomes (Lasaro et al., 2009; Hay and Zhu, 2015; Wang et al., 2017; Jia et al., 2022). During the development and formation of biofilm, the transcription profile of bacteria changes, and some genes with obviously variable expression levels, which are proved by previous experiments, often play an important role in biofilm formation. Therefore, it is important for researchers to obtain these data and analyze gene and protein expression profile in the background of biofilms. However, those important transcriptome and proteome data have been uploaded separately to various databases, which make biofilm researchers pain to have a comprehensive access to these data. Although several resources provide biofilm data, such as Quorumpeps (Wynendaele et al., 2013) for QS-derived signaling peptides, BiofOmics (Lourenço et al., 2012) for biofilm experimental information, BaAMPs (Di Luca et al., 2015), aBiofilm (Rajput et al., 2018), dpABB (Sharma et al., 2016) for antibiofilm Agents, BSD8 (Urbance et al., 2020) for structural information. There is an urgent need to combine multi-omics data for the prediction and analysis of biofilm-associated proteins.

Here, we developed BBSdb, an online database focusing on experimentally validated biofilm-associated proteins. In addition, BBSdb provided a predictor for prediction of biofilm-associated protein, in which users could upload their interested protein sequence to predict candidate biofilm-associated proteins and browse corresponding entries of DEG. BBSdb can serve as a useful resource to make researchers pain-free to obtain transcriptomes, proteomes in biofilm research, query information of experimentally validated biofilm-associated proteins, and utilize developed predictor for protein prediction.

2 Materials and methods

2.1 Data collection, pre-processing and analysis

To give users a clear study design of the data collection, pre-processing, analysis and integration in BBSdb, the overview of study design is illustrated in Figure 1.

For data collection, we collected and curated transcriptome datasets from biofilm research, including 105 RNA-seq projects (1205 samples) from 16 different bacteria species, from the European Nucleotide Archive (EBI ENA, https://www.ebi.ac.uk/ena/browser/home) (Amid et al., 2020) and Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra/) (Kodama et al., 2012). As the related meta-data of corresponding experiments, projects and literature were obtained from NCBI PubMed and GEO databases (Barrett et al., 2013). For the processing of raw sequencing reads, FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used to evaluate the overall quality of the raw sequencing reads, followed by the Trim_galore to remove sequencing vectors and low-quality bases (Utturkar et al., 2020) and performed transcript quantification using Salmon (Patro et al., 2017), which adopted TPM (Transcript Per Million) for normalization (Figure 1B), a better unit for RNA abundance than RPKM and FPKM since it respects the invariance property and is proportional to the average relative RNA molar concentration (Mortazavi et al., 2008; Zhao et al., 2020). It is essential to emphasize that the "mapping" step is based on salmon, which maps the reads obtained by next-generation sequencing onto the transcript of bacteria to achieve the estimation of gene expression. The batch effect correction between different experimental conditions is mainly performed by the ComBat function of sva package in R. In addition, all the transcriptomics are from Illumina sequencing, including single-end reads and pair-end reads. For pair-end reads, the use of FastQC and Trim_Galore requires the inspection and pruning of reads at both ends respectively, and the use of mapping tool (Salmon.) need to compare read1 and read2 at the same time to ensure that the reads are positioned correctly. For proteome datasets, we directly collected them from supplementary data of articles. We analyzed cleaned transcriptome and proteome data using differential expressed analysis, and obtaining 57,385 DEGs and 13,605 DEPs, in which we used a cutoff of $|\log2 FC| > 1.5$ (FC, fold change) and p-value <0.05 to define differentially expressed genes and proteins between experiments (Zhang et al., 2019). Please note, In order to make the definition of log2FC clear, we took the case of biofilm vs planktonic as an example, is FC planktonic/biofilm, and it is always the second/the first as shown in the experimental conditions. In addition, we proposed 'Threshold-based DEGs', which are required to differential express in more than 80% experiment conditions and will be helpful to obtain universal biofilm associated genes, and calculated 'Top 5% differentially expressed genes' of 16 different bacteria species, which was proposed by our previous research (Zhang et al., 2022).

2.2 Text-mining analysis

To obtain experimentally validated biofilm-associated proteins, we performed text-mining analysis. The main steps included: 1) obtaining literature, which must focus on bacterial biofilm at the gene or protein level; 2) obtaining sentences as supported evidence, which must describe gene or protein and contain the following keyword: biofilm; 3) extracting information of potential biofilm-associated gene or protein. The retrieved information was further verified by three rounds of manual inspection (Figure 1C). In

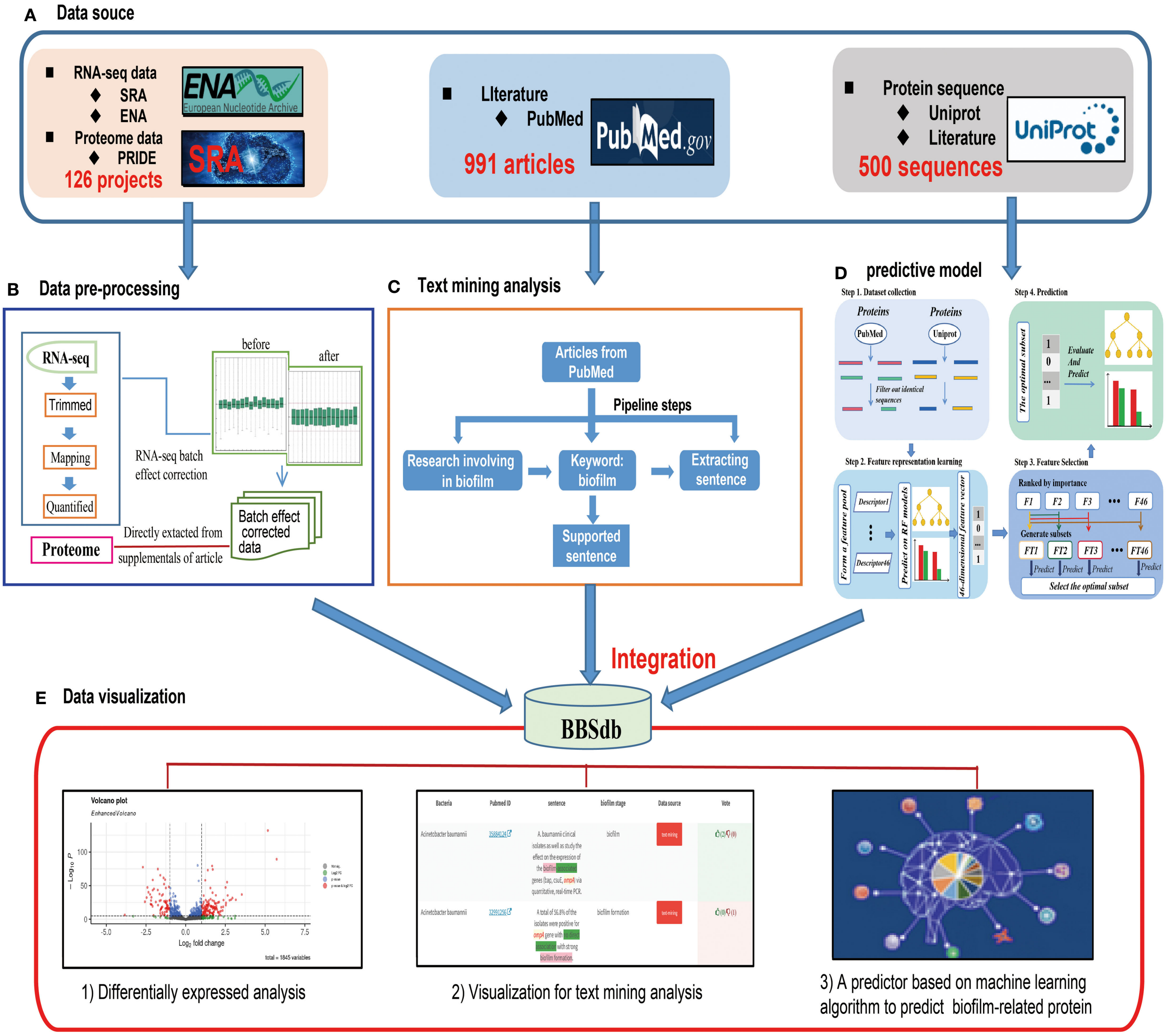
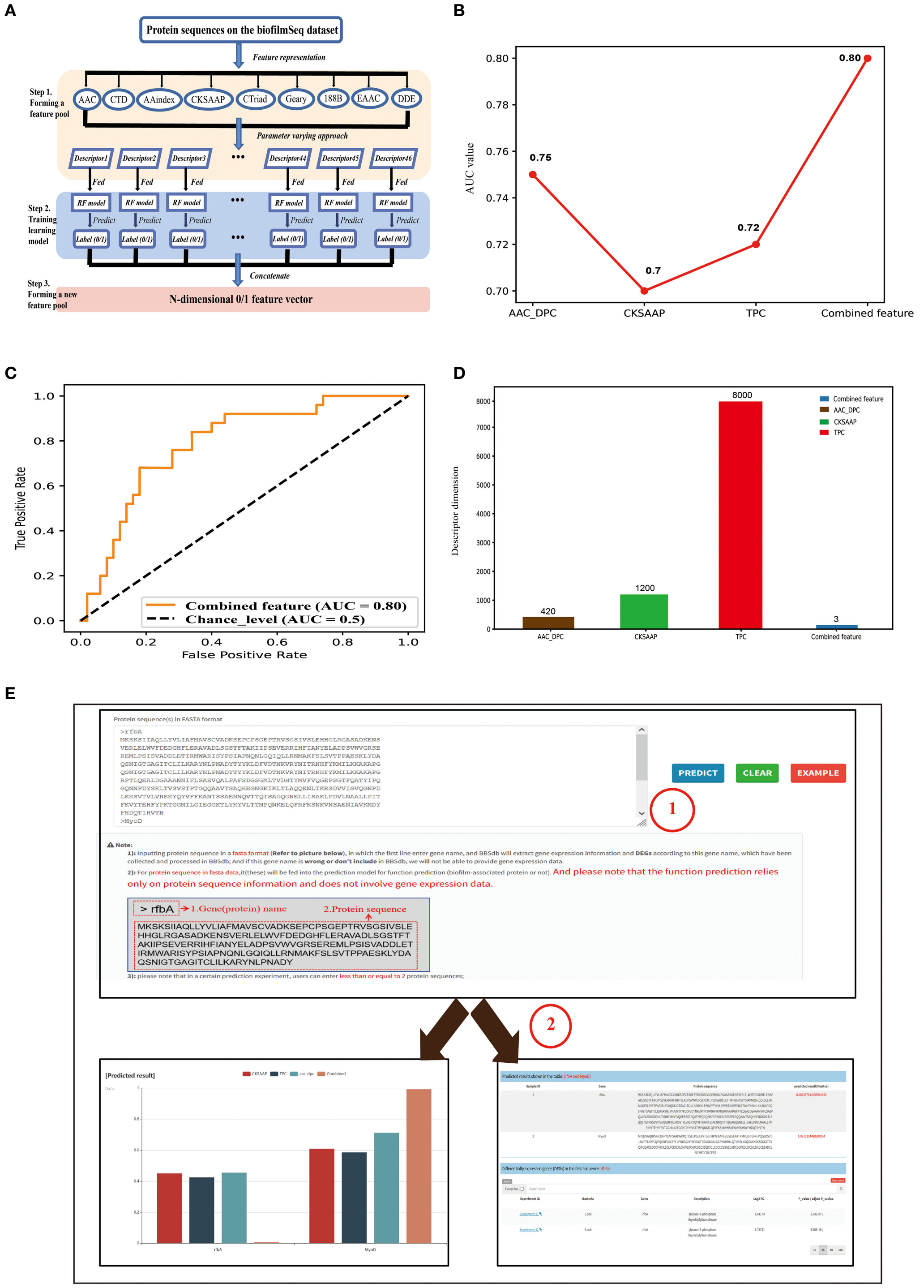

A Data souce
RNA-seq data
SRA
ENA
Proteome data
PRIDE
126 projects
LIterature
PubMed
991 articles
Protein sequence
Uniprot
Literature
500 sequences
B Data pre-processing
RNA-seq
Trimmed
Mapping
Quantified
Proteome
before
after
RNA-seq batch effect correction
Directly extacted from supplementals of article
Batch effect corrected data
C Text mining analysis
Articles from PubMed
Pipeline steps
Research involving in biofilm
Keyword: biofilm
Extracting sentence
Supported sentence
D predictive model
Step 1. Dataset collection
Step 2. Feature representation learning
Step 3. Feature Selection
Step 4. Prediction
Integration
BBSdb
E Data visualization
1) Differentially expressed analysis
2) Visualization for text mining analysis
3) A predictor based on machine learning algorithm to predict biofilm-related protein

FIGURE 1
Overview of BBSdb. **(A)** The data source. All data including the raw sequencing data, information of genome annotation, literature, and experimental validated biofilm-associated proteins were collected from public databases, such as NCBI GEO, ENA, Uniprot, PubMed and so on. **(B)** The data pre-processing. The RNA-seq raw data perform quality control, mapping, transcript quantification and normalization to obtain high-quality data for further analysis. **(C)** Text mining analysis. A customized Python pipeline was used by searching for biofilm-associated proteins (genes) in the titles or abstracts of literature from the NCBI PubMed database. **(D)** Construction of predictive model. Firstly, the targeted protein sequences are subjected to the feature representation learning scheme, and as a result, a 46-dimensional feature vector will be generated. Secondly, the feature vector generated at the previous step is optimized to a 3-dimensional optimal feature vector. Ultimately, the proteins are predicted and scored by the well-trained RF model. **(E)** The data integration. BBSdb integrated polytype data with a biofilm-associated proteins predictor, which placed in “Bacteria”, “Genes(Proteins)”, “Diff. Expr.” and “Prediction” modules.

addition, Text-mining analysis only search titles and abstracts of literature. We obtained 1,781 experimentally validated biofilm-associated proteins, which were validated by 2,514 entries of supported evidence from 942 articles and public databases. We selected above-mentioned protein sequences with sequence similarity < 50% (CD-HIT) as positive datasets for effectively training a machine learning model with strong robustness and wide adaptability. And reviewed non biofilm-associated proteins from Uniprot database (https://www.uniprot.org/) (UniProt, Consortium, 2021) were extracted as negative datasets (http://124.222.145.44/#!/terms).

2.3 Predictor development

The work used a feature representation learning scheme that integrated different types of sequence-based feature descriptors to develop predictive model (Figure 2A). For protein sequence-based feature descriptors, we utilized different feature extraction methods (Rajput et al., 2015; Wei et al., 2020) for feature representation and obtained 46 feature descriptors in total (Supplementary S1). We compared all 46 feature descriptors using RF (Random Forest) classifier, and the top 3 best feature descriptors CKSAAP (Tung, 2013), TPC (Duan et al., 2008), and AAC_DPC with the



Protein sequence(s) in FASTA format

```
>rfbA
MKSKSIIAQLLYVLIAFMAVSCVADKSEPCPSGEPTRVSGSIVSLEHHGLRGASADKENS
VERLELWVFDEDGHFLERAVADLSGSTFTAKIIPSEVERRIHFIANYELADPSVWVGRSE
REMLPSISVADDLETIRMWARISYPSIAPNQNLGQIQLLRNMAKFSLSVTPPAESKLYDA
QSNIGTGAGITCLILKARYNLPNADYTYYKLDFVDTNKVRYNITRNHFYKMILKKAKAPG
QSNIGTGAGITCLILKARYNLPNADYTYYKLDFVDTNKVRYNITRNHFYKMILKKAKAPG
RPTLQEALDGAAANNIFLSAEVQALPAFSDGSGMLTVDHTYMVFVQGEPSGTFQATYIPQ
GQNNPDYSKLTVSVSTPTGQQAAVTSAQHEGNGKIKLTLAQQENLTKRSDVVIGVQGNPD
LKRSVTVLVREKYQYVFFKANTSSAENNQVTTQISAGQGNELLISAKLPDVLNAALLPIT
FKVYTEHFYPKTGGMILGIEGGKTLYKYVLTTMPQNKELQFRFKSNKVNSAENIAVKMDY
FHDQTIHVTN
>MyoD
```

⚠ Note:

1): Inputting protein sequence in a fasta format (**Refer to picture below**), in which the first line enter gene name, and BBSdb will extract gene expression information and **DEGs** according to this gene name, which have been collected and processed in BBSdb; And if this gene name is **wrong or don't include** in BBSdb, we will not be able to provide gene expression data.

2): For protein sequence in fasta data,it(these) will be fed into the prediction model for function prediction (biofilm-associated protein or not). And please note that the function prediction relies only on protein sequence information and does not involve gene expression data.

> rfbA → 1.Gene(protein) name   2.Protein sequence

```
MKSKSIIAQLLYVLIAFMAVSCVADKSEPCPSGEPTRVSGSIVSLE
HHGLRGASADKENSVERLELWVFDEDGHFLERAVADLSGSTFT
AKIIPSEVERRIHFIANYELADPSVWVGRSEREMLPSISVADDLET
IRMWARISYPSIAPNQNLGQIQLLRNMAKFSLSVTPPAESKLYDA
QSNIGTGAGITCLILKARYNLPNADY
```

3): please note that in a certain prediction experiment, users can enter less than or equal to 2 protein sequences;

FIGURE 2
Introduction of predictor for biofilm-associated protein. **(A)** Pipeline of the feature representation learning scheme. Firstly, a feature pool with 46 feature descriptors is constructed by nine feature-encoding algorithms. Afterwards, each descriptor is trained and evaluated using the RF classifier on the biofilm-associated protein sequence datasets. Finally, the predicted class label for each trained RF model is regarded as an attribute to form a new feature vector. **(B)** Comparative analysis of single feature descriptor with combined feature (feature subsets). **(C)** ROC curves of the best-performing feature descriptors using RF classifier. **(D)** Feature dimension of the optimal feature (combined feature) and The original three feature descriptors. **(E)** The introduction of "Prediction" module. Users can enter their protein sequences of interest (in fasta format) into the input box on the module page, and then click the 'Predict' button on the right. At this point, the entered protein sequence is fed into the prediction model for function prediction, while the protein name is used to search for corresponding gene expression information in BBSdb database.

RF classifier obtained 0.7 0.72, 0.75 AUC scores, respectively (Figure 2B). Then we use the feature selection strategy to select the features with the greatest contribution from the 46-dimensional features. The results show that the performance of the model built based on 3-dimension combined feature is similar to that of the model based on 46-dimensional features, but the dimension is lower. We constructed predictive model based on a 3-dimensional feature vector, which consisted of prediction result of CKSAAP, TPC, and AAC_DPC descriptors on datasets, and obtained an AUC score 0.80 (Figures 2C, D). BBSdb provided 'Prediction' module to make users free for biofilm-associated protein prediction.

2.4 Database design and implementation

BBSdb, which integrated data and a predictor, was designed as a relational database. All data were loaded into a MySQL database. The frontend of the website was coded using JavaScript and HTML, while the backend was coded using Python with a Flask framework to support queries to the MySQL database and provide representational state transfer (REST) application programming interfaces (APIs) for programmable access to our data. The AngularJS framework was used to bride the front and back-ends. Echarts.js and plotly.js were used for visualizations at the front end.

3 Results

3.1 Summary of BBSdb

The BBSdb database provides a user-friendly, open access web interface for searching, browsing and downloading data, which includes 105 transcriptome projects from 16 different bacteria species and 21 proteome projects from 5 different bacteria species and 1,781 entries of experimentally validated biofilm-associated protein via text-mining analysis, which were validated by 2,514 entries of supported evidence from 942 articles and public databases. We analyzed RNA-seq and proteome datasets and performed differentially expressed analysis, in total obtaining 33,180 DEGs, 13,605 DEPs. We calculated 'Threshold-based DEGs' and 'Top 5% DEGs' of 16 different bacteria species, and obtaining 1,930 'Top 5% DEGs' and 444 'Threshold-based DEGs'. All data shown in Table 1.

TABLE 1 Summary in BBSdb database.

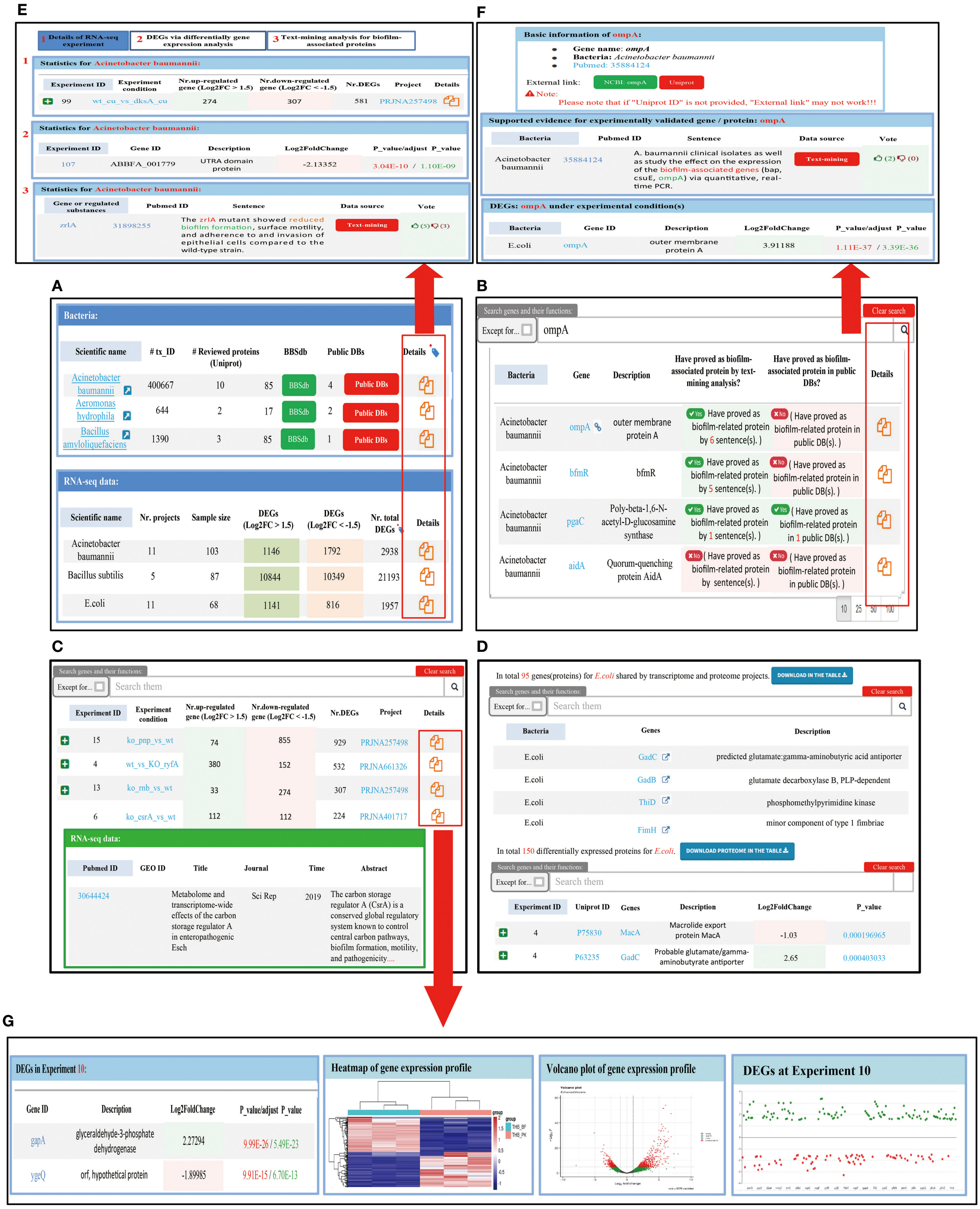
Types	Entries	BBSdb
Data summary	Bacteria	48
	Transcriptome projects	105
	Referring bacteria in transcriptomes	16
	Transcriptome samples	1,205
	DEGs	57,823
	Proteome projects	21
	Referring bacteria in proteomes	5
	DEPs	13,605
	Experimentally validated proteins	1,003
	Literature	1,781
	Supported sentences	2,514
	Top 5% differentially expressed genes	1,930
	Threshold-based DEGs	444
Analysis summary	Text-mining analysis	Yes
	Data visualization	Yes
	Online predictor	Yes
	Differentially expressed gene analysis	Yes

3.2 Effective online predictor

'Prediction' module aims to provide an online predictor for identification of potential biofilm-associated protein. Users can input protein sequence in a fasta format, in which the first line enter gene name, and BBSdb will extract gene expression information and DEGs according to inputted gene name, which have been collected and processed in BBSdb (Figure 2E); And if this gene name is wrong or don't include in BBSdb, gene expression data will not be able to be provided. For protein sequence of the second line, it will be fed into the prediction model for function prediction (biofilm-associated protein or not). And please note that the function prediction relies only on protein sequence information and does not involve in gene expression data.

3.3 Functional modules

The BBSdb database mainly includes four functional modules, 'Bacteria', 'Genes (Proteins)', 'Diff. Expr.', and 'Proteome'. The 'Bacteria' module consists of two tables (Figures 3A, E), with the first table displaying biofilm-associated protein information from 48 bacteria species, as reported in the literature or recorded in public databases. The second table summarizes transcriptomic information from 16 bacteria species, including the number of experimental samples, the number of DEGs, and links providing more detailed information. Users can obtain transcriptomic experimental information of bacteria, DEGs, and experimentally validated biofilm-associated proteins by clicking on the link. The 'Genes (Proteins)' module aims to provide researchers with experimentally validated biofilm-associated proteins and corresponding evidence obtained through data-mining analysis, allowing users to select the bacteria and genes of interest to retrieve information (Figures 3B, F). In addition, we provided a user-feedback mechanism, allowing users to vote on each text mining entry. Users can use the thumb-up and thumb-down buttons to vote on the creditability of the entry, the



E

1 Details of RNA-seq experiment | 2 DEGs via differentially gene expression analysis | 3 Text-mining analysis for biofilm-associated proteins

1 Statistics for Acinetobacter baumannii:

	Experiment ID	Experiment condition	Nr.up-regulated gene (Log2FC > 1.5)	Nr.down-regulated gene (Log2FC < -1.5)	Nr.DEGs	Project	Details
⊞	99	wt_cu_vs_dksA_cu	274	307	581	PRJNA257498	

2 Statistics for Acinetobacter baumannii:

Experiment ID	Gene ID	Description	Log2FoldChange	P_value/adjust P_value
107	ABBFA_001779	UTRA domain protein	-2.13352	3.04E-10 / 1.10E-09

3 Statistics for Acinetobacter baumannii:

Gene or regulated substances	Pubmed ID	Sentence	Data source	Vote
zrlA	31898255	The zrlA mutant showed reduced biofilm formation, surface motility, and adherence to and invasion of epithelial cells compared to the wild-type strain.	Text-mining	(5) (3)

F

Basic information of ompA:

- Gene name: *ompA*
- Bacteria: *Acinetobacter baumannii*
- Pubmed: 35884124

External link: NCBI: ompA | Uniprot

Note:
Please note that if "Uniprot ID" is not provided, "External link" may not work!!!

Supported evidence for experimentally validated gene / protein: ompA

Bacteria	Pubmed ID	Sentence	Data source	Vote
Acinetobacter baumannii	35884124	A. baumannii clinical isolates as well as study the effect on the expression of the biofilm-associated genes (bap, csuE, ompA) via quantitative, real-time PCR.	Text-mining	(2) (0)

DEGs: ompA under experimental condition(s)

Bacteria	Gene ID	Description	Log2FoldChange	P_value/adjust P_value
E.coli	ompA	outer membrane protein A	3.91188	1.11E-37 / 3.39E-36

A

Bacteria:

Scientific name	# tx_ID	# Reviewed proteins (Uniprot)		BBSdb		Public DBs	Details
Acinetobacter baumannii	400667	10	85	BBSdb	4	Public DBs	
Aeromonas hydrophila	644	2	17	BBSdb	2	Public DBs	
Bacillus amyloliquefaciens	1390	3	85	BBSdb	1	Public DBs	

RNA-seq data:

Scientific name	Nr. projects	Sample size	DEGs (Log2FC > 1.5)	DEGs (Log2FC < -1.5)	Nr. total DEGs	Details
Acinetobacter baumannii	11	103	1146	1792	2938	
Bacillus subtilis	5	87	10844	10349	21193	
E.coli	11	68	1141	816	1957	

B

Search genes and their functions: | Clear search
Except for... □ | ompA

Bacteria	Gene	Description	Have proved as biofilm-associated protein by text-mining analysis?	Have proved as biofilm-associated protein in public DBs?	Details
Acinetobacter baumannii	ompA	outer membrane protein A	✓Yes Have proved as biofilm-related protein by 6 sentence(s).)	✗No (Have proved as biofilm-related protein in public DB(s).)	
Acinetobacter baumannii	bfmR	bfmR	✓Yes Have proved as biofilm-related protein by 5 sentence(s).)	✗No (Have proved as biofilm-related protein in public DB(s).)	
Acinetobacter baumannii	pgaC	Poly-beta-1,6-N-acetyl-D-glucosamine synthase	✓Yes Have proved as biofilm-related protein by 1 sentence(s).)	✓Yes (Have proved as biofilm-related protein in 1 public DB(s).)	
Acinetobacter baumannii	aidA	Quorum-quenching protein AidA	✗No (Have proved as biofilm-related protein by sentence(s).)	✗No (Have proved as biofilm-related protein in public DB(s).)	

10 | 25 | 50 | 100

C

Search genes and their functions: | Clear search
Except for... □ | Search them

	Experiment ID	Experiment condition	Nr.up-regulated gene (Log2FC > 1.5)	Nr.down-regulated gene (Log2FC < -1.5)	Nr.DEGs	Project	Details
⊞	15	ko_pnp_vs_wt	74	855	929	PRJNA257498	
⊞	4	wt_vs_KO_ryfA	380	152	532	PRJNA661326	
⊞	13	ko_rnb_vs_wt	33	274	307	PRJNA257498	
	6	ko_csrA_vs_wt	112	112	224	PRJNA401717	

RNA-seq data:

Pubmed ID	GEO ID	Title	Journal	Time	Abstract
30644424		Metabolome and transcriptome-wide effects of the carbon storage regulator A in enteropathogenic Esch	Sci Rep	2019	The carbon storage regulator A (CsrA) is a conserved global regulatory system known to control central carbon pathways, biofilm formation, motility, and pathogenicity....

D

In total 95 genes(proteins) for *E.coli* shared by transcriptome and proteome projects. DOWNLOAD IN THE TABLE

Search genes and their functions: | Clear search
Except for... □ | Search them

Bacteria	Genes	Description
E.coli	GadC	predicted glutamate:gamma-aminobutyric acid antiporter
E.coli	GadB	glutamate decarboxylase B, PLP-dependent
E.coli	ThiD	phosphomethylpyrimidine kinase
E.coli	FimH	minor component of type 1 fimbriae

In total 150 differentially expressed proteins for *E.coli*. DOWNLOAD PROTEOME IN THE TABLE

Search genes and their functions: | Clear search
Except for... □ | Search them

	Experiment ID	Uniprot ID	Genes	Description	Log2FoldChange	P_value
⊞	4	P75830	MacA	Macrolide export protein MacA	-1.03	0.000196965
⊞	4	P63235	GadC	Probable glutamate/gamma-aminobutyrate antiporter	2.65	0.000403033

G

DEGs in Experiment 10:

Gene ID	Description	Log2FoldChange	P_value/adjust P_value
gapA	glyceraldehyde-3-phosphate dehydrogenase	2.27294	9.99E-26 / 5.49E-23
ygeQ	orf, hypothetical protein	-1.89985	9.91E-15 / 6.70E-13

FIGURE 3
Introduction of four main functional module of BBSdb database. **(A)** 'Bacteria' module of BBSdb database. This module summarized experimentally validated biofilm-associated protein information from 48 different bacteria species and transcriptomic information from 16 different bacteria species. **(B)** 'Genes (Proteins)' module of BBSdb database. This module provided researchers with experimentally validated biofilm-associated proteins and evidence obtained through data-mining analysis. **(C)** 'Diff. Expr.' module of BBSdb database. This module provided transcriptomic data and DEGs from 16 different bacteria species, and visualized these data to facilitate users' querying and mining of DEGs. **(D)** 'Proteome' module of BBSdb database. This module provided proteomic data and DEPs from 5 different bacteria species. In addition, BBSdb performed multi-omics combined analysis, obtained overlapping genes and proteins between transcriptomics and proteomics were integrated in this module. **(E)** The bacteria detail page. Users can obtain detailed transcriptomic experimental information of bacteria, DEGs, and experimentally validated biofilm-related proteins. **(F)** The Genes (Proteins) detail page. **(G)** The Diff. Expr. detail page.

background color of the table cell will be changed automatically according to the voting results. In 'Diff. Expr.' module, we provided DEGs and specific information on transcriptomic experiments and visualized these data to facilitate users' querying and mining (Figures 3C, G). The 'Proteome' module collected proteomic data from 5 bacteria species and provided the analyzed differentially expressed proteins. We placed the results of the combined analysis of bacterial transcriptomics and proteomics in this module, and provided their overlapping genes or proteins (Figure 3D), which help us to find new regulatory factors under the bacterial biofilm phenotype through data mining.

3.4 Multi-omics data mining

We classified 16 bacteria species into two groups: (1) Human microbial pathogens, in total 15: *E.coli*, *Burkholderia cenocepacia*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Porphyromonas gingivalis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Mycobacterium Tuberculosis*, *Vibrio cholerae*, and *Listeria monocytogenes*; (2) others: *Bacillus subtilis*. For human pathogenic bacteria, we analyzed the gene expression of different bacteria species, and found that in the background of biofilm, the number of DEGs of different bacteria species are quite different, which may be due to the number of genes in the bacterial genome itself is different. The proportion of DEGs to the number of genes in the bacterial genome range from 0.3% to 53% (Figure 4A). We also analyzed gene expression in single type of bacteria under different conditions. According to differential expression analysis, the shared genes that were differentially expressed under different experimental conditions and 'Threshold-based DEGs', 'Top 5% DEGs' were obtained, which might play important role in biofilm phenotype. Due to there being only one transcriptome dataset for *Vibrio cholerae* and *Mycobacterium Tuberculosis*, their 'Threshold-based DEGs' and 'Top 5% DEGs' could not be obtained. For the 13 different bacteria species *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *E.coli*, *Bacillus subtilis*, *Acinetobacter baumannii*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Porphyromonas gingivalis*, *Burkholderia cenocepacia*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus pneumoniae*, *Salmonella enterica*, the number of 'Top 5% DEGs' are 297, 283, 230, 221, 190, 171, 152, 148, 73, 73, 52, 28, 12, separately, and the number of 'Threshold-based DEGs' are 8, 0, 5, 112, 7, 34, 3, 196, 0, 4, 72, 3, 0, separately (Figure 4B), which are likely involved in the regulatory development of the biofilm as genes encoding biofilm-associated proteins. Subsequently, we performed a multi-omics analysis based on transcriptomic and proteomic data, and identified 95, 418, 367, 1958 shared proteins (genes) in *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, respectively (Figure 4C); By enriching these shared genes (proteins) in the 'Top 5% differentially expressed genes' datasets of the bacteria, we obtained 26, 47, 34, 128 entries respectively (Figure 4D); STRING (Szklarczyk et al., 2023), GO (Gene Ontology, Consortium, 2015), and KEGG (Kanehisa et al., 2017) analysis revealed that for *Pseudomonas aeruginosa*, the 34 genes are enriched in biofilm formation and the type VI secretion system pathways (Figure 4E), suggesting their involvement in the regulatory development of biofilms, providing researchers with clues for a more comprehensive understanding of the biofilm developmental mechanism. *Staphylococcus aureus* and *Bacillus subtilis* were not enriched to related pathways.

3.5 Case study

Users could use BBSdb to obtain existing biofilm-associated protein information and also to systematically explore potential biofilm-associated proteins utilizing collected data and developed tool (Supplementary Figure S1). We used *E. coli* as an example to represent how to screen out biofilm-associated proteins using transcriptome data and developed predictor. Firstly, we calculated DEGs of 3 RNA-seq experiments for *E. coli* (Experiment_1: aerobic biofilm cultures versus aerobic planktonic cultures, Experiment_2: anaerobic biofilm cultures versus anaerobic planktonic cultures, and Experiment_3: biofilm versus planktonic cultures), in which the expression abundances were normalized as TPM values, using a cutoff of $|\log2\ FC| > 1.5$ (FC, fold change) and p-value <0.05 to define DEGs between experiments. We found that the number of DEGs in Experiment_1, Experiment_2, and Experiment_3 were 604, 1475, and 2217, respectively. Among these experiments, the number of shared genes of Experiment_1 with Experiment_2, Experiment_2 with Experiment_3, and Experiment_1 with Experiment_3 were 188, 1329, and 279, respectively (Figure 5A). In total, 168 shared genes were obtained from the 3 RNA-seq experiments. Meanwhile, we constructed protein-protein interaction (PPI) networks for 168 shared genes using STRING (Figure 5B), a current collection of known and predicted direct physical binding and indirect functionally related interactions between proteins/genes. The results indicated that these genes were involved in propanoate, butanoate and pyruvate metabolism. When exploring the top expression genes for the three experiments, we found that they shared 23 genes in the top 500 DEGs, including one commonly down-regulated gene *garP* (Figure 5C), which was associated with the plasma membrane, and 22 commonly upregulated genes (*b1551, infA, tyrU, cspI, groS, csrA, exbD, argQ, greA, sraF, metU, b4140, yifE, rplU, yheL, secG, zapA, wzzB, ydfK, cspB, cspG*, and *yjcB*) in three experiments (Figure 5D), which are mainly were involved in RNA-binding activity. Subsequently, we filtered out 4 tRNA genes (*tyrU, sraF, metU, argQ*) and then used the constructed classifier to predict 19 remaining DEGs, in total, 10 genes (*garP, yjcB, yifE, yheL, secG, b1551, csrA, zapA, ydfK, b4140*) were predicted to be biofilm-associated proteins (Figure 5E). For gene *zapA*, we noted that it is described in the literature as "Biofilm production was significantly associated with the expression of *zapA* ($P < 0.05$)", which indicated *zapA* may be involved in biofilm formation, although its experimental subject was *Proteus mirabilis* (Sun et al., 2020). SecG belongs to the accessory Sec system (Bandara et al., 2016), and deleting member *secA2* of the Sec system caused a substantial reduction in biofilm formation. According to research, Asp5, which is necessary for early stage

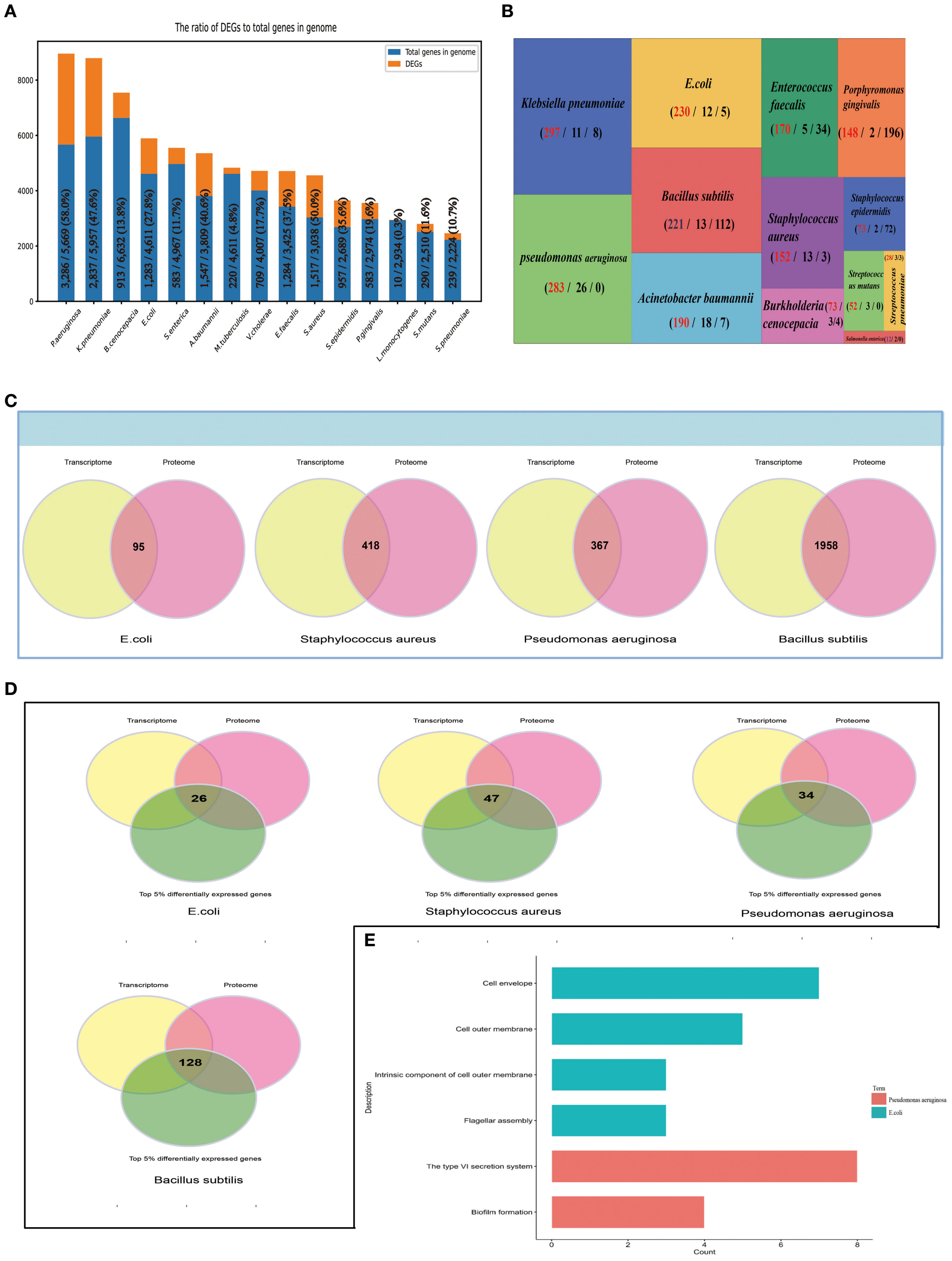


FIGURE 4
Multi-omics Data mining. **(A)** The number of DEGs of 15 Human microbial pathogens, and the proportion of DEGs to the number of genes in the bacterial genome. **(B)** Summary of 'Top 5% differentially expressed genes' from 14 different bacteria species. BBSdb calculates the numbers of the experimental condition of each gene when differentially expressed in experiments, and ranks the corresponding gene by the calculated number of experimental conditions. The first number represents "Top 5% differentially expressed genes", the second number represents the number of experiments associated with the biofilm phenotype and the third number represents "Threshold-based DEGs". **(C)** A multi-omics combined analysis between transcriptomics and proteomics to obtained shared genes (proteins) from 4 different bacteria species, including *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. **(D)** The overlapping analysis between above-mentioned shared genes. **(E)** STRING, GO, KEGG analysis.

biofilm formation in *Streptococcus gordonii*, is homologous to SecG which indicated a similar SecG function (Takamatsu et al., 2005; Bandara et al., 2016). In addition, gene *csrA* plays an important role in biofilm formation by *E. coli* (Jackson et al., 2002) and *yifE* is involved in biofilm formation (https://www.uniprot.org/uniprotkb/P0ADN2/entry).

4 Discussion

In this work, we provided a comprehensive database for biofilm research, which could be an infrastructure for the biofilm research community. In addition, we developed a predictor assisting researchers to explore potential biofilm-associated proteins.

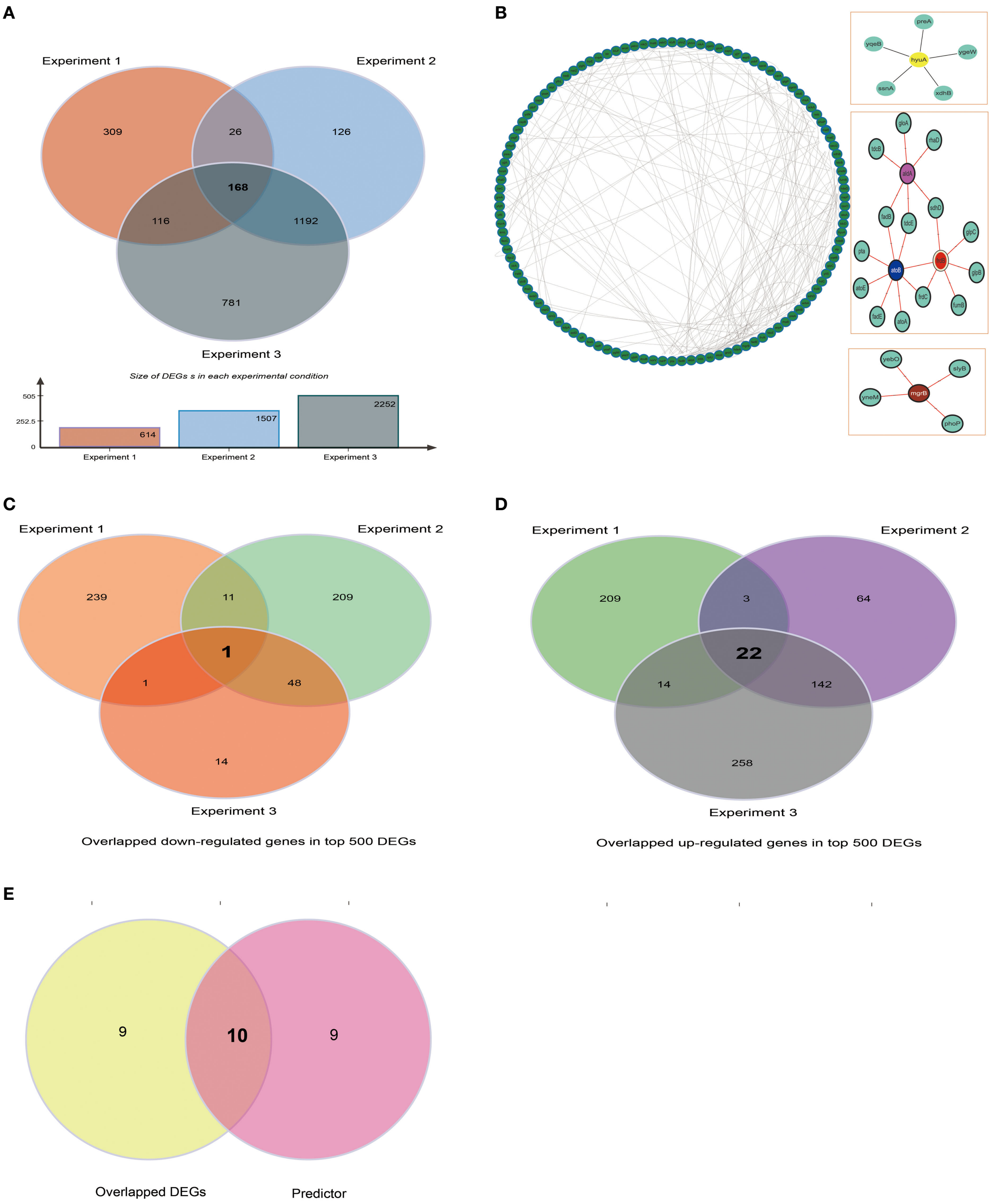


FIGURE 5
Case study. **(A)** The number of DEGs in Experiments 1, 2, and 3 of 3 RNA-seq experiments for *E. coli* were 604, 1475, and 2217, respectively **(B)** The shared and specific DEGs of 3 RNA-seq experiments for *E. coli.* **(C)** The analysis of protein-protein interaction (PPI) interaction networks for 173 shared genes obtained from 3 RNA-seq experiments. **(D)** Top 500 DEGs were obtained by differentially expressed analysis for each RNA-seq experiment, then, up-regulated genes were retained to screen out overlapped up-regulated genes of three experiments. **(E)** Same as above, the down-regulated genes were retained to screen out overlapped down-regulated genes of three experiments.

However, several questions remain to be addressed. For example, the BBSdb database only collected bacterial proteomes through Supplementary Materials in literature without collecting, processing, and analyzing the original bacterial proteome data in public databases such as PRIDE (Perez-Riverol et al., 2022). Regarding the existence of multiple post-transcriptional regulation, proteomes will provide useful information for researchers, therefore more proteomes should also be integrated in the BBSdb.

Biofilm-associated proteins are participating in the biofilm formation process of their respective bacteria referring to previous research (Lasa and Penadés, 2006). In this study, we defined biofilm-associated protein as those that have been reported in the literature and validated experimentally as components of the biofilm structure or involved in the regulation of biofilm development. In practical applications, we need to take into account the differences between different biofilm model systems and many other factors, such as the duration of the experiment and the specific strain, in order to obtain condition-dependent biofilm-associated proteins (Edel et al., 2019; Flemming et al., 2023). These proteins can effectively explain the diversity of mechanisms in bacterial biofilm development. In addition, homologous genes and proteins of different bacteria species can perform different functions, it is necessary to develop a bacteria-specific predictive model for better guiding practice in the next release.

In the future, other questions also remain to be addressed, including the long-term maintenance and update of BBSdb and improvement of predictive ability upon big data accumulation.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Author contributions

ZZ: Data curation, Formal analysis, Methodology, Resources, Software, Writing – original draft, Writing – review & editing. YP: Writing – original draft, Resources, Software, Visualization. WH: Formal analysis, Validation, Writing – original draft. GC: Formal analysis, Validation, Visualization, Writing – original draft. EL: Validation, Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2024.1428784/full#supplementary-material

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