BOX-PCR genomic fingerprints (for differentiating and determining diversity among bacterial isolates).

BOX-PCR is performed as described by Rademaker and De Bruijn (37) using the BOXA1R primer 5'-CTA CGG CAA GGC GAC GCT GAC G-3'.

Each 25 uL PCR reaction contains:

Component	**Volume (µL)**
10X PCR buffer	2.5
25 mM $MgCl_2$	1.25
BOXA1R primer (3µM)	5
BSA (5 mg/ml)	2.0
dNTP mix (10 mM)	0.5
Taq DNA polymerase (5 U/µL)	0.2
ddH_2O	13.55, 14.05 or 17.55
Template: Either picked colony or 4 µL of overnight culture Or 0.5 µl of extracted DNA	0, 0.5 or 4

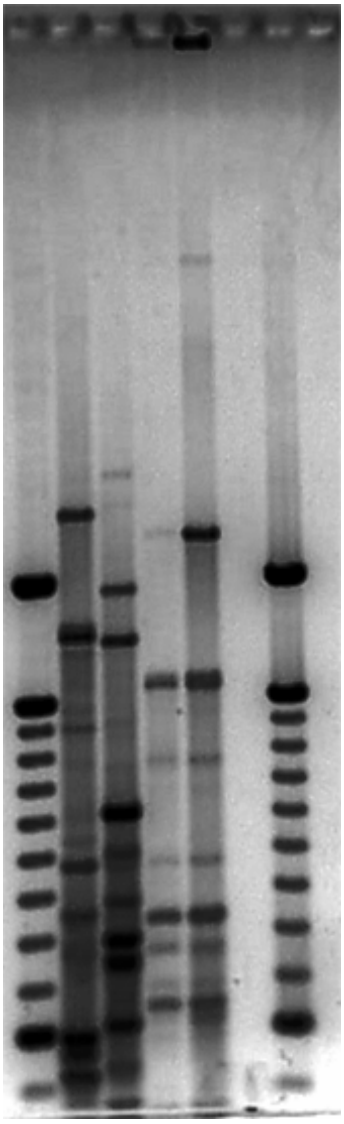
Initial denaturation at 95°C for 6 min

35 cycles of: 94°C for 1 min, 53°C for 1 min, 65°C for 8 min, final extension at 65°C for 16 min.

A 15-µl aliquot of amplified PCR product is resolved on a 15 cm long 1.5% agarose gel in 1× Tris-acetate-EDTA buffer at 30 V (2 V/cm of gel) for 16 h in a cold room (4°C). A 100 bp DNA size ladder is used at both ends and in the middle of the gels. The gels are post stained with Sybr Green at manufacturer's recommended concentration of 1:10,000 for 30 min with shaking at 25 rpm. Gels are viewed under UV transillumination and photographed with Kodak digital camera. Images are then

saved as .TIFF files and analyzed by Kodak digital science software (or preferably Gelcompar if we can get it).

Example of gel image: Lane 1 - E.coli; Lane 2 – Ps.stutzeri; Lane 3 – Culture A; Lane 4 – Culture B.



From banding patterns we can see that the unknown cultures are identical.