

Supplementary Data for

**Genomic and metabolic characterization of plant growth-promoting rhizobacteria isolated from nodules of clover grown in non-farmed soil**

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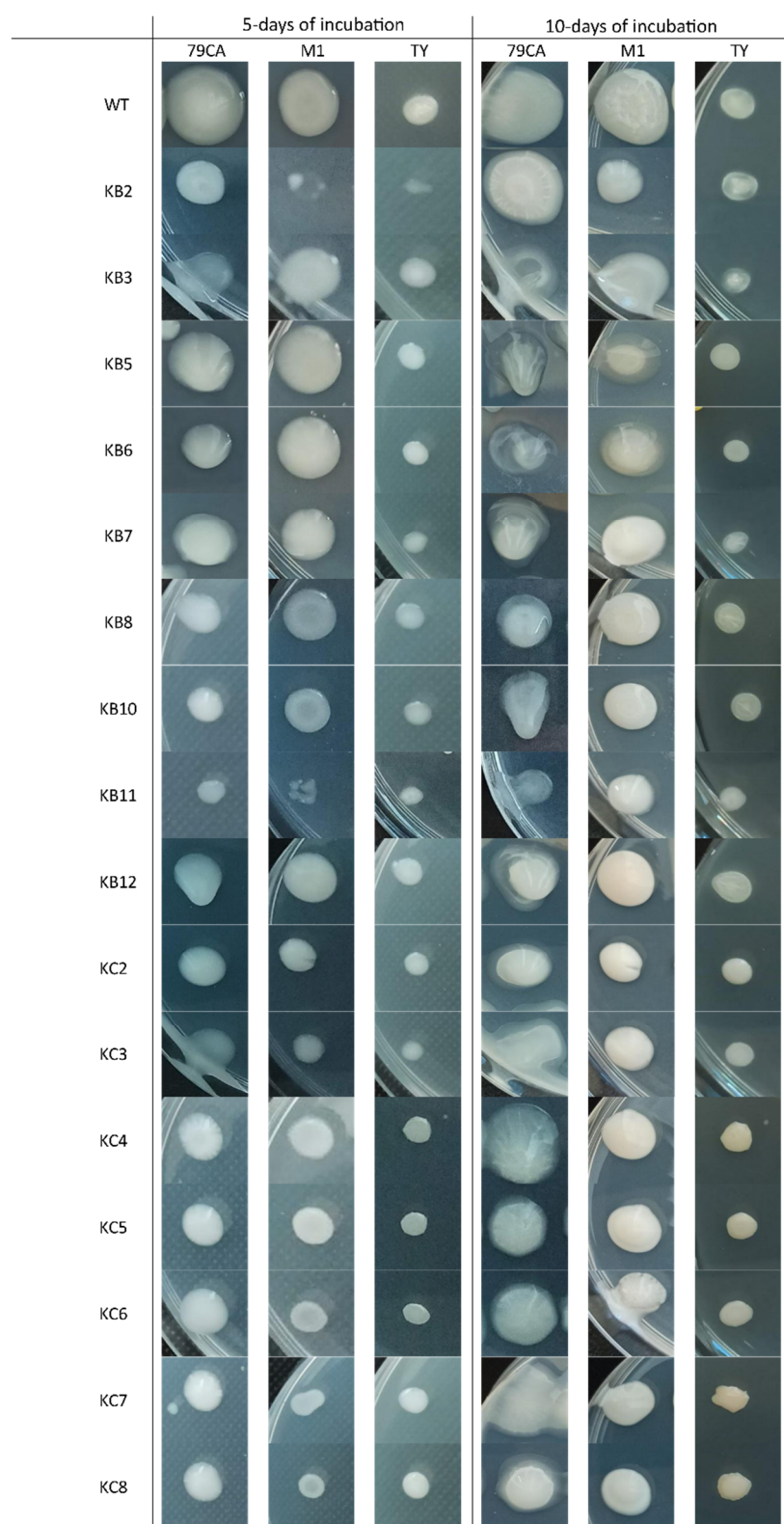
Supplementary Tables S1 to S2  
Supplementary Figures S1 to S5

Supplementary Table S1. Sequencing data processing results.

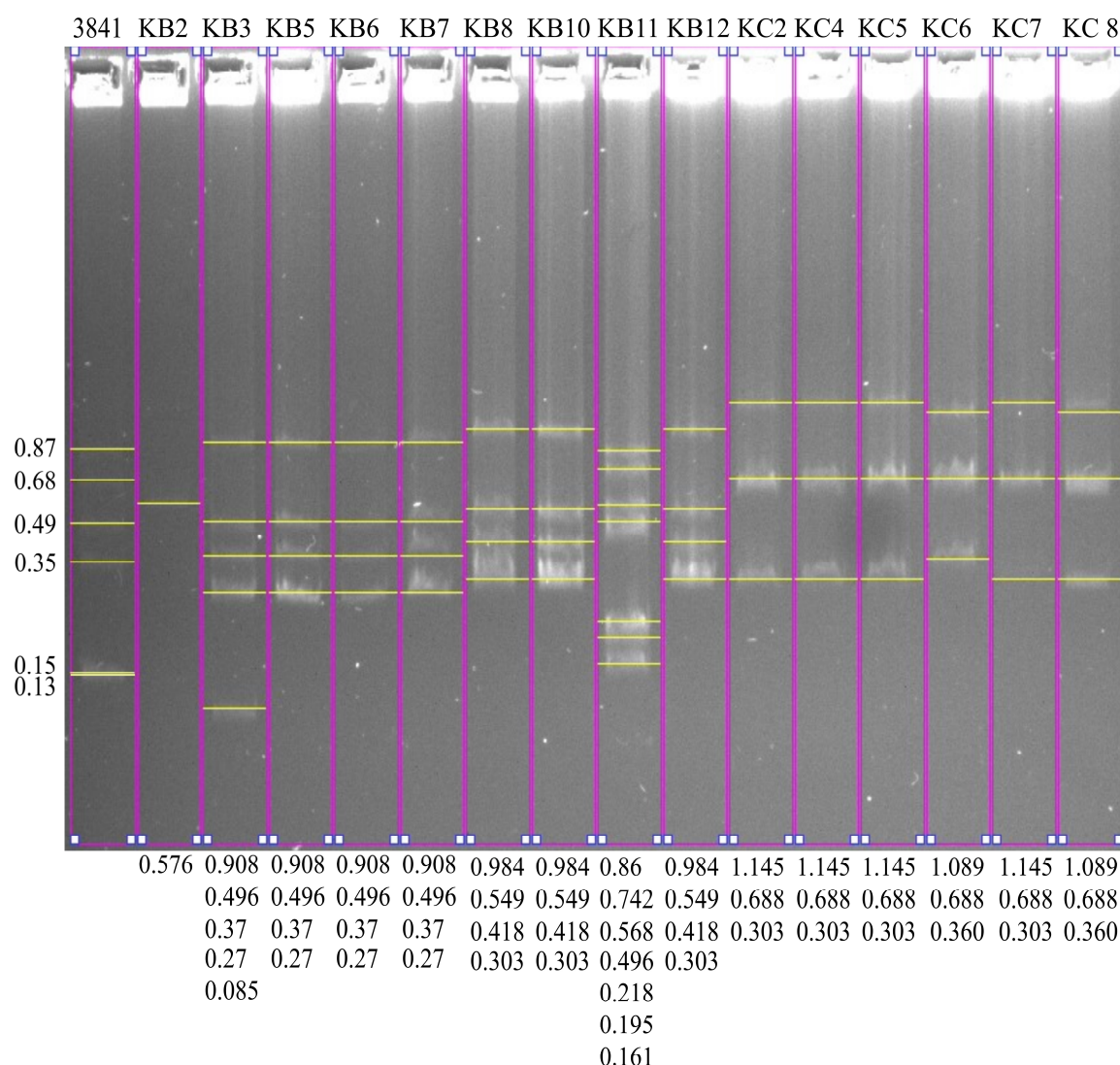
Clover rhizosphere microbiota	Sample name	Input sequences	Sequences after preprocessing	Sequences after chimera removal	Sequences assigned to OTUs	Sequences assigned to taxa
Non-farmed soil	A1	142,741	142,728	142,202	67,660	67,660
	A2	117,392	117,378	117,294	62,174	62,174
	A3	133,225	133,216	132,867	55,417	55,417
Agricultural soil	B1	154,950	154,933	154,578	68,491	68,491
	B2	126,259	126,253	125,933	53,292	53,292
	B3	135,907	135,896	135,600	67,351	67,351
	TOTAL	810,474	810,404	808,474	374,385	374,385

Supplementary Table S2. Comparison of alpha diversity indices within microbiota of soil rhizosphere of clover grown in non-farmed (A) and agricultural soil (B).

Clover rhizosphere microbiota	Sample	Chao1	Simpson	Shannon
Non-farmed soil	A1	42.2	0.892	3.242
	A2	51.8	0.898	3.302
	A3	41.2	0.890	3.222
Agricultural soil	B1	48.6	0.896	3.282
	B2	47.6	0.894	3.262
	B3	42.2	0.892	3.242

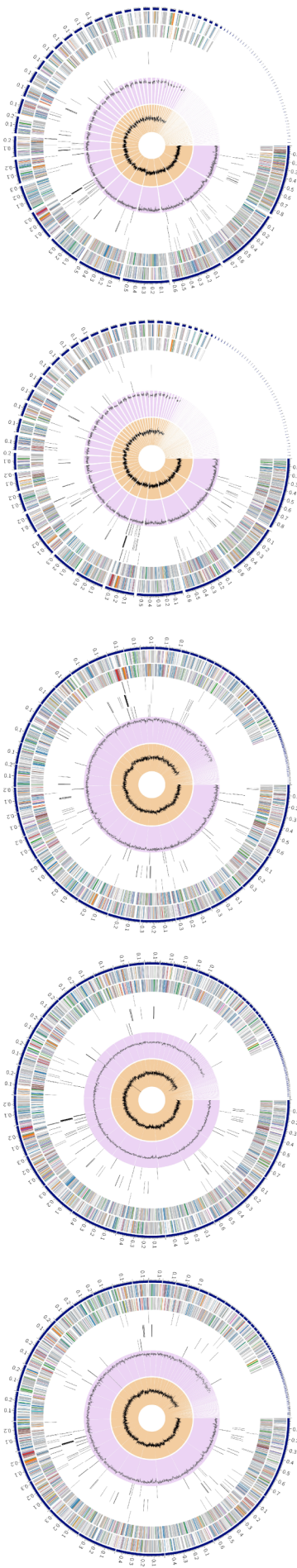


Supplementary Figure S1. Macroscopic characteristics of the nodule isolates derived from white (KB2-12) and red clover (KC2-8) growing in the buffer zone of Białowieża National Park compare with the wild type strain (*Rhizobium leguminosarum* bv. *trifolii* TA1 (marked as WT)). The strains were cultivated in solid 79CA, M1, and TY media. Bacterial suspensions in the bottom panel were washed and standardized to the same starting optical density. Photographs were taken 5 and 10 days post-inoculation (left and right, respectively).

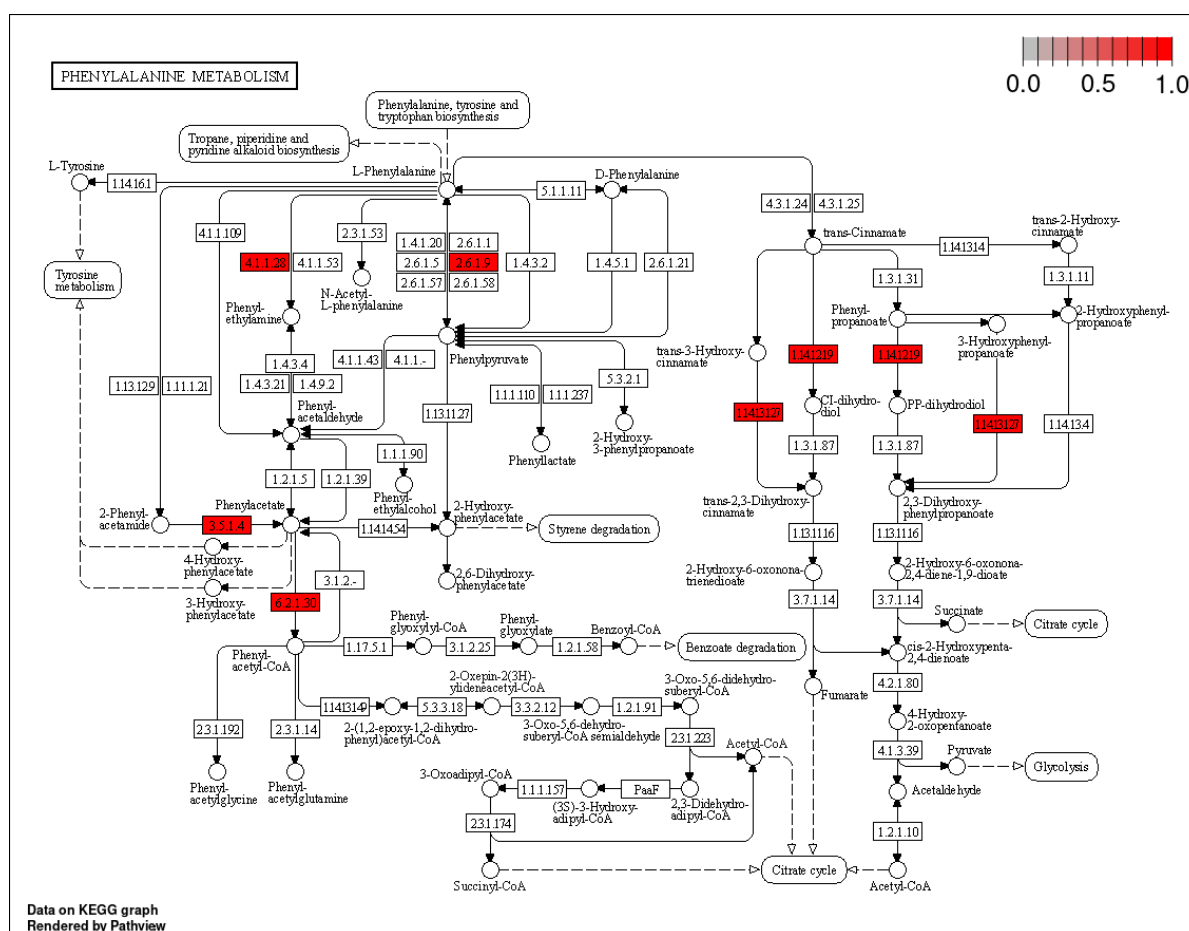


Supplementary Figure S2. Plasmid profiling of *Rhizobium* strains by the Eckhardt method. Lanes: 1: *Rhizobium leguminosarum* bv. *viciae* 3841<sup>T</sup> (T-type strain, ECR size marker); 2-17: *Rhizobium* isolates derived from white (KB2-12) and red clover (KC2-8) growing in the buffer zone of Białowieża National Park. The numbers on the left indicate the size of the replicons (in Mb) of the reference strain, which was the standard for determining the ECR size of the KB and KC strains. The calculated size of KB and KC ECR (in Mb) determined on the basis of electrophoretic mobility relative to the reference strain, arranged from the largest to the smallest, were shown below the lanes.

<i>Rhizobium leguminosarum</i> K5		<i>Rhizobium leguminosarum</i> K4		<i>Rhizobium leguminosarum</i> K87		<i>Rhizobium leguminosarum</i> K88		<i>Rhizobium leguminosarum</i> K812	
Length: 7373243bp, Contigs: 81		Length: 7341322bp, Contigs: 86		Length: 7098746bp, Contigs: 101		Length: 7412147bp, Contigs: 103		Length: 7412133bp, Contigs: 103	
General Info		General Info		General Info		General Info		General Info	
Genome ID	384.1015	384.1014		379.691		379.692		384.1013	
Genome Name	<i>Rhizobium leguminosarum</i> K5	<i>Rhizobium leguminosarum</i> K4		<i>Rhizobium leguminosarum</i> K87		<i>Rhizobium leguminosarum</i> K88		<i>Rhizobium leguminosarum</i> K812	
Taxonomy Info									
Taxon ID	384	384		379		379		384	
Kingdom	Bacteria	Bacteria		Bacteria		Bacteria		Bacteria	
Phylum	Proteobacteria	Proteobacteria		Proteobacteria		Proteobacteria		Proteobacteria	
Class	Alphaproteobacteria	Alphaproteobacteria		Alphaproteobacteria		Alphaproteobacteria		Alphaproteobacteria	
Order	Hyphomicrobiales	Hyphomicrobiales		Hyphomicrobiales		Hyphomicrobiales		Hyphomicrobiales	
Family	<i>Rhizobiaceae</i>	<i>Rhizobiaceae</i>		<i>Rhizobiaceae</i>		<i>Rhizobiaceae</i>		<i>Rhizobiaceae</i>	
Genus	<i>Rhizobium</i>	<i>Rhizobium</i>		<i>Rhizobium</i>		<i>Rhizobium</i>		<i>Rhizobium</i>	
Species	<i>Rhizobium leguminosarum</i>	<i>Rhizobium leguminosarum</i>		<i>Rhizobium leguminosarum</i>		<i>Rhizobium leguminosarum</i>		<i>Rhizobium leguminosarum</i>	
Genome Statistics		Genome Statistics		Genome Statistics		Genome Statistics		Genome Statistics	
Contigs	81	86		101		103		103	
Genome Length	7373243	7341322		7098746		7412147		7412133	
GC Content	60.731064	60.747234		60.78547		60.722973		60.72313	
Contig L50	6	7		11		8		8	
Contig N50	395773	310761		221519		295871		295871	
Annotation Statistics		Annotation Statistics		Annotation Statistics		Annotation Statistics		Annotation Statistics	
tRNA	45	45		46		47		47	
rRNA	3	3		3		3		3	
CDS	7515	7478		7202		7570		7567	
CDS Ratio	1.019258	1.0186176		1.0145454		1.0212965		1.0208937	
Hypothetical CDS	2144	2099		1955		2102		2104	
Hypothetical CDS Ratio	0.38310048	0.37951323		0.37017494		0.37701452		0.37729615	
PFAM CDS	7078	7086		6733		7004		7000	
PFAM CDS Ratio	0.94184985	0.94757956		0.9348792		0.92523116		0.9250694	
Genome Quality		Genome Quality		Genome Quality		Genome Quality		Genome Quality	
Coarse Consistency	99.5	99.7		99.3		99.3		99.3	
Fine Consistency	95.8	96.3		96.3		95.4		95.4	
CheckM Completeness	100	100		100		100		100	
Genome Quality	Good	Good		Good		Good		Good	

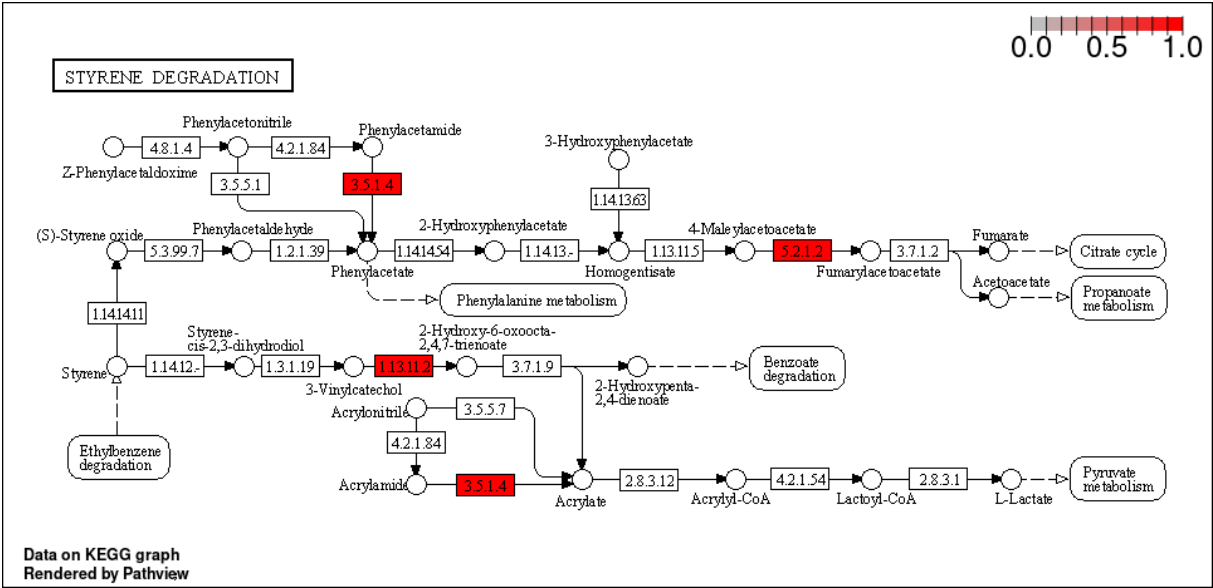


A)





B)





**QUORUM SENSING**

The diagram illustrates the quorum sensing pathways in various bacterial species, categorized by the type of event (Biosynthetic, Activation, Sensing, Signaling) and the specific signaling molecules involved.

**Species and Pathways:**

- Vibrio:** Shows the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Pseudomonas:** Details the LasI/LexA and SdiI/SdiR systems, which regulate the production of proteases, elastase, and other virulence factors.
- Burkholderia:** Illustrates the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Escherichia:** Shows the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Rhodospirillum:** Details the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Klebsiella:** Illustrates the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Agrobacterium:** Shows the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.
- Bacillus:** Details the production of autoinducers (AI-1, AI-2) and their role in regulating virulence factors and biofilm formation.

**Key Signaling Molecules and Pathways:**

- AI-1 (N-acyl homoserine lactone):** Produced by LuxI/LexA, SdiI/SdiR, and other synthase/receptor pairs. It regulates various physiological processes.
- AI-2 (2,2'-diacetylserine):** Produced by LuxS/SymK and other synthase/receptor pairs. It regulates various physiological processes.
- c-di-GMP:** A second messenger involved in biofilm formation and virulence regulation.
- Autoinducers:** Various signaling molecules produced by different bacterial species, including autoinducers, autoinducers, and autoinducers.

**Regulation of Gene Expression:**

- Virulence factors:** Regulated by various signaling molecules, including autoinducers, autoinducers, and autoinducers.
- Biofilm formation:** Regulated by various signaling molecules, including autoinducers, autoinducers, and autoinducers.
- Sporulation:** Regulated by various signaling molecules, including autoinducers, autoinducers, and autoinducers.

Supplementary Figure S4. Mapping the KB unique genes to the KEGG: A) phenylalanine metabolism (00360), B) styrene degradation (00643), C) quorum sensing (02024), pathway maps. Chemical compounds are represented as circles and gene products are represented as rectangles.



[illegible]

Supplementary Figure S5. Mapping the KC unique genes to the KEGG A) benzoate degradation (00362), B) fluorobenzoate degradation (00364), C) quorum sensing (02024) pathway maps. Chemical compounds are represented as circles and gene products are represented as rectangles.