How to convert wild-type spreading growth to colonial.

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Background

The need for restricting the rapid linear growth of morphologically normal strains in order to to obtain colonies on microbiological plating medium led Tatum *et al.* (1949) to test various colonializing agents, including the keto-hexose sorbose and the nonionic detergent tergitol. Sorbose and tergitol were the most effective colonializers. Sorbose was preferred because the colonies showed less aerial growth and conidiation. Use of sorbose medium for platings soon became standard routine.

Tergitol has been found useful for a special application, in experiments where protoplasted conidia of a morphological mutant were transformed using DNA from a genomic library and transformed colonies were sought that showed the wild-type phenotype (Springer 1991). Tergitol is better than sorbose for this purpose because it induces vegetative growth that is colonial in agar medium or on the surface without affecting the growth or morphology of aerial hyphae.

Before colonializing agents were known, semicolonial growth had been obtained either by using an inositol-requiring strain and plating on limiting inositol (0.3 mg.ml, Giles and Lederberg 1948) or by using *col*-4, a slow spreading morphological mutant (Giles 1951). Several conidiating colonial strains are now available that are suitable for plating and/or replication. **(**See *How to do replica plating*.) These have been used effectively for determining recombination frequencies (D. G. Catcheside 1966, D. E. A. Catcheside 1970), obtaining new mutations (Schroeder 1970), and measuring recessive lethal mutation rates (Stadler *et al.* 1979, 1984**).**

Procedure

Sorbose. Vogel's medium N. with 1% or 2% sorbose, 0.05% glucose, and 0.05% fructose is commonly used. Sorbose is added to the basal mediumwhile sucrose is omitted and replaced by glucose and fructose. This prevents the toxicity observed when sorbose and sucrose are together and reduces the variability of colony size caused by hydrolysis of sucrose during sterilization (Brockman and de Serres 1963). Colony size is affected by the sorbose concentration (smaller with higher sorbose) and by the basal medium [smaller on Westergaard/Mitchell synthetic crossing medium (SC) than on Vogel's (Brockman and de Serres 1963)]. There are a variety of formulas, varying in whether Vogel's or SC is used and in the ratio of sugars. For example, Pittenger (1964) used SC with 1% sorbose, 0.01% glucose, and no fructose. Catcheside (1966) used Vogels with 0.5% sorbose, 0.0125% glucose and 0.025% fructose.

If 4% agar is used, 0.1 to 0.3 ml of spore suspension may be spread on the surface using a glass spreader. Alternatively, a sterile bottom layer of sorbose medium (2% agar) is allowed to solidify. Conidia (or activated ascospores) are then added in an overlayer using 0.75% agar, 3 ml to 5 ml per plate. With 5 ml, the colonies will conidiate later than with 3 ml. Delayed conidiation is good if counting or isolating colonies is the object, but not if albino or other visible traits are to be scored..

Browning of the medium occurs when Vogel's medium with sorbose is autoclaved. This has little or no effect on colony growth. Browning can be avoided completely by autoclaving the sugars separately from the salt solution. Browning is also said to be minimized by using SC (which substitutes nitrate for ammonium) or by using 1% glucose and omitting fructose.

Tergitol. The following is copied from Springer (1991). "This medium is based on standard Vogel's minimal medium plus 1.5% sucrose and 1.5% agar. Tergitol NP-10 (available from Sigma) is added to

autoclaved bottom agar medium at a concentration of 0.005%. Medium containing 0.001% tergitol allows some spreading growth, while viability suffers at concentrations of 0.01% and above. Sterilization of the concentrated tergitol has not been necessary. Care should be taken when mixing the detergent into the medium to avoid excessive foaming. It is normal for the resulting medium to be slightly turbid.

Plating transformants directly in top agar containing tergitol will kill the spheroplasts. Instead, the top agar should consist of Vogel's minimal medium plus 1.5% sucrose, 1.5% agar, and 1 M sorbitol for osmotic stabilization. Tergitol medium has successfully been used with both benomyl and hygromycin to differentiate wild-type transformants from aconidial mutant transformants.

Unlike sorbose, which restricts both mycelial and aerial hyphal growth, tergitol only restricts the growth of hyphae that are in contact with the medium. Aerial growth, which commences about one day after plating, proceeds vigorously until aerial hyphae and conidiophores completely fill the space between the agar and the lid of the Petri dish. The colonies must therefore be checked about twice a day to avoid overgrowth. For this reason, standard sorbose medium is still preferable for colonial growth where the morphology of the transformants is inconsequential. However, the preservation of differences in aerial morphology makes tergitol medium quite useful for the study of morphological mutants."

Mutants.used to obtain colonial growth

cot-1 allele c102t: The conditional mutant*colonial temperature-sensitive-1*, which shows tight colonial growth above 30°C but wild type growth at 25°, is effective on ordinary media without sorbose or tergitol. . It has also been used extensively in combination with sorbose to obtain the tiny colonies desired for large scale high resolution studies of recombination and of genes that affect the frequency of recombination (e.g., Catcheside 1966, Catcheside 1970).

rg cr-1: The conidiating double mutant *ragged crisp-1* forms small colonies that are suitable for replication (Maling 1960). It has been used to study crossing over (Maling 1960), to detect new UV sensitive mutants (Schroeder 1970), and to measure recessive lethal mutation (Stadler and Crane 1979). Crosses homozygous for *rg cr-1* sre female-sterile but *rg cr-1* can be used as fertilizing parent..

cr-1; sn: The combination *crisp-1; snowflake* is phenotypically similar to *rg cr-1* but is fertile both as female and as male (Perkins 1971). It has been used effectively in quantitative studies of recessive lethal mutation (Stadler and Macleod 1984).

cr-1: The single mutant forms large colonies which conidiate profusely close to the surface. It is suitable for replication using a needle replicator such as that employed for Aspergillus (Maling 1960).

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