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**Development of a fluorescence polarization assay for multi-determination of 10 aminoglycosides in pork muscle sample based on ribosomal protein S12 and studying its recognition mechanism**

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## 1. MATERIALS AND METHODS

### 1.1 Biological materials

All the enzymes and biological reagents were molecular biology grade. IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside), and restriction enzymes (*Eco*R I, *Hind* III) were from Takara Company (Dalian, China). EasyPure Quick Gel Extraction Kit, pET-32a, Rosetta-gami(DE3), and Luria-Bertani culture medium (liquid and solid) were from TransGen Biotech (Beijing, China). SDS-PAGE gel preparation kit was from Beijing ComWin Biotech Co. Ltd (Beijing, China).

### 1.2 Expression procedures of *RpsL12*

The plasmids containing the express vector pET32a-*RpsL12* were added into 500  $\mu$ L of *Escherichia coli* Rosetta-gami(DE3) solution. The mixture was heat shocked for 90 seconds at 42 °C, kept in ice water for 2 min, and cultured on Luria-Bertani (LB) liquid culture media at 37 °C for 1 h. Then, 150  $\mu$ L bacteria solution was transferred on LB solid culture media for culturation overnight (37 °C). The single bacterial colony was transferred into 2.5 mL LB liquid culture media for culturation overnight (37 °C, 220 rpm). When the OD<sub>600</sub> value reached 0.6, 0.5 mM IPTG was added to induce protein expression (26 °C 2 h). The bacteria solution was centrifuged at 4,000 rpm for 10 min. The supernatant was discarded, and the left sediment was resuspended in 500  $\mu$ L breakage buffer (50 mM Tris, 300 mM NaCl, 0.2% Triton X-114, 0.5 mM EDTA, 2 mM DTT, pH 8.0). After the bacteria solution was sonicated for 6 min and centrifuged for 5 min (4 °C, 12,000 rpm), the supernatant was purified with nickel-doped agarose gel FF column to obtain the *RpsL12* protein.

### 1.3 Surface plasmon resonance (SPR) test

The 10 AGs diluted with DMSO were printed on the Photo-Cross-Linker Sensor CHIP™ respectively by using Biodot™ AD1520 Array Printer. At the same time, rapamycin as the positive control and DMSO as the negative control were also printed on the chip. The chip was dried under vacuum and subjected to photocrosslinking treatment under UV irradiation. Then the chip was washed with DMF, ethanol and water in turn, dried by using nitrogen stream, and inserted into the Berthold bScreen LB 991 Microarray System for SPR test. The *RpsL*12 protein was diluted with PBS to prepare the test solutions (200, 400, 800, 1600 and 3200 nM). These solutions were injected into the Berthold bScreen LB 991 Microarray System for analysis respectively. The operation conditions were: flow rate 0.5 µL/s, association time 600 s, dissociation time 360 s, temperature 4 °C.

our seq.seq	MPTINQLVRKPRQSKITKSKSPALNKGYN SFKKSLTDVKS	PQKRGVCTRV	50
Bacillus subtilis.seq	MPTINQLIRKGRVSKVENSKSPALNKGYN SFKKEHTNVSS	PQKRGVCTRV	50
Escherichia coli.seq	MATVNQLVRKPRARKVAKSNVPAL-----EA--C----	PQKRGVCTRV	37
Enterococcus faecalis.seq	MPTINQLVRKPRKSKVEKSDSPALNKGYN SFKKTQTNVNS	PQKRGVCTRV	50
Thermus thermophilus HB8.seq	MPTINQLVRKGREKVRKSKVPAL-----KG--A----	PFRRGVCTVV	37
Acinetobacter baumannii .seq	MATVNQLVRKPRARKVAKSNVPAL-----EA--C----	PQKRGVCTRV	37
our seq.seq	GTMTPRKPNSALRKYARVRLTNQIEVTAYIPGEGHNLQEHSVVLIRGGRV		100
Bacillus subtilis.seq	GTMTPKKPNSALRKYARVRLTNGIEVTAYIPGIGHNLQEHSVVLIRGGRV		100
Escherichia coli.seq	YTTTPKKPNSALRKYCRVRLTNGFEVTSYIGGEGHNLQEHSVILIRGGRV		87
Enterococcus faecalis.seq	GTMTPKKPNSALRKYARVRLSNLIEVTAYIPGIGHNLQEHSVLLRGGRV		100
Thermus thermophilus HB8.seq	RTVTPKKPNSALRKYAVRLTSGYEVTAIPGEGHNLQEHSVVLIRGGRV		87
Acinetobacter baumannii .seq	YTTTPKKPNSALRKYCRVRLTNGFEVTSYIGGEGHNLQEHSVILIRGGRV		87
our seq.seq	KDLGVRYHIVRGALDTAGVNGRLQSR SKYGT	KRPKEKK	139
Bacillus subtilis.seq	KDLPGVRYHIVRGALDTAGVENRAQGR SKYGT	KKPKAK	138
Escherichia coli.seq	KDLPGVRYHTVRGALDCSGVKDRKQAR SKYGV	KRPKA	124
Enterococcus faecalis.seq	KDLPGVRYHIVRGALDTAGVNDKQSR SKYGT	KRPKA	137
Thermus thermophilus HB8.seq	KDLPGVRYHIVRGVYDAAGVKDRKKSRSKYGT	KKPKAAKTA	132
Acinetobacter baumannii .seq	KDLPGVRYHTVRGALDCSGVKDRKQAR SKYGV	KRPKA	124

**Figure S1.** Amino acid sequences of the present *RpsL12* (*Lysinibacillus sphaericus*, PDB: 7P7S) and the *RpsL12* proteins of *Bacillus subtilis* (PDB: 3J9W), *Escherichia coli* (PDB: 5JTE), *Enterococcus faecalis* (PDB: 7P7U), *Thermus thermophilus* HB8 (PDB: 2F4V), and *Acinetobacter baumannii* (PDB: 7RYF). The amino acids highlighted in dark blue are the 100% conserved residues.

**Table S1.** Ka, Kd, KD and KA values of the *RpsL12* for the 10 AGs.

Drug	Ka (1/Ms)	Kd (1/s)	KD (M)	KA
Amikacin	1.69E+03	7.20E-05	4.25E-08	24.487
Apramycin	2.02E+04	2.81E-04	1.39E-08	26.102
Gentamicin	2.18E+03	1.03E-02	4.72E-06	17.693
Micronomicin	1.41E+02	5.43E-03	3.85E-05	14.666
Neomycin	3.15E+03	4.70E-05	1.49E-08	25.997
Spectinomycin	2.11E+05	1.35E-02	6.39E-08	23.900
Streptomycin	8.20E+03	3.07E-04	3.75E-08	24.670
Netilmicin	3.71E+01	1.41E-03	3.80E-05	14.684
Paromomycin	2.63E+05	1.74E-02	6.61E-08	23.851
Etimicin	5.12E+01	1.95E-02	3.81E-04	11.358

Ka, association constant.

Kd, dissociation constant.

KD = Kd/Ka, equilibrium dissociation constant.

KA=Abs (Log2(KD)), absolute affinity constant.