PSOSP uncovers pervasive SOS-independent prophages with distinct genomic and host traits in the bacterial genomes

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Yali Hao, Mujie Zhang, Xinjuan Lei, Chengrui Zhu, Taoliang Zhang, Yanping Zheng, Xiang Xiao, Huahua Jian. 2025. PSOSP uncovers pervasive SOS-independent prophages with distinct genomic and host traits in the bacterial genomes. ***iMeta*** 4: e70073. https://doi.org/10.1002/imt2.70073

Introduction

- the **classical** lysogenic-lytic switch of prophage relies on the **bacterial SOS pathway**
- recently, more **SOS pathway-independent induction** of prophages has been reported
- induction experiments using MMC on environmental microorganisms have shown **highly variable induction rates, with widespread insensitivity**

eg: **237 human intestinal** lysogenic bacterial strains : **1/3** of the prophages can be activated by MMC and H_2O_2

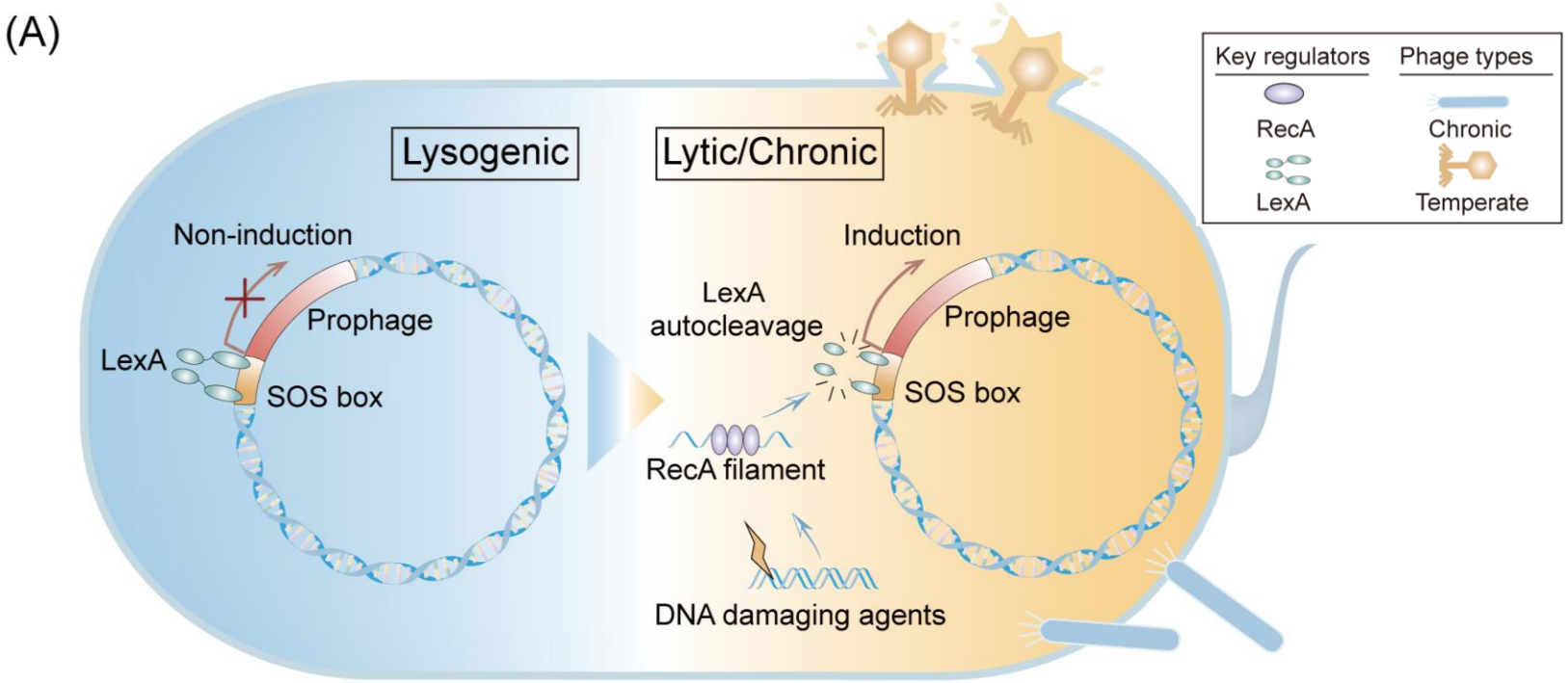
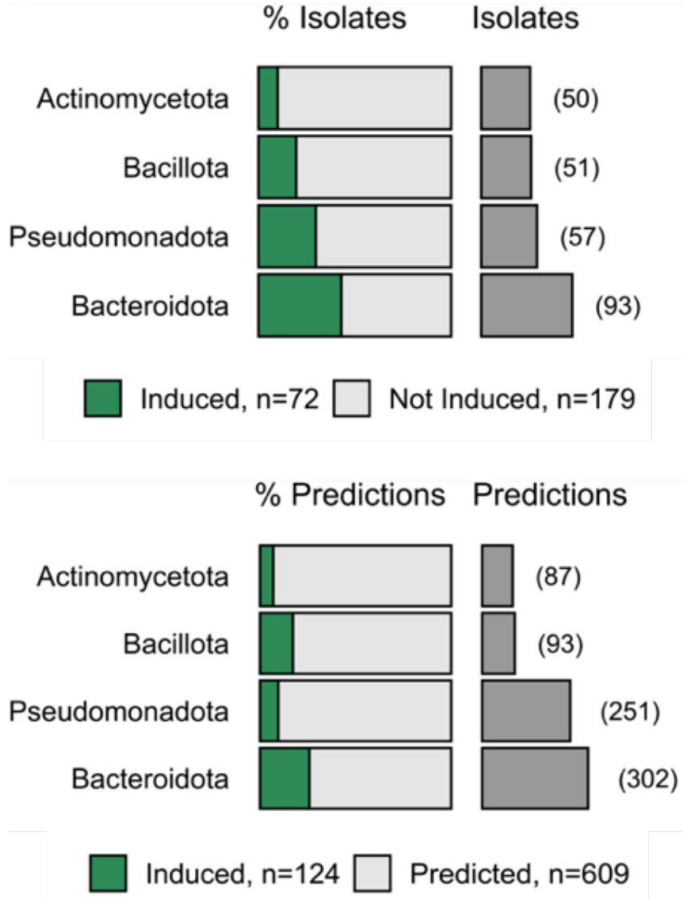


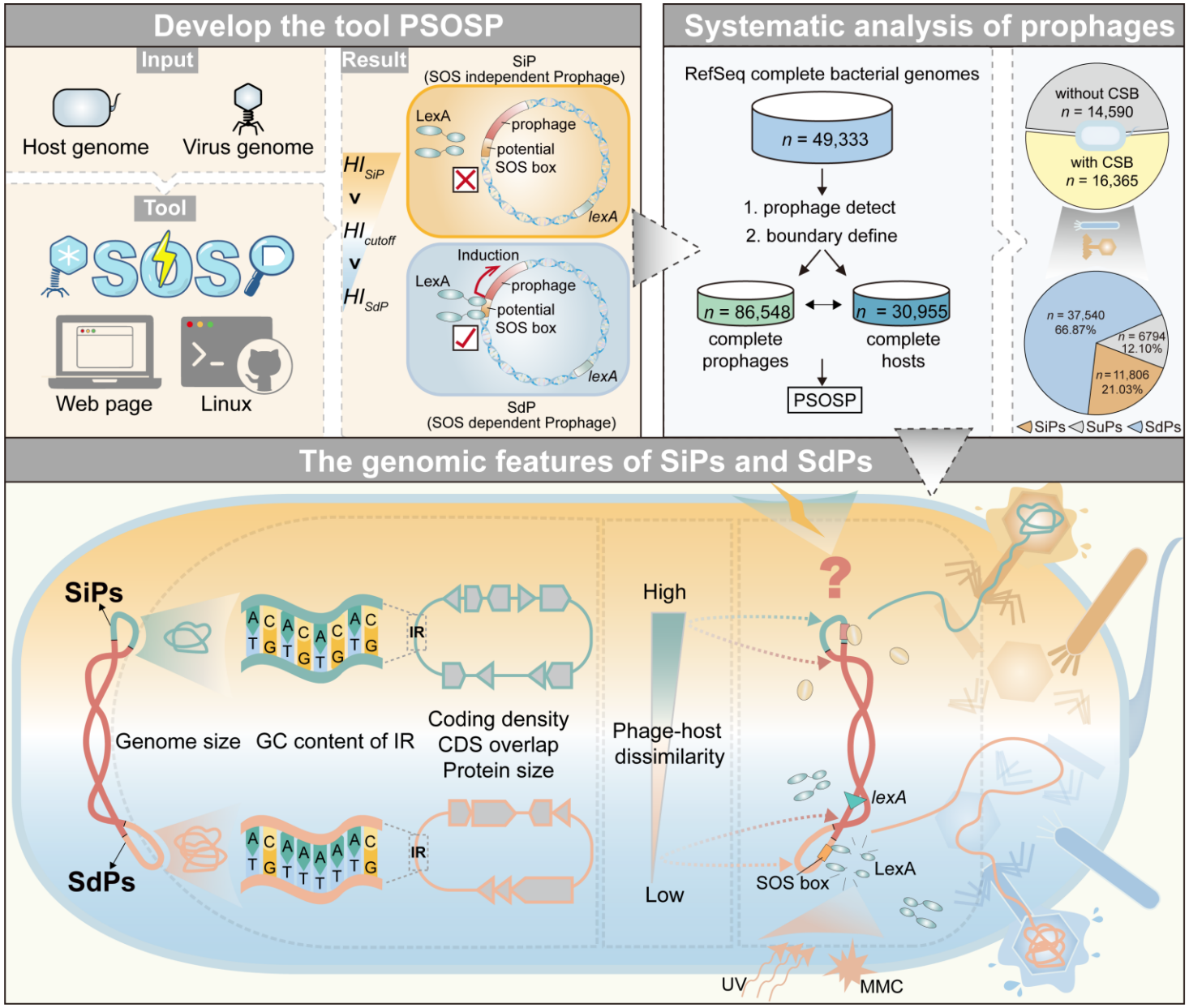
Figure 1(A) Schematic diagram of the currently known lysogenic-lytic switch mechanism in temperate phages.



(S. Dahlman et al. bioRxiv. 2023)

<u>Limitation</u>: due to the lack of specialized tools for determining prophage induction modes, the distribution proportions and genomic characteristics of SOS-independent prophages (SiPs) remain largely unexplored

Highlights



- Develop a novel bioinformatics tool **PSOSP that predicts prophages induction modes;**

Website:https://vee-lab.sjtu.edu.cn/PSOSP/

Github:https://github.com/mujiezhang/PSOSP

- **Identify 11,806 SiPs** by applying PSOSP to 49,333 complete bacterial genomes;
- Uncover that **SiPs and SdPs exhibit distinct genomic and host traits**, suggesting the **potential for mutual conversion** between certain SiP and SdP groups;
- Refine the conventional understanding of **temperate phage induction mechanisms** and **provide novel tools** and insights for exploring the lysogenic-lytic switch of phages

Result 1: *HI* reliably predicts LexA binding potential

- **The workflow of PSOSP:**

(1) scanning the host genome to identify LexA protein and canonical SOS boxes (CSBs) located upstream of the *lexA* gene;
(2) identifying potential SOS boxes (PSBs) across bacterial genomes, calculating the Heterology Index (*HI*) for each PSB and establishing classification thresholds (HI_{C1} and HI_{C2}) via Mean Shift clustering results;
(3) scanning PSBs within prophage promoter regions and determining of the minimum *HI* (HI_{min});
(4) evaluating the ability of LexA binding to prophage promoter regions by comparing HI_{min} with thresholds (HI_{C1} and HI_{C2}), and subsequently classifying the induction modes of prophage

- The binding interactions between previously reported LexA protein with **PSBs (*n* = 24) in *E. coli* K12** (as documented by *Lewis* et al.) could be **precisely predicted based on *HI***

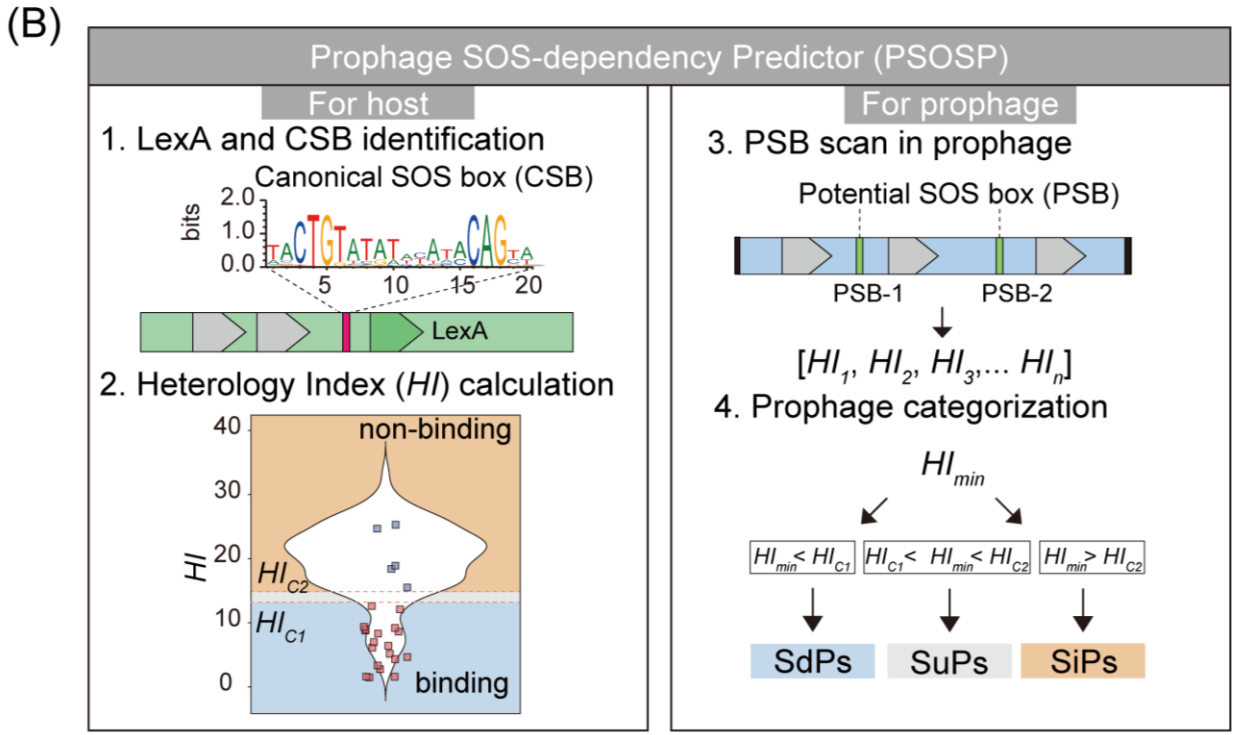


Figure 1(B) Schematic diagram of the PSOSP workflow

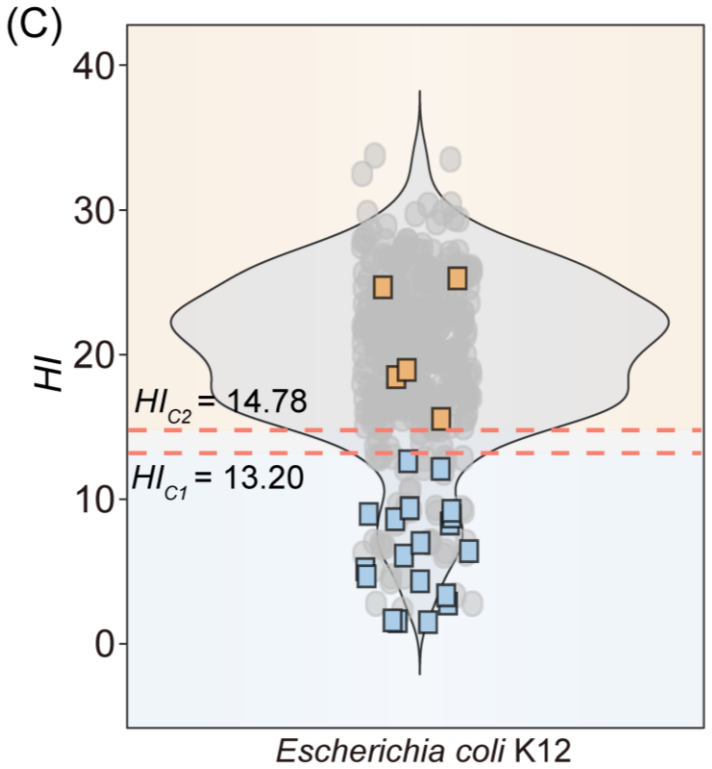


Figure 1(C) Distribution of *HI* for all PSBs in *E. coli* K12

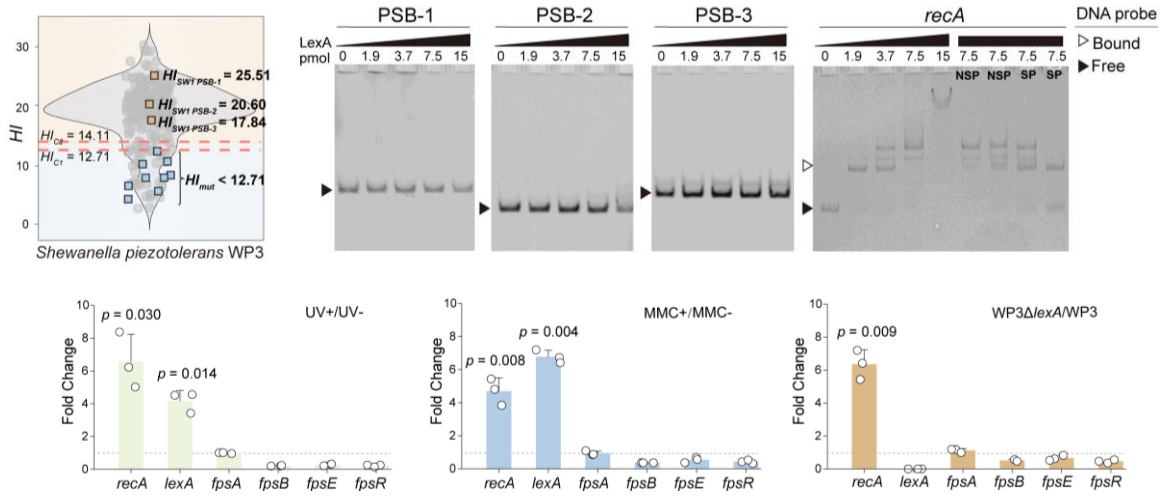
Result 2: PSOSP: determining the regulatory mode of prophages based on *HI*

Table S4.Experimentally validated induction-mode bacteriophages and hosts in this study.

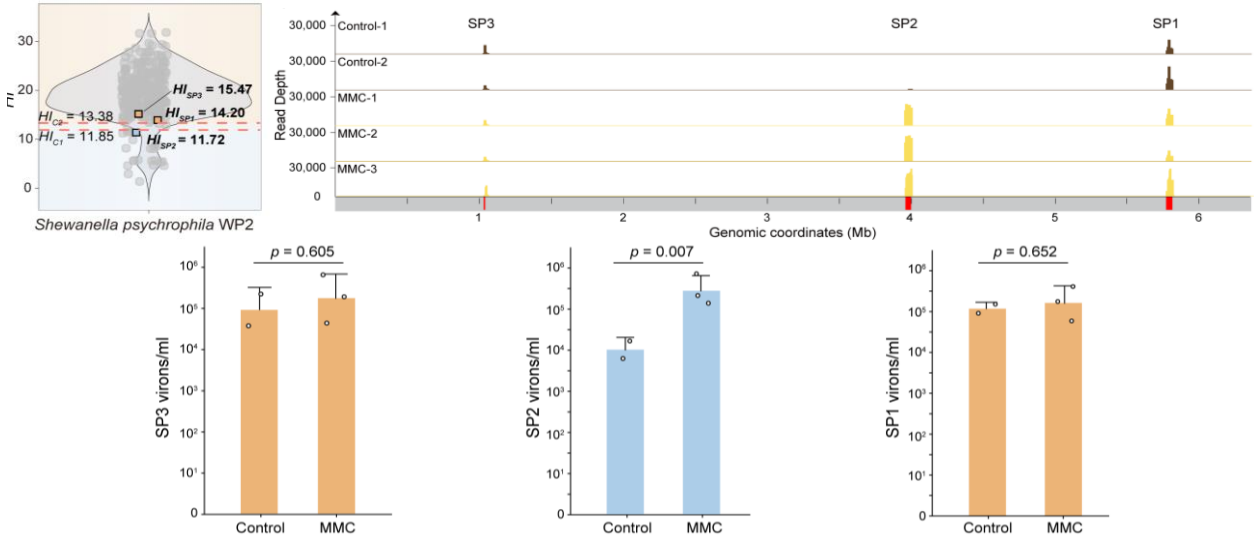
Prophage	Host taxonomy (Genus)	Phage genome size (bp)	Experimentally validated induction mode	PSOSP predicted induction mode
P22	*Salmonella*	41,724	SdP	SdP
Fels-1	*Salmonella*	42,723	SdP	SdP
Fels-2	*Salmonella*	33,693	SdP	SdP
phiECO1	*Escherichia*	31,478	SdP	SdP
ST-8624	*Escherichia*	62,822	SdP	SdP
VALGphi6	*Vibrio*	8,530	SiP	SiP
B3	*Pseudomonas*	38,439	SiP	SiP
vB_SspS_OS31	*Serratia*_J	42,280	SdP	SdP
vB_SspM_BZS1	*Serratia*_J	44,995	SdP	SdP
yong1	*Hafnia*	43,329	SdP	SdP

- **Test set**: **ten prophages** (8.5–62.8 kb) belonging to **eight viral taxonomic families**, with hosts spanning **seven different genera**.
- PSOSP achieve **100% sensitivity and 100% specificity** in the test set

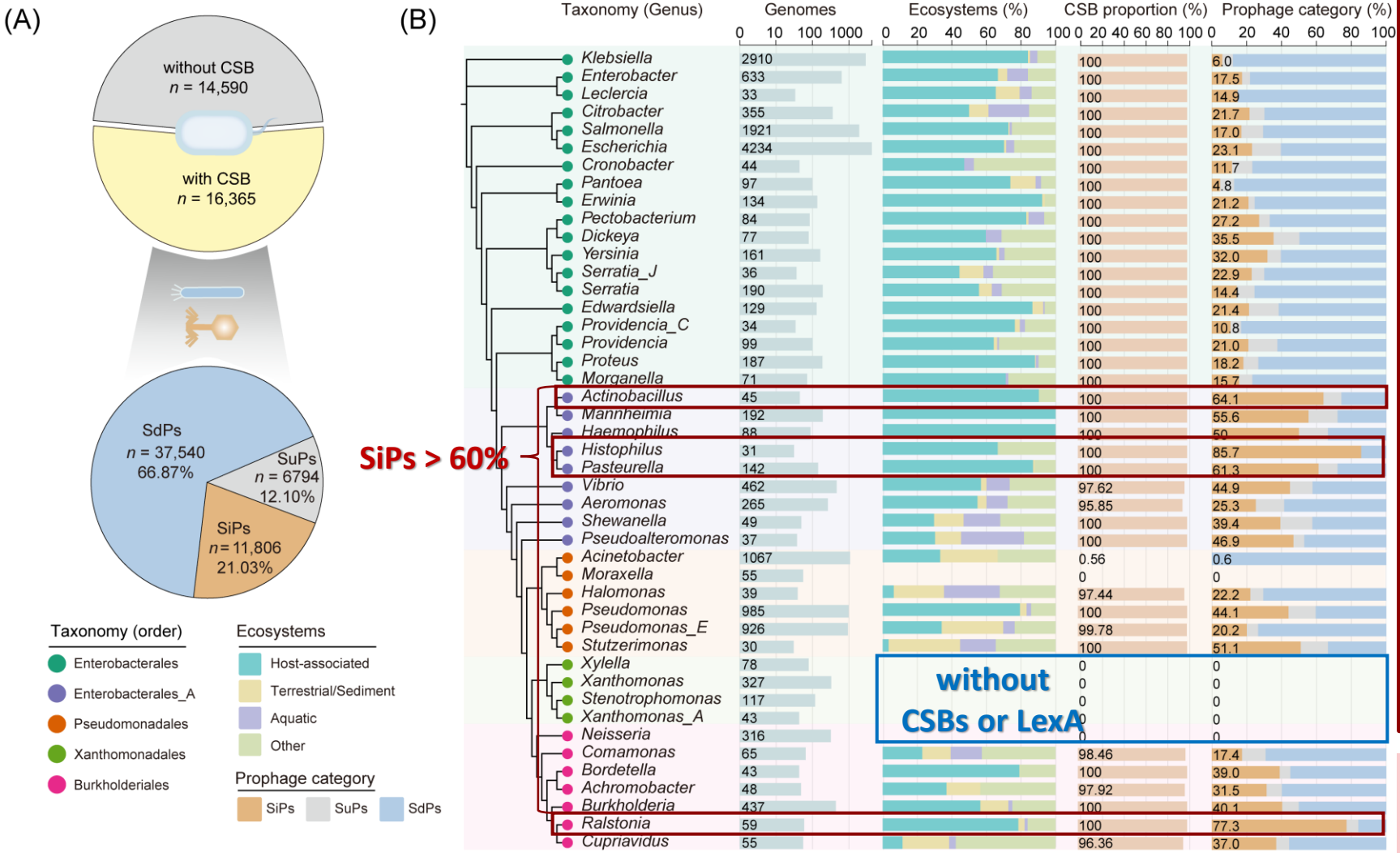
The validation of prophage SW1 in *Shewanella piezotolerans* WP3



The validation of prophage SP1, SP2, SP3 in *S. psychrophile* WP2



Result 3: Systematic analysis of SiPs and SdPs in bacterial genomes

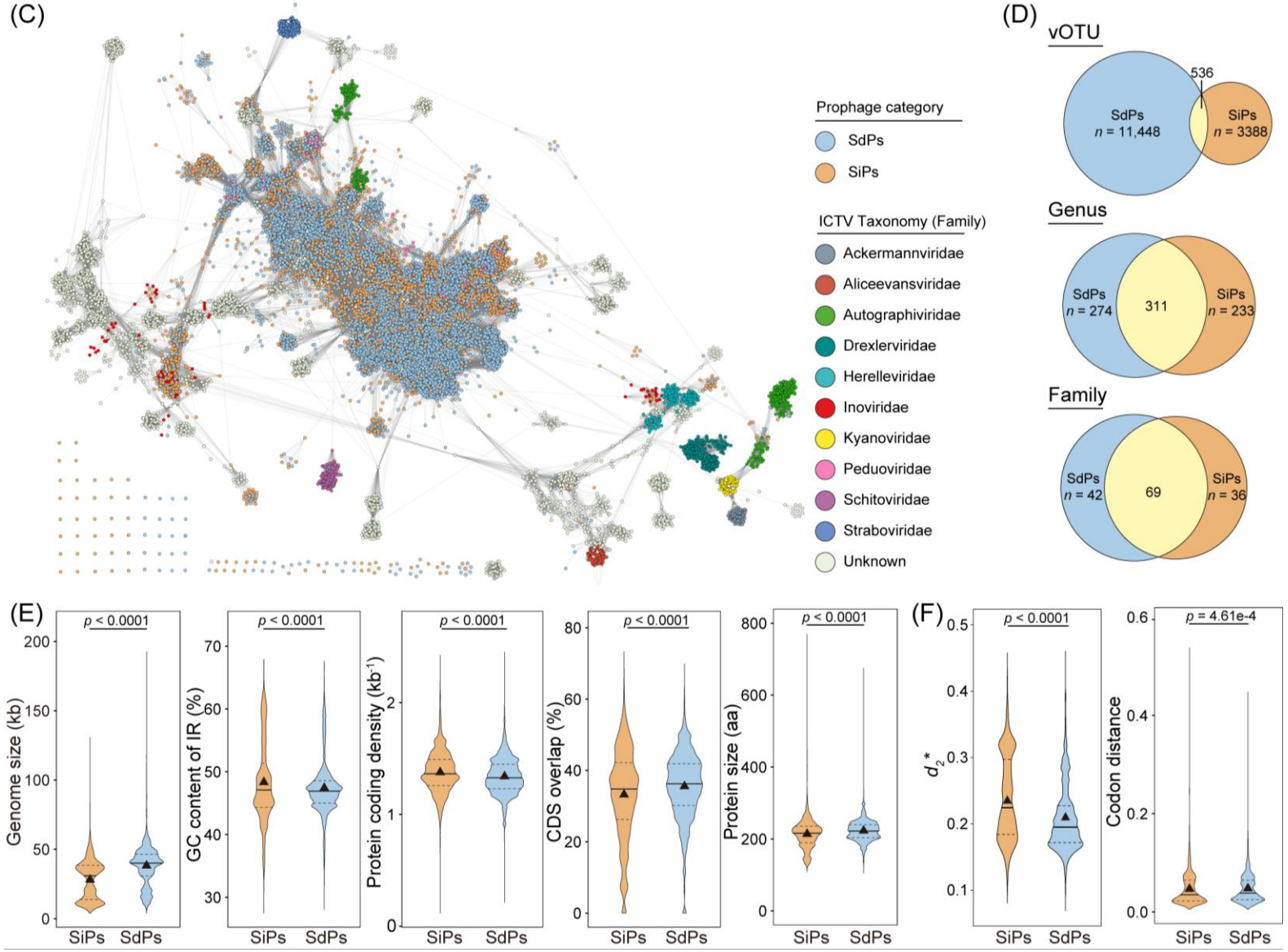


- 16,365 bacteria harbored CSBs, among which **11,806 SiPs (21.03%) were identified**
- **within Gammaproteobacteria**, **SiPs** were found **widely distributed across multiple genera**
- in certain genera, such as *Histophilus*, *Ralstonia*, *Actinobacillus*, and *Pasteurella*, their **proportion exceeded 60%**
- bateria genomes **without CSBs and LexA can't be predicted** by PSOSP

【Discussion1】The limitations and perspectives of PSOSP

Figure 2. Widespread existence of SiPs and the comparison between SiPs and SdPs

Result 3: Systematic analysis of SiPs and SdPs in bacterial genomes



- SiPs and SdPs exhibit both distinct **clustering** and **overlapping** patterns
- at different taxonomic levels, **the degree of shared features between SiPs and SdPs increased with higher taxonomic ranks**

【**Discussion2**】The clustering feature may suggest **the potential for mutual conversion** between certain SiP and SdP groups

- compared to SdPs, SiPs have a significantly **lower** proportion of CDS overlap (PCO) and smaller protein sizes , but **higher** GC content of intergenic region (IR) and protein-coding density (PCD)
- **SiPs exhibited greater nucleotide feature divergence from their hosts** compared to SdPs, suggesting **lower compatibility with their hosts**

Figure 2. Widespread existence of SiPs and the comparison between SiPs and SdPs

Summary

- We developed **a novel bioinformatics tool PSOSP** to predict prophage induction modes. PSOSP was **experimentally validated** to accurately distinguish SdPs from SiPs.

- We discovered that **SiPs were widely distributed within bacterial genomes and exhibited distinct genomic features** compared to the more well-studied SdPs. Correspondingly, **the hosts** of these two prophage types are hypothesized to **differ in their physiological characteristics**.

- These PSOSP-enabled findings provide not only novel insights into **diverse induction mechanisms** but also **a critical methodology** for future studies on phage-host interactions and prophage isolation strategies.

- PSOSP website: https://vee-lab.sjtu.edu.cn/PSOSP/
  PSOSP Github: https://github.com/mujiezhang/PSOSP

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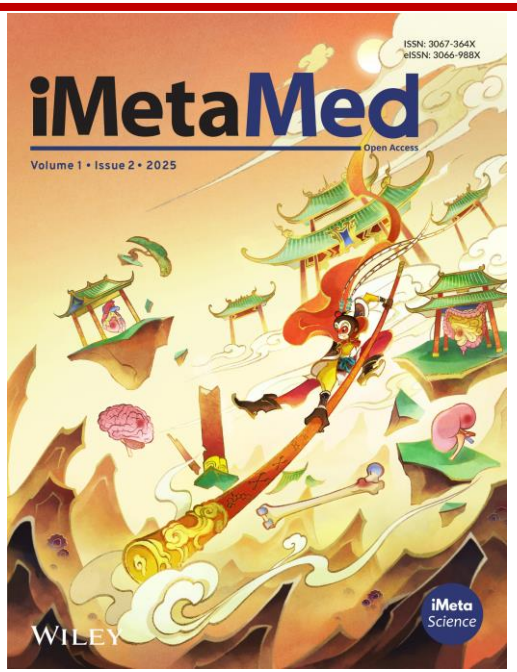

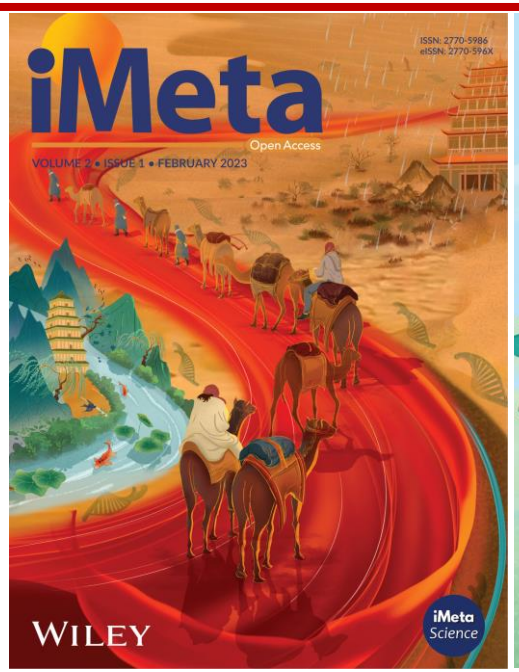

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