

TABLE S1**Strains used in the study**

Designation		Relevant characteristics	Reference
In the lab strain collection	In this study		
JB124	WT	Wild-type	Lab collection
JB1853	R1	IleRS (H594Y, <u>C</u> AC1780 -> <u>T</u> AC)	(PAULANDER <i>et al.</i> 2007a)
JB1855	R2	IleRS (F596L, <u>TTC</u> 1788 -> <u>TTG</u>)	“
JB1850	R3	IleRS (WV631L, <u>TGGGTG</u> 1889-1891 ->TTG)	“
DA8947	C1	Evolved from R1: IleRS (H594Y) and expression mutation C (-591)->A	(PAULANDER <i>et al.</i> 2007a) and this study
DA11020	C2	Evolved from R1: IleRS (H594Y) and expression mutation C (-1036)->T	“
DA11022	C3	Evolved from R1: IleRS (H594Y) and 4-fold amplification of <i>ileS</i>	“
DA8992	C4	Evolved from R2: IleRS (F596L) and expression mutation C (-577)->T	“
DA9162	C5	Evolved from R2: IleRS (F596L) and expression mutation G (-22) ->T	“
DA8995	C6	Evolved from R2: IleRS (F596L) and expression mutation C (-1036)->T	“
DA9163	C7	Evolved from R2: IleRS (F596L) 2-fold amplification of <i>ileS</i> and expression mutation T (-421)->C	“
DA9167	C8	Evolved from R2: IleRS (F596L) 2-fold amplification of <i>ileS</i> and expression mutation T (-405)->C	“
DA9171	C9	Evolved from R2: IleRS (F596L) 2-fold amplification of <i>ileS</i> and expression mutation T (-405)->C	“
DA9169	C10	Evolved from R2: IleRS (F596L) and 4-fold amplification of <i>ileS</i>	“
DA9150	C11	Evolved from R3: IleRS (WV631L), 2-fold amplification of <i>ileS</i> and expression mutation C (-567)->T	“
DA9155	C12	Evolved from R3: IleRS (WV631L) and 6-fold amplification of <i>ileS</i>	“
DA13883	C12-1	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	This study
DA13884	C12-3	Evolved from C12: IleRS (WV631L, Q420H)	“
DA13867	C12-4	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13855	C12-5	Evolved from C12: IleRS (WV631L, Q420H)	“

DA13885	C12-6	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13868	C12-7	Evolved from C12: IleRS (WV631L, Q420H)	“
DA13886	C12-8	Evolved from C12: IleRS (WV631L, P184T)	“
DA13856	C12-9	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13869	C12-10	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13887	C12-11	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13870	C12-12	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13871	C12-13	Evolved from C12: IleRS (WV631L) and 5-fold amplification of <i>ileS</i>	“
DA13888	C12-14	Evolved from C12: IleRS (WV631L) and 3-fold amplification of <i>ileS</i>	“
DA13874	C12-16	Evolved from C12: IleRS (WV631L) and 3-fold amplification of <i>ileS</i>	“
DA13876	C12-17	Evolved from C12: IleRS (WV631L) and 2-fold amplification of <i>ileS</i>	“
DA13872	C12-18	Evolved from C12: IleRS (WV631L) and 4-fold amplification of <i>ileS</i>	“
