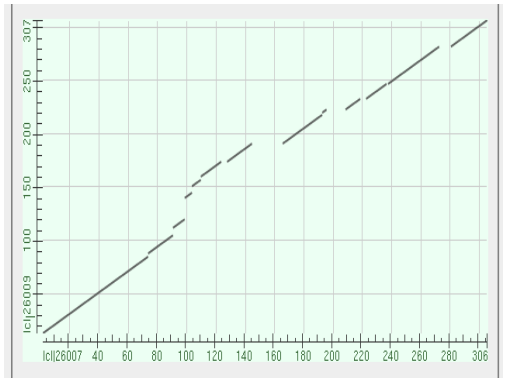
A

BLASTP Parameters:

Expect Threshold: 10
Word size: 3
Max matches in a query range: 0
Matrix: BLOSUM62
Gap Costs: existence 10, extension 1*
Compositional Adjustments: Conditional compositional score matrix adjustment

*Default values are: existence 11, extension 1.

B

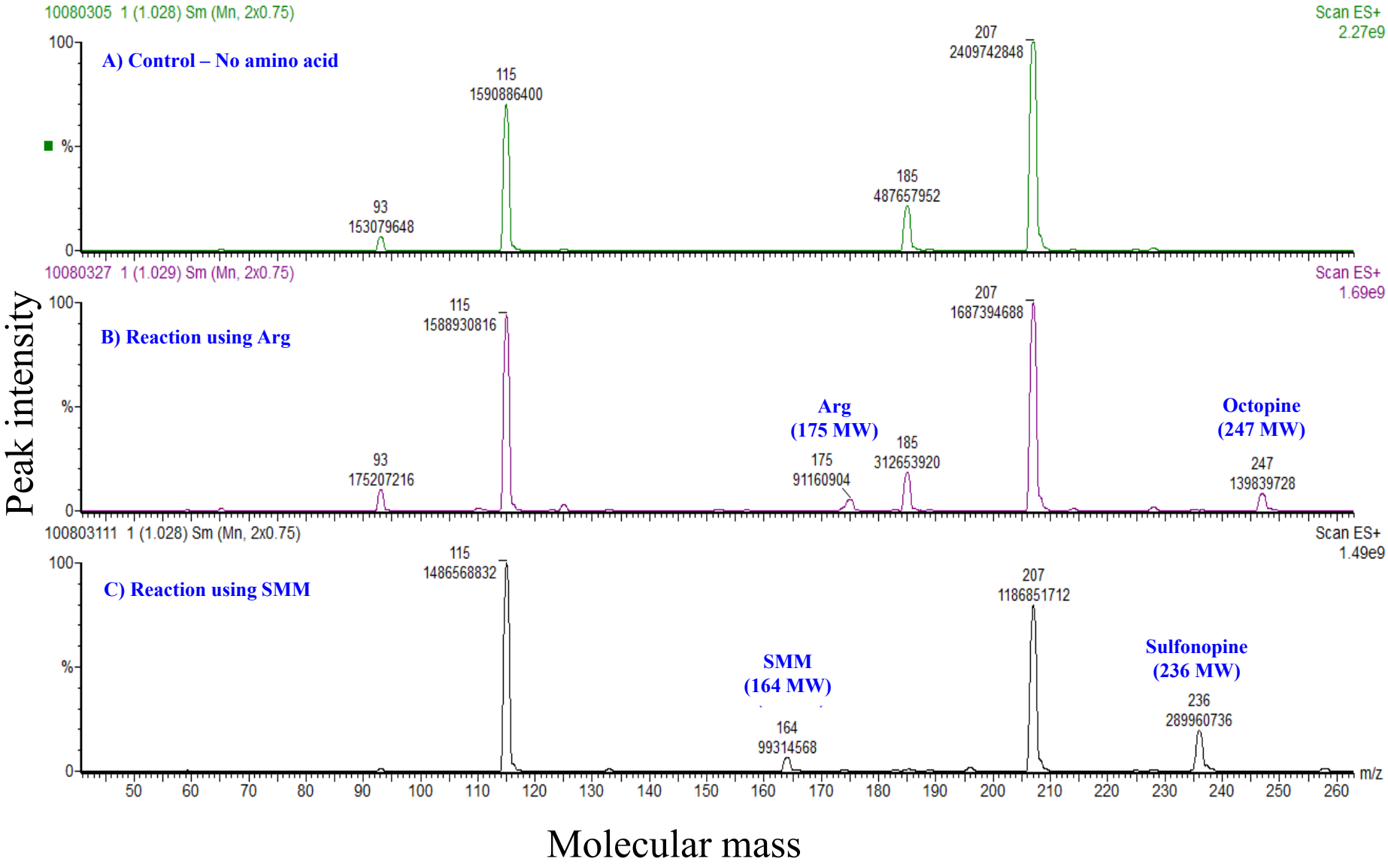


C

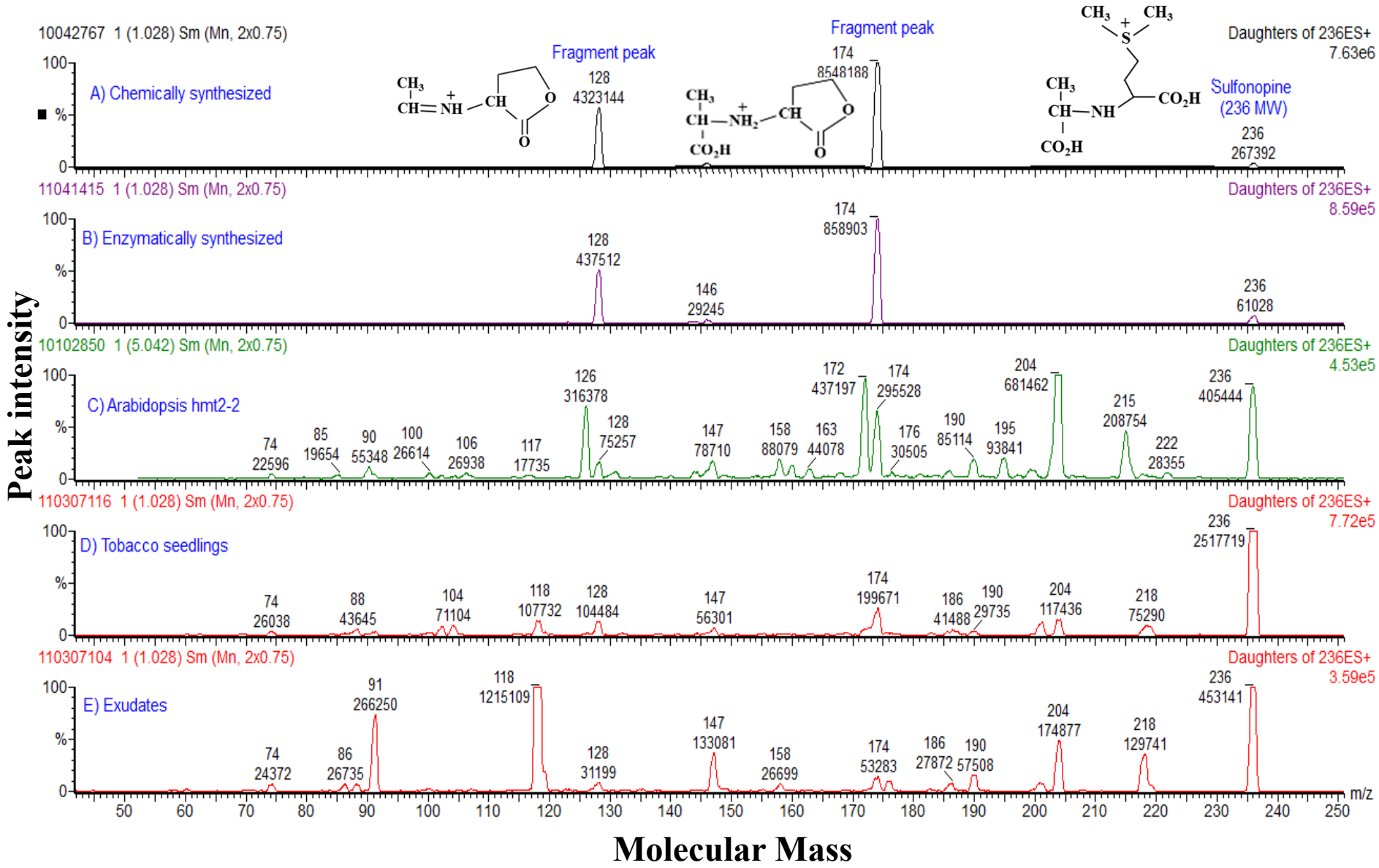
Identities = 80/345 (23%), Positives = 118/345 (34%), Gaps = 92/345 (26%)

```
 Msh 3   SKVTILDGGMGRELLRNGAPFRQPEWSALSLIEAPEFVKMAHDAFVAAGAEVITTNSYAL  62
          + + LDG M  EL   G  +    WSA  L+E PE+++  H  +  AGA+   T SY
Mmum 14  QDILLLDGAMATELEARGCNLADSLWSAKVLVENPELIREVHLDYYRAGAQCAITASYQA  73

 Msh 63  VPFHIGDQALPH---MGLLSPIYPAARSCRRA-------EGTTVCTG------------  99
          P     + L       L+      AR  R A        GT +  G
Mmum 74  TPAGFAARGLDEAQSKALIGKSVELARKAREAYLAENPQAGTLLVAGSVGPYGAYLADGS 133

 Msh 100 -------CRLSA------PRLRLL---PARFVRCRQGACHSRYPRQSPKLLTSISGSPRP 143
                C + A      PR+  L    A ++ C      S    +   L   ++  PR
Mmum 134 EYRGDYHCSVEAFQAFHRPRVEALLDAGADLLACETLPNFS----EIEALAELLTAYPRA 189

 Msh 144 RAPSRRSKQIRNDIGLRLRGPAVGFLYARRTTKSVADVICLVSVPTLVSG--EAVAQRRS 201
         RA                    F +  R ++ ++D   L  V  L++G  + V
Mmum 190 RA--------------------WFSFTLRDSEHLSDGTPLRDVVALLAGYPQVV----- 223

 Msh 202 QSSAACAGALLFNCSQAEIMEAGVKSANQALKADNLDIPIGVYANAFVPKPETEESLAAN 261
               AL  NC   E       +A Q L    + +P+ VY N+         ++   +
Mmum 224 --------ALGINCIALE----NTTAALQHLHGLTV-LPLVVYPNSGEHYDAVSKTWHHH 270

 Msh 262 DGLSGLRDDLNPSGYLQFAQRWVSAGATIIGGCCGIGPEHIAELK 306
               D  P        +W +AGA +IGGCC   P IA  LK
Mmum 271 GEHCAQLADYLP--------QWQAAGARLIGGCCRTTPADIAALK 307
```

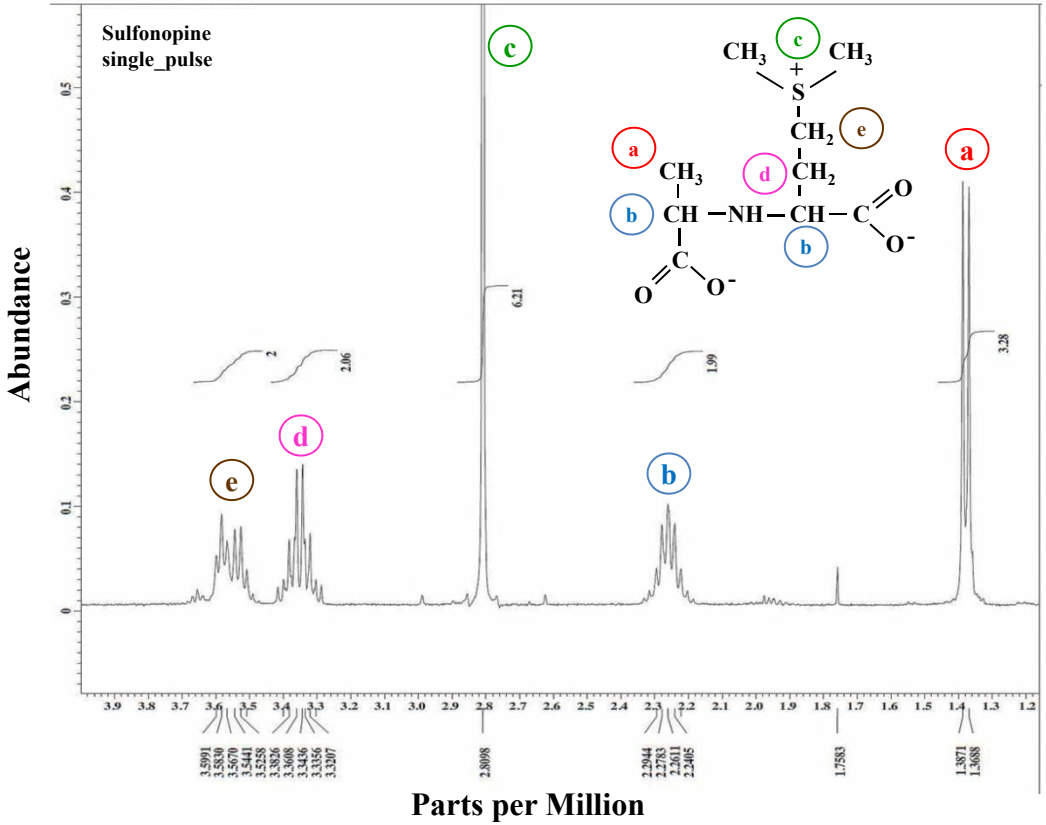
Supplementary Figure S1 End-to-end sequence similarity between Msh and the MmuM protein of *E. coli*. (A) BLAST parameters. (B) Msh and Mmum amino acid sequence alignment. (C) Scatter plot of the amino acid comparison between Msh and MmuM.



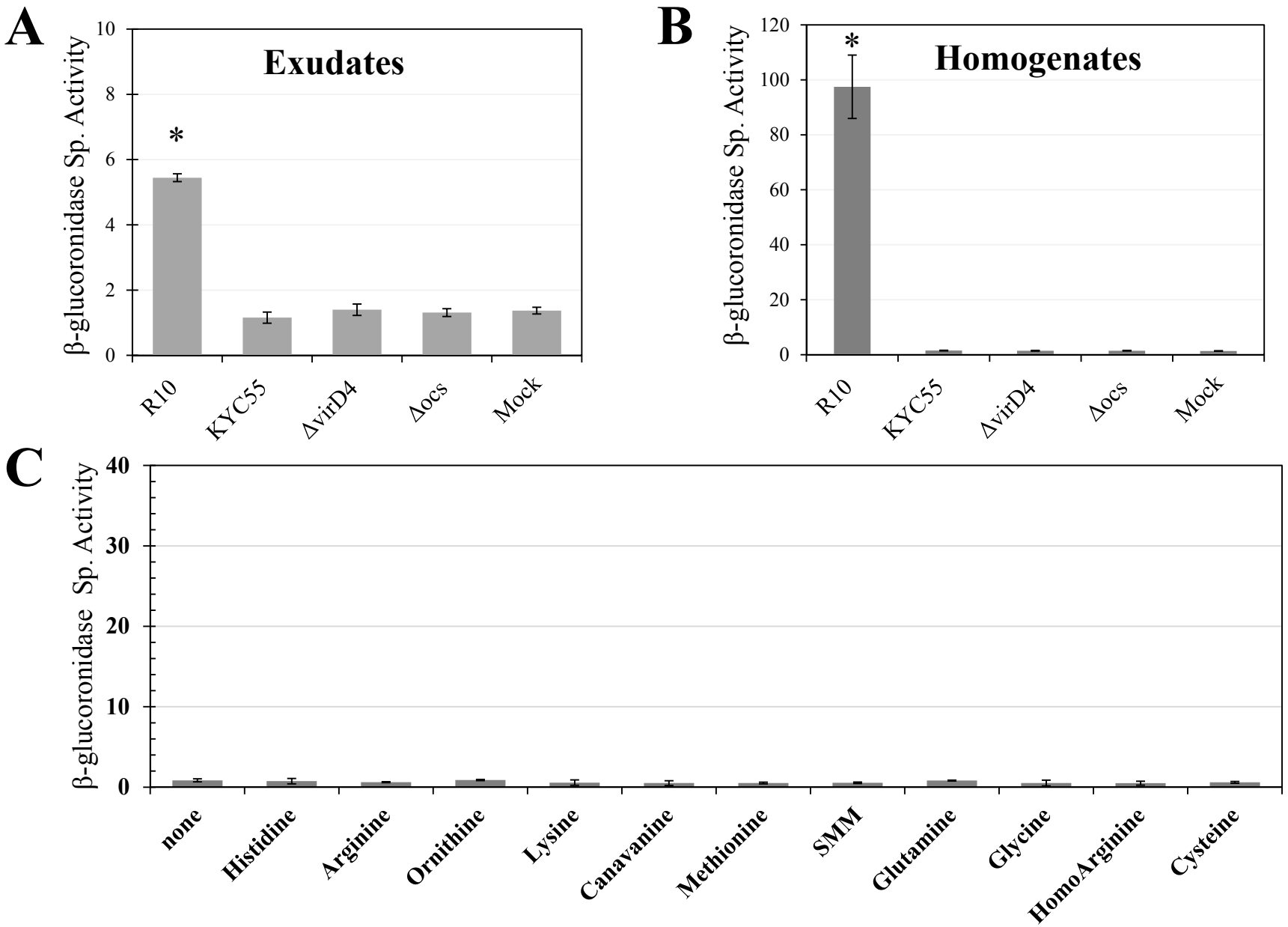
Supplementary Figure S2. Detection of octopine and sulfonopine by ESI-MS/MS in the Ocs enzymatic reaction. The enzymatic reactions contain 150 mM PIPES pH6.6 buffer, 10 mM of pyruvate, 10 mM of NADPH and 10 mM of amino acid (arginine or SMM) and 0.08 uM Ocs. (A) Control enzymatic reaction- no amino acid. (B) Reaction with arginine. (C) Reaction with SMM. X-axis molecular weight and y-axis peak intensity. Peaks 93, 115 and 207 are compounds from the enzymatic reaction.



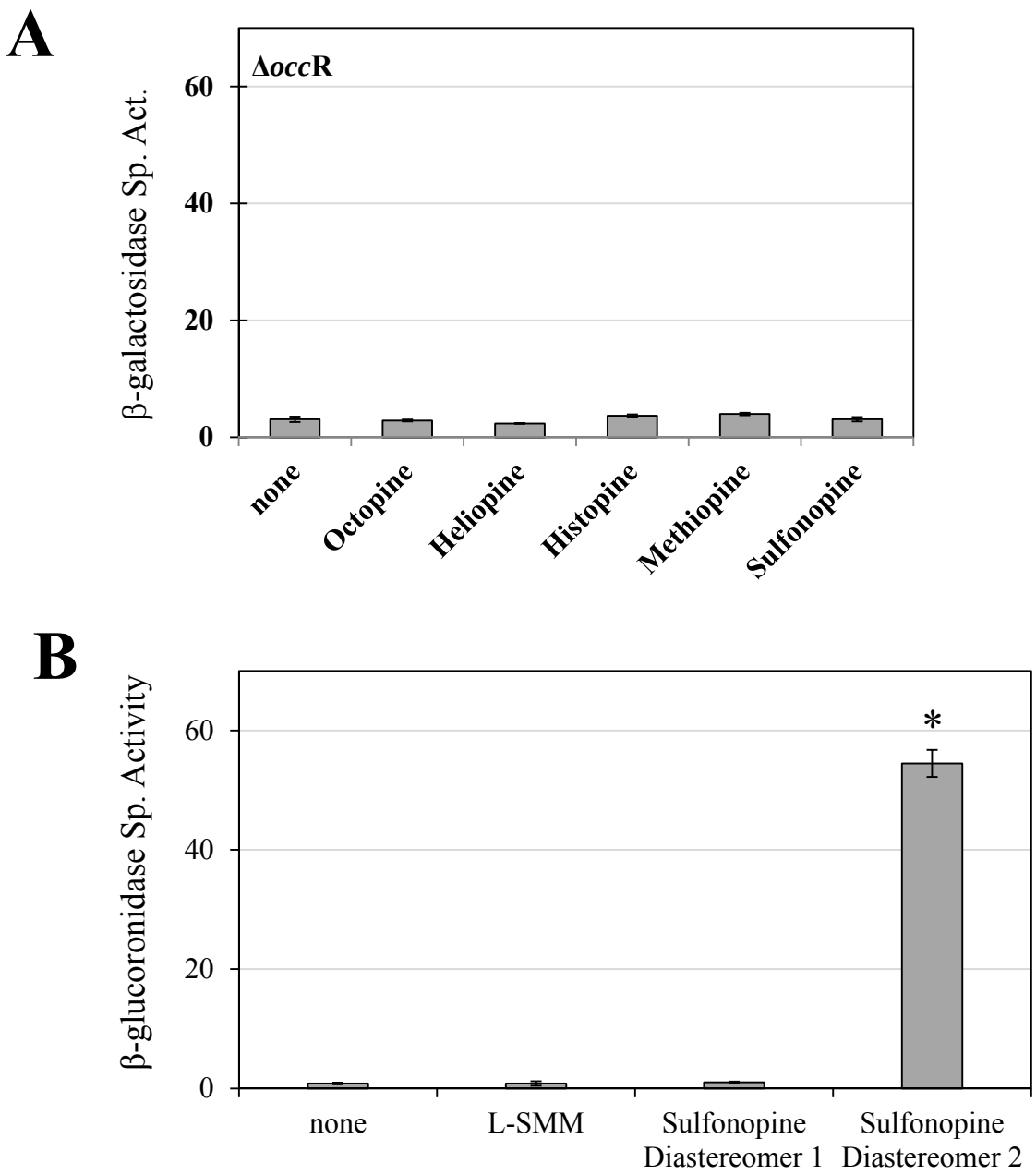
Supplementary Figure S3. Authentication of sulfonopine production: (A) chemically synthesized, (B) enzymatically synthesized, (C) *Arabidopsis* tissue, (D) tobacco seedling tissue and (E) in tobacco seedling exudates. The authentication of sulfonopine was done by fragmenting it and comparing the fragments with the authentic compound chemically synthesized. Sulfonopine has two fingerprint fragments, which have a molecular weight of 174 and 128.



Supplementary Figure S4. ^{1}H NMR spectrum of Sulfonopine in D_20. x-axis parts per Million. y-axis abundance.



Supplementary Figure S5. Induction of *occ* operon by octopine-type opines and amino acids. β-glucoronidase specific activity of *ooxA-uidA* strain KYC16 cultured in the presence of cultured in the presence of (A) exudates or (B) tissue homogenates from infected tobacco seedlings or in the presence of amino acids (C). Mean ± SD of n=3. *P<0.001, *t* test relative to none sample.



Supplementary Figure S6. Induction of *occ* operon by octopine-type opines. (A) β-galactosidase activity of *A. tumefaciens* KYC1203 (pKYC148) (Δ *occ*R) cultured in the presence of different opines. pKYC148 contains an *occ*Q-lacZ translational fusion. (B) β-glucoronidase specific activity of *ooxA-uidA* strain KYC16 cultured in the presence of SMM and Sulfonopine. Mean ± SD of n=3. *P<0.001, *t* test relative to none sample.

Table S1. Bacterial Strains and plasmid used in this study

Strains or plasmids	Relevant features	References
BL21/DE3	*E. coli* P_{lac}-gene 1 of bacteriophage T7	(Studier et al., 1990)
S17-1/λpir	RK2, *tra* regulon, *pir*, host for *pir*-dependent plasmid	(Simon et al., 1983)
R10	*A. tumefaciens* R10	(S. K. Farrand)
KYC55	*A. tumefaciens* R10, Ti plasmid less	(Cho et al. 1997)
KYC16	R10 (*oox*A::Tn5*gus*A7), Km^R	(Cho et al. 1996)
ALFM20	R10::pAFM110, *ocs*-lacZ, Km^R	This study
ALFM21	R10::pAFM111, *vir*D4-lacZ, Km^R	This study
Plasmids		
pVIK107	*lac*ZY for translational fusions, Km^R	(Kalogeraki and Winans, 1996)
pMCSG19	P_{T7}-MBP-TVMV-his_6-TEV, Amp^R	(Donnelly et al. 2005)
PT7-groE	P_{T7}-*gro*ESL, ColE1; Cm^R	(Yasukawa et al., 1995)
pRK1037	P_L-tetO-TVMV protease, Km^R	(Donnelly et al. 2005)
pAFM04	*ocs* cloned into pMCSG19, Amp^R	This study
pAFM11	*msh* cloned into pMCSG19, Amp^R	This study
pAFM110	pVIK107, *ocs* internal fragment, in-frame translational fusion	This study
pAFM111	pVIK107, *vir*D4 internal fragment, in-frame translational fusion	This study

Table S2. Oligonucleotides used in this study

Oligonucleotide Name	DNA Sequence
ALFM21	5’-CGCGGATCCATGTCATCGAAAGTC-3’
ALFM27	5’-ATA GTTTAGCGGCCGCTC AGGCTGCGG C-3’
ALFM28	5’-TACTTCCAATCCAATGCAATGGCTAAAGTGGCA-3’
ALFM29	5’-TTATCCACTAATTCAAACTCCATTGAG-3’
ALFM218	5’-GGGGTACCTCGGTCCTCGTAGCATTGCCC-3’
ALFM219	5’-CGCGGATCCTCTTGGAGTTTCGATATCAGC-3’
ALFM220	5’-GGGGTACCATTGGCGAAATGCAGCATGCT-3’
ALFM221	5’-CGCGGATCCTAGGCTTTCCTCCGCGAGTTG-3’