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E. Polak
Mikrobiologisches Institut

U. Kües
Mikrobiologisches Institut

M. Aebi
Mikrobiologisches Institut

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Abstract

Asexual spores (oidia) of *Coprinus cinereus* adhere to surfaces such as metal or velveteen. We used this feature to develop a new method for replica plating and demonstrate its value in screening for auxotrophic mutants and mutants in sporulation.

Replica plating of *Coprinus cinereus* colonies using asexual spores

Eline Polak, Ursula Kües and Markus Aebi - Mikrobiologisches Institut, ETH Zürich, Schmelzbergstrasse 7, CH-8092 Zürich, Switzerland.

Asexual spores (oidia) of *Coprinus cinereus* adhere to surfaces such as metal or velveteen. We used this feature to develop a new method for replica plating and demonstrate its value in screening for auxotrophic mutants and mutants in sporulation.

Monokaryons of the basidiomycete *Coprinus cinereus* produce large numbers of asexual spores (oidia) in sticky liquid droplets on specialized aerial structures (Brodie 1931 Annals Bot. **45**:315-344). Specific mutations in the mating type loci lead to so called *AmutBmut* homokaryons (Swamy *et al.* 1984 J. Gen. Microbiol. **130**:3219-3224) which produce oidia in a light dependent manner (Polak *et al*. submitted). Oidia of both monokaryons and *AmutBmut* homokaryons are uninucleate, haploid and hyaline (Brodie 1931 Annals Bot. **45**: 315-344; Polak *et al*. submitted). Therefore they were found to be very useful for mutagenesis (Moore and Pukkila 1985 J. Biol. Education **19**:31-40). Here we demonstrate the value of oidia in replica plating and screening for mutants with auxotrophies and mutants in oidiation.

Principle of the method: The sticky character of the oidial droplets causes the oidia to adhere to a smooth glass surface when the aerial mycelium of an oidiating *C. cinereus* colony is carefully touched with this surface (Brodie 1931 Annals Bot. **45**: 315-344; Polak *et al*. submitted). We found that oidia also adhere to the metal surface of a steel cylinder (8 cm Ø) or to velveteen covering this surface. With both materials oidia can easily be taken off from colonies grown on solid media in Petri dishes. Oidia attached to metal or velveteen can be released upon gently pressing onto the surface of a fresh agar plate.

Four *C. cinereus* strains were used to test the possible applications of these observations in replica plating. Monokaryons FA2222 (*A5B6*, *trp-1.1,1.6*, *acu-1*) and PG78 (*A6B42*, *trp1-1.1,1.6*, *pab-1*) were provided by L.A. Casselton (Oxford University). Homokaryon AmutBmut (*A43mut B43mut, pab-1*), provided by P. J. Pukkila (University of North Carolina), produces large numbers of oidia in light (comparable to monokaryons) but about 200 times less oidia when kept in dark (manuscript in preparation). Strain E-2095 (*A43mutB43mut, pab-1*+) is a REMI mutant generated in our lab from homokaryon *AmutBmut* (Granado *et al.* Mol. Gen. Genet. in press) that constitutively produces high numbers of oidia. The media used were YMG (Rao and Niederpruem 1969 J. Bacteriol. **100**:1222-1228) supplemented with tryptophan (100 mg/l; YMG/T) and MM (Shahriari and Casselton 1974 Mol. Gen. Genet. **134**:85-92) supplemented with tryptophan (100 mg/l; MMtrp) and/or *para*-aminobenzoic acid (5mg/l; MMpab) where appropriate. Media were solidified with 1% agar. All cultures were incubated at 37 C.

Efficiency of the method: In a first experiment, strain E-2095 was replica plated from fully grown MM plates onto fresh YMG/T plates. Either the bare metal surface of the cylinder or the velveteen was used for replica plating. In each case, the masterplate and successively four replica plates were pressed onto the surface of the stamp. After 2 days of incubation all replica plates were covered with mycelium derived from germinated oidia. This shows that, independently of

the material used for replicating, the oidia produced on the masterplate were efficiently transferred to the replica plates even over four passages.

The sensitivity of the method was further tested by replica plating from a YMG/T masterplate containing five colonies of strain E-2095 and five colonies of strain *AmutBmut* grown in the dark (colony diameter +/- 1 cm). Velveteen was used for the transfer. Oidia were evidently transferred to the YMG/T replica plates from all colonies on the masterplate. However, on the replica plates the density of germination per original E-2095 colony was much higher than the density per original *AmutBmut* colony. This is consistent with the high oidia production of strain E-2095 and the low oidia production of homokaryon *AmutBmut* in dark. Replica plating therefore clearly distinguished between the amounts of oidia produced by the different colonies on the master plate. This feature provides us with a fast and easy screening method for mutants in oidiation.

Screening for auxotrophic phenotypes: The F1 generation of a cross between strain E-2095 (*pab-1+*) and strain PG78 (*trp-1.1,1.6, pab-1*) was screened for *para*-aminobenzoic acid (pab) auxotrophy. Single colonies of the F1 generation were inoculated and grown on YMG/T masterplates (five different colonies/plate). The colonies were replica plated with velveteen onto MMtrp, MMtrp/pab and YMG/T medium, respectively (in this order). After three days of incubation, all 98 colonies tested grew on the YMG/T and on the MMtrp/pab plates. Only the 40 pab prototrophic colonies grew on the MM/trp plates.

Similarly, by replica plating onto MM/trp, MM/pab and MM/trp/pab (in this order) single colonies of monokaryon FA2222 (*trp1.1,1.6*) were detected in a background of *AmutBmut* (*pab-1*) colonies (100-400 colonies per YMG/T master plate; diameter of the colonies +/- 2 mm). Due to the small colony size of 2 mm only 1-10% of all colonies of homokaryon *AmutBmut* were successfully transferred in these experiments.

Comparison to an earlier method for replica plating of C. cinereus: A method for replica plating *C. cinereus* presented before uses 'Velcro', a hooked material which nicks out tiny pieces of agar with mycelial fragments. 'Velcro' efficiently transfers colonies with a size of 3-4 mm independently of oidia production, adhesion or germination efficiency. It is therefore a useful method for screening for mutants with auxotrophies. A disadvantage is that 'Velcro' is only available in stripes of maximally 2 inches and that the agar may be ripped by the 'Velcro' (Kaplan and Walls 1971 Genet. Res., Camb.**17**: 279-281). Most important, mutants in oidiation will not be detected by the 'Velcro' technique.

Kaplan and Walls state in their paper that velveteen and asexual spores of *C. cinereus* can not be used for replica plating. Our experiments gave different results. Replica plating with oidia and velveteen functioned with all four *C. cinereus* strains tested. It is efficient in identification of mutants with auxotrophies and in screening for mutants in oidiation. It is possible both to detect clones producing abundant oidia amongst clones with poor sporulation (see replica plating of FA2222 and *AmutBmut* colonies) and to identify poor sporulators in a collection of heavy sporulators (see replica plating of strain E-2095 and homokaryon *AmutBmut*).

Replica plating using asexual spores of *C. cinereus* has the advantage that it can also be done with a bare metal surface and therefore replica plating of microtiter dishes with the help of a

multi-pin-replicator (with blunt pins!) becomes possible. In our lab, we use such 24-well microtiter dishes to store mutant collections made by REMI and UV mutagenesis.

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