Method Sheet 31

Measuring enhancement of sensitivity to existing antibiotics

Overview

This method sheet explains how to test whether your hit extract is capable of enhancing the capacity of existing antibiotics to kill the bacterial strain you have been studying. This is a potentially useful approach for therapy, as there is much effort underway currently to find ways of defeating bacterial mechanisms of antibiotic resistance, which may in turn restore the clinical effectiveness of existing antibiotics.

Reagents

- One or more hit natural extracts at 10 mg/ml in DMSO
- An established antibiotic compound (e.g. ampicillin, tetracycline) at 10 mg/ml

Equipment

- Multichannel pipette (8- or 12-channel) capable of dispensing 1 - 10 µl
- Sterile pipette tips (compatible with each pipette)
- Static (non-shaking) 37°C incubator
- Waste container for used tips

Preparation of dilutions

1) Fully defrost one of the hit natural extracts and one aliquot of an antibiotic of interest (which should be at 10 mg/ml).
2) Prepare a 96-well plate containing bacterial cell cultures at 80 µl per well (follow Method Sheet 03, this should be done early in the morning on the same day as challenge).
3) Label 10 sterile microtubes with the antibiotic concentrations 0, 0.5, 1, 2, 4, 8, 16, 32, 64 and 128.
4) Add 50 µl of LB medium into each of these tubes, except the tube labelled 128, into which you should pipette 87 µl of LB medium.
5) Supplement the tube labelled 128 with 12.8 µl of antibiotic and mix by pipetting gently.
6) Prepare doubling dilutions of the antibiotic by transferring 50 µl of suspension from the 128 tube into the 64 tube, mix by pipetting gently, then repeat this process by transferring 50 µl of the resulting suspension into the next tube in the row.
7) Continue preparing the series of dilutions in this way until the tube labelled 0.5, but do not add anything to the tube labelled 0, this will be the zero antibiotic control.
8) Now label a fresh microtube with ‘DMSO’ and another with ‘Extract’.
9) Pipette 25 µl of DMSO into 225 µl of LB medium in the ‘DMSO’ tube.
10) Pipette 25 µl of hit extract into 225 µl of LB medium in the ‘Extract’ tube.

Challenging the bacterial cultures

1) Supplement all of the 20 wells shown in the plate map below with 10 µl of the 'DMSO' control suspension (the first two rows).
2) Then supplement all of the 20 wells shown in the plate map below with 10 µl of the 'Extract' suspension (the second two rows).
3) Supplement each column of 4 wells in the treated section with 10 µl per well of the appropriate antibiotic dilution, as shown by the numbers in the plate map below:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		DMSO 0	DMSO 0.5	DMSO 1	DMSO 2	DMSO 4	DMSO 8	DMSO 16	DMSO 32	DMSO 64	DMSO 128	
C		DMSO 0	DMSO 0.5	DMSO 1	DMSO 2	DMSO 4	DMSO 8	DMSO 16	DMSO 32	DMSO 64	DMSO 128	
D		Extract 0	Extract 0.5	Extract 1	Extract 2	Extract 4	Extract 8	Extract 16	Extract 32	Extract 64	Extract 128	
E		Extract 0	Extract 0.5	Extract 1	Extract 2	Extract 4	Extract 8	Extract 16	Extract 32	Extract 64	Extract 128	
F												
G			Values indicate final concentrations of antibiotic in µg/ml									
H												

4) Note that we are avoiding use of the outer wells of the plate to minimise errors arising from the 'edge effect'.
5) The other wells on the plate receive no supplement (i.e. they remain only 80 µl culture).
6) Move the culture plate backwards and forwards, then side to side, gently several times to mix the suspension into each culture.
7) Avoid jarring movements that may splash liquid between wells and cause cross-contamination.
8) Measure the absorbance of the plate straight after challenge (to obtain the baseline, time = 0 h readings) using a microplate reader (follow Method Sheet 05).
9) Place the culture plate in a static 37ºC incubator to allow growth to begin (does not need to be a shaking incubator).
10) Measure the growth once more on day 2, at 24 hours after the first measurement (i.e. at the same time as the first measurement was taken the day before).
11) The plate can now be discarded.
12) Repeat this experiment a further three times in separate plates to obtain four experimental replicates.
13) Proceed to analyse the data as shown in Method Sheet 32.

Notes

- You can choose to test any antibiotic you have available in the laboratory, but good options to try in which enhancement can often be observed are tetracycline and chloramphenicol.
- This protocol suggests measuring growth at a single time point (24 h), but it will be possible to measure the absorbance of the plate at multiple timepoints to generate more data from the same experiment.
- This protocol yields a final concentration of DMSO of 1% vol/vol and a final concentration of extract at 100 µg/ml.