D-Xylose Blocks the Broad Negative Regulation of XylR on Lipid Metabolism and Affects Multiple Physiological Characteristics in Mycobacteria

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Figure S1. Bacterial one-hybrid assay for XylR and *xylR*p interaction.

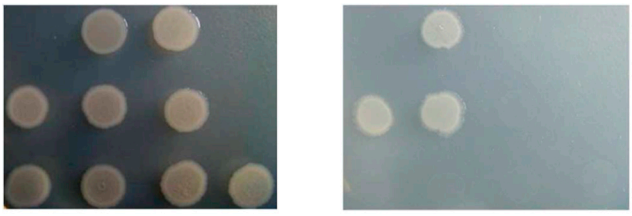
Figure S2. EMSA assays for XylR binding to DNA substrates of mutant IR sequence.

Figure S3. The assays of β-galactosidase activity.

Figure S4. ITC assays for interaction between XylR and L-Ara.

Figure S5. The assays for the growths of wildtype and recombinant *M. smegmatis* strains under the induction of D-xylose.

Table S1. Transcriptomic assays for the differential gene expression of the *xylR*-overexpressing compared to the Msm/pMV261 strain (provided as an Excel table, separately)



-3AT -Str^r +3AT +Str^r

	CK$^+$	CK$^-$	
pTRG-*xylR*/ pBX-*Ms6021*p	pTRG-*xylR*/ pBX-*xylR*p	pTRG-*xylR*/ pBX-*Ms6020*p	
pTRG/ pBX-*Ms6021*p	pTRG/ pBX-*xylR*p	pTRG/ pBX-*Ms6020*p	pTRG-*xylR*/ pBXcmT

Figure S1. Bacterial one-hybrid assay for XylR and *xylR*p interaction. A pair of pBXcmT/pTRG plasmids was co-transformed into the reporter strain and its growth was monitored together with that of the self-activation controls on selective medium. Co-transformants containing the pBX-*Rv2031*/pTRG-*Rv3133c* plasmids served as positive controls (CK$^+$) and co-transformants containing the empty vectors pBX and pTRG served as negative controls (CK$^-$).

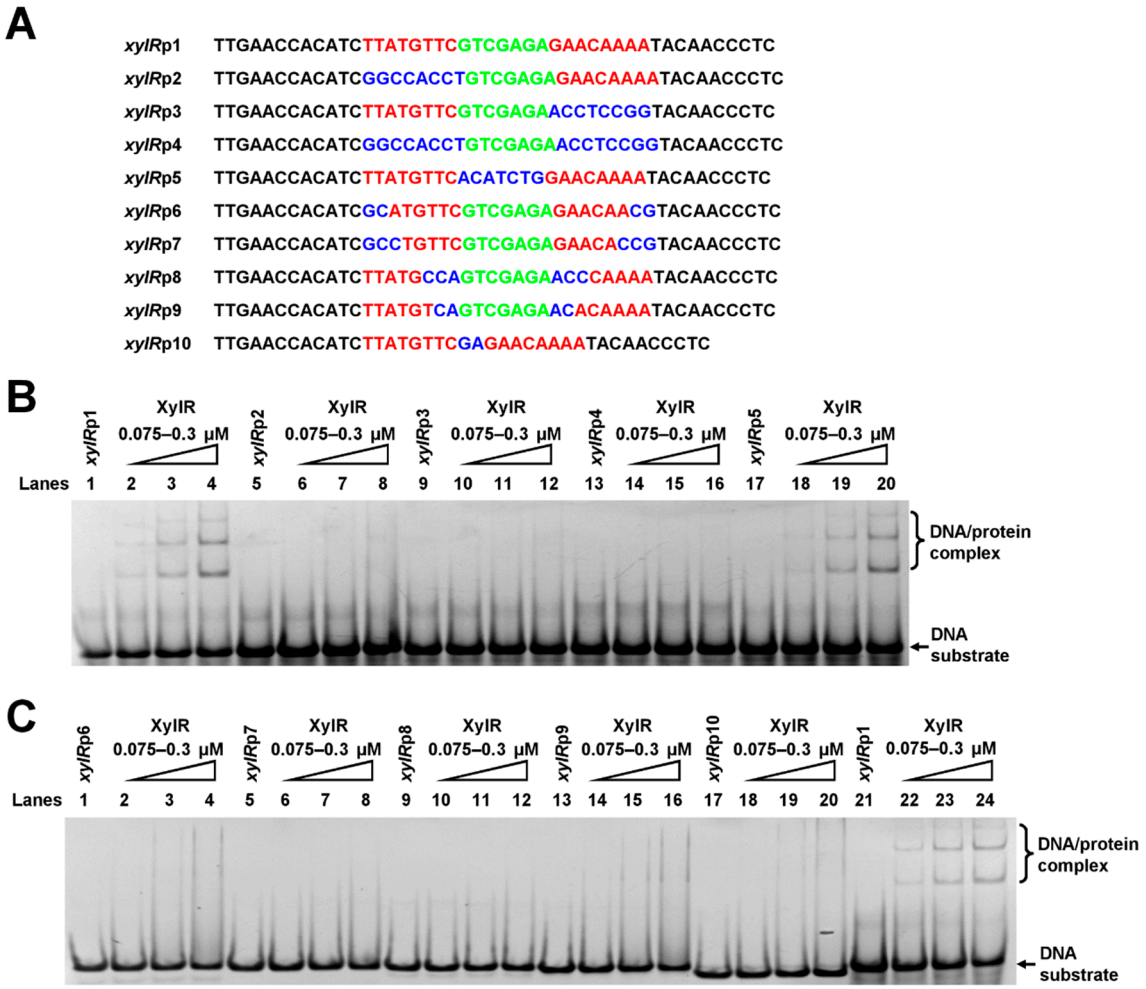


Figure S2. EMSA assays for XylR binding to DNA substrates of mutant IR sequence. **(A)** The wild-type IR sequence (*xylR*p1) and mutated sequences (*xylR*p2-p10) were listed. The DNA-binding activity of XylR on wild-type IR sequence (lanes 1-4) **(B)** and mutated sequences (lanes 5-20) **(B)**, (lanes 1-20) **(C)** were shown in figures. The DNA substrates were co-incubated with 0.075-0.3 μM of XylR protein.

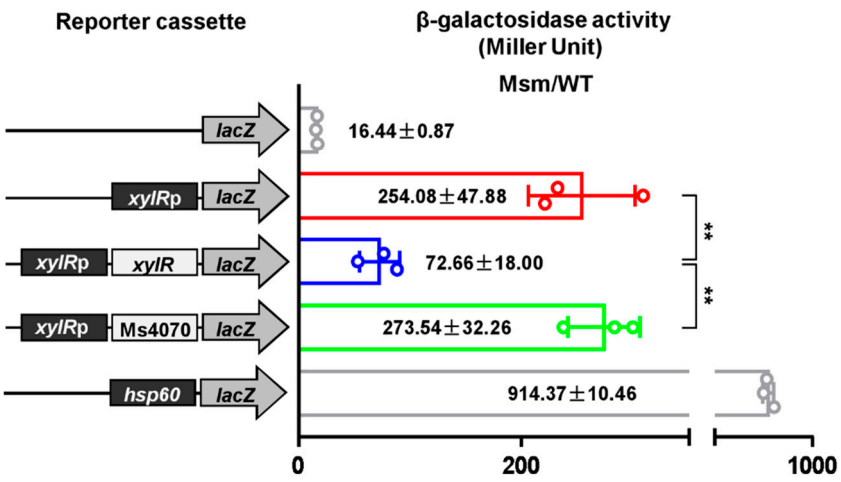


Figure S3. The assays of β-galactosidase activity. The effect of XylR on the gene expression was assayed by constructing a series of *lacZ* alone or promoter-*lacZ* and promoter-*xylR*-*lacZ* co-expression plasmids. These plasmids were transformed into Msm/WT strains. The activity of β-galactosidase was further examined and presented as Miller units (right panel). Left column: schematic representation of each clone used to generate recombinant strains. Null promoter-*lacZ*, *xylR*p-*Ms4070*-*lacZ* and *hsp60*-*lacZ* were used as controls. Right column: β-galactosidase activity was expressed as Miller units. The values presented were the averages of three independent experiments. For statistical analysis, two-way analysis of variance with Bonferroni multiple comparison tests were performed using a *P*-value of < 0.01. The *P*-values of the results (<0.01) are indicated by two asterisk (**) on top of the column in the figure.

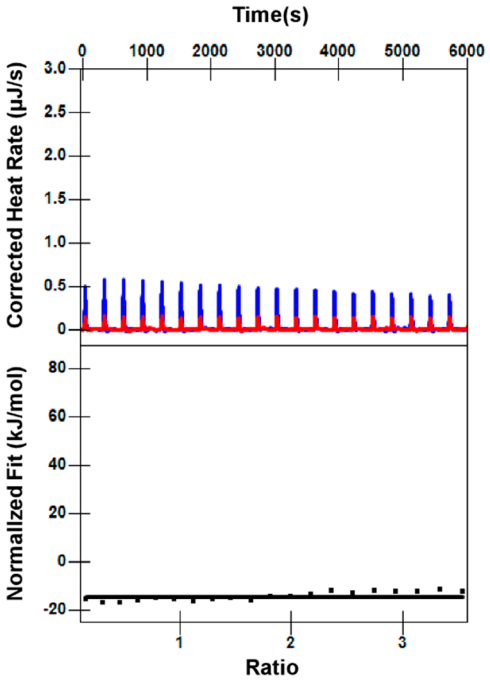


Figure S4. ITC assays for interaction between XylR and L-Ara. Original titration data and integrated heat measurements are shown in the upper and lower plots, respectively.

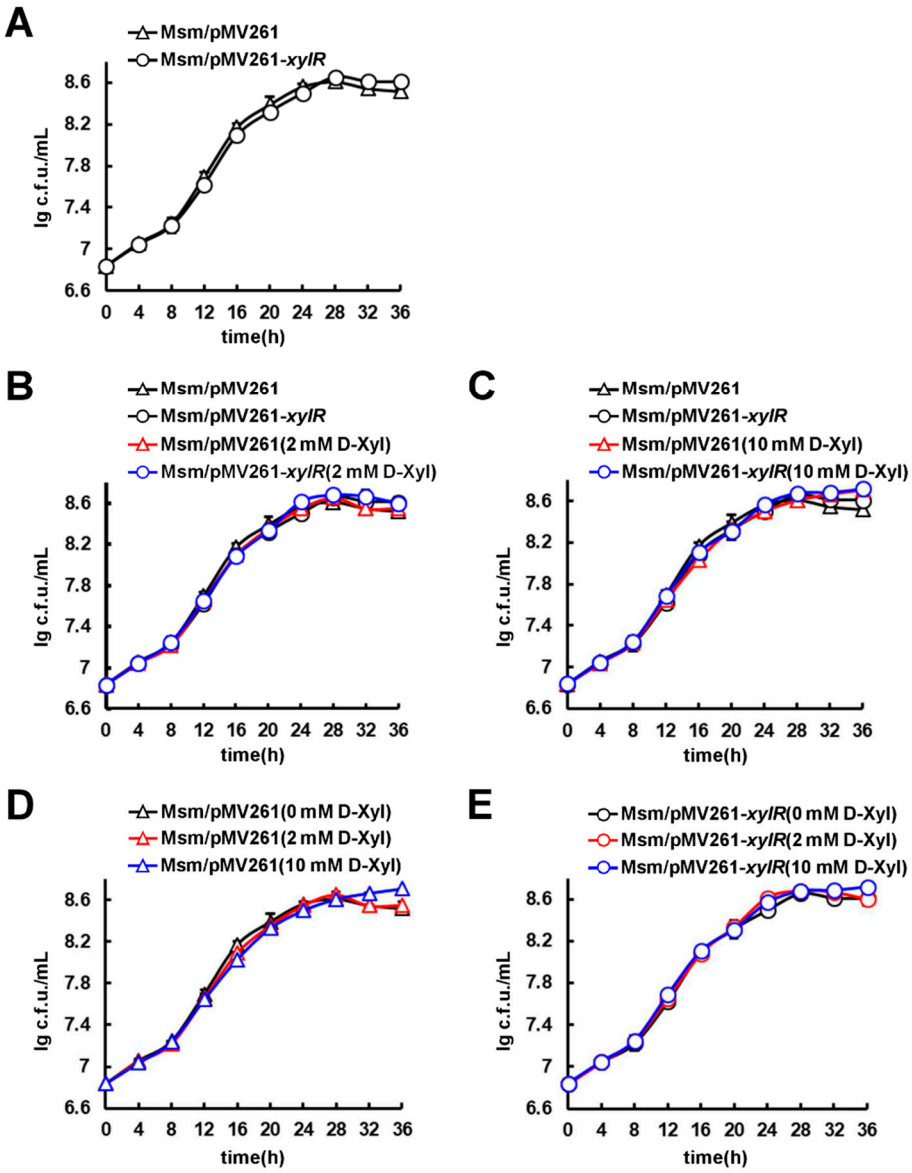


Figure S5. The assays for the growths of wildtype and recombinant *M. smegmatis* strains under the induction of D-xylose. Growth curves of the recombinant *M. smegmatis* strains were determined as described in 'Materials and Methods' section. The *xylR*-overexpression and wild-type strains **(A)** were grown in 7H9 medium without antibiotic stress and no obvious growth difference was observed. When 2 or 10 mM D-xylose was added into the medium as a stress inducer, no growth difference between the wild type *M. smegmatis* and *xylR*-overexpression strains **(B-E)**.