Supporting Information

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SI Text

Assessments of the Prerequisite Conditions for the Influx Assay

The prerequisite conditions for the influx assay were (*i*) no leakage of AmpC enzyme, (*ii*) the movement of the substrates is in a steady state, at which it is possible to consider that $V_h = V_{in}$ in LA51A, and $V_h = V_{in} - V_e$ in LA51, where V_{in} and V_e denote the rate of influx and efflux, respectively. If the assay condition satisfies both, V_{in} or V_e can be determined from the value of V_h.

To check the condition (*i*), the supernatant of the cell suspension in 50 mM potassium phosphate buffer (pH 7.0) containing 5 mM $MgCl_2$ was collected after 15-min incubation at room temperature (RT), and the hydrolytic activity was examined (Fig. S1*B*). No detectable activity was found, satisfying condition (*i*). Following this, the hydrolytic pattern of the intact cell was examined during 15-min incubation. Fig. S1*A* shows a typical result. Both for ampicillin (AMP) and benzylpenicillin (PEN), linear increases in the amount of hydrolyzed product were observed. This indicates that C_p was constant during the assay, and V_{in} and V_h are thus considered to be in a steady state. Similar experiments were done for every substrate concentration used, and all of them confirmed that the steady-state assumption was valid. This satisfies condition (*ii*).

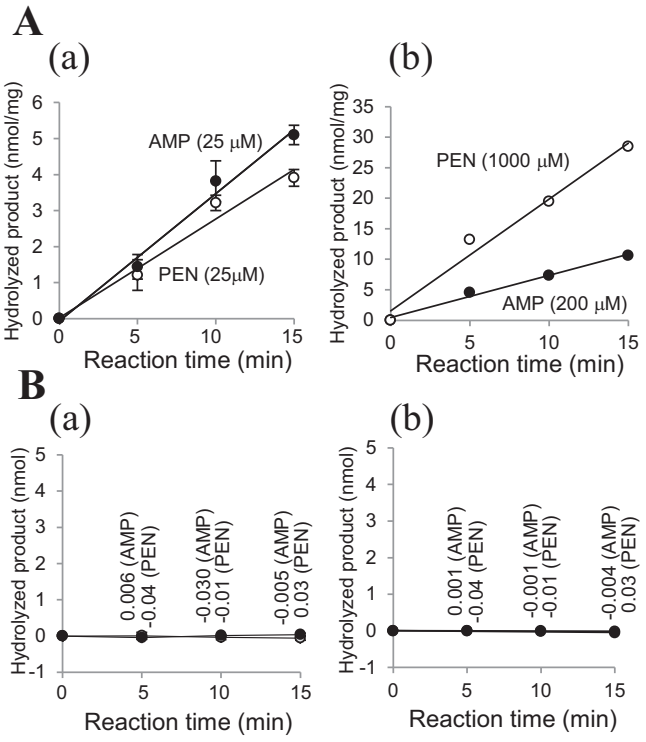

Fig. S1. Evidence for steady state and no leakage of AmpC into the supernatant. (*A*) One of the typical results of the assay using LA51 (*a*) or LA51A (*b*) is shown. Hydrolytic activity of the intact cell was examined under the extracellular substrate concentrations shown in the graphs. (*B*) Hydrolytic activity of the supernatant taken from intact cell suspension of LA51 (*a*) or LA51A (*b*) after 15-min incubation at RT, examined under 50 μM AMP or PEN. Values represent the average of triplicate experiments.

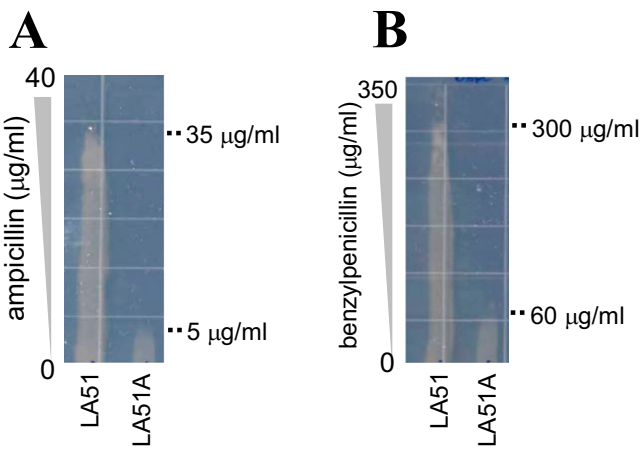

Fig. S2. Minimum inhibitory concentration (MIC) determination by gradient plating method. MIC values of AMP (*A*) and PEN (*B*) for LA51 and LA51A were measured under 0–40 μg/mL gradient of AMP and 0–350 μg/mL gradient of PEN. Measured MIC values are shown in the figure.