Random ascospore isolation

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Abstract
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Conidia germinated into visible colonies after 7 days, 0.1% sorbose in the medium, plus 0.01% 0.02% 0.05% 0.1% 0.25% fructose.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Conidia plated</th>
</tr>
</thead>
<tbody>
<tr>
<td>S⁺³/₁</td>
<td>202</td>
</tr>
<tr>
<td>S⁺³/₃</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>162 181 175 158 194</td>
</tr>
</tbody>
</table>

Substrains have been isolated from colonies of slow and fast-growing strains. They have been re-checked on the same medium and their growth features are identical with the original strains. However, mutation to faster or slower growth occurs spontaneously. The mutation rate from slow to fast is ca. 1 in 200 germinating conidia.

The advantages of the new plating method are: 1) There are no agar-impurities or decomposition products to be taken into account when explaining any results, 2) All colonies grow on the same level (optical level and level of oxygen tension), 3) Individual colonies can be marked microscopically at an early stage and followed through their further development, 4) The medium can be replaced or changed without difficulties, sustaining the colonies in their original position.

The disadvantages are that plates with liquid medium are not easily handled, and that growth of wild-type is slow. The peculiarities of certain mutants in these and related media are under investigation.

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Nelson, N. Cross-feeding experiments to test for biological activity. These cross-feeding experiments were originally performed to determine whether any of the II groups of adenine mutants induced in 74A and differentiated on the basis of heterokaryon tests would accumulate a diffusible precursor capable of supporting the growth of any of the other groups. Petri plates of Fries minimal agar plus 5 gamma/ml. adenine sulfate (20 ml. medium/petri plate) were inoculated with testers from each of the II heterokaryon groups. After 24 hours growth, cores were cut with a 1 cm. stainless steel cork borer from the growing hyphae and one core/plate was placed in a hole of identical size in the middle of a petri plate of unsupplemented Fries minimal agar (20 ml./plate). A circle of sterile dialyzer tubing, 3 inches in diameter, was used as the diffusible membrane. These circles were laid over the transplanted core. A core of the second tester strain was placed directly above the first core with the mycelial surfaces facing each other but separated by the membrane layer. If the top core showed stimulation, mycelial growth was on the surface of the membrane; while if the lower core grew, hyphae penetrated the agar. Tests were made in all combinations in this manner, and controls showed that the residual growth from the cores was negligible. In these experiments only the ad-8 mutants (adenine specific) produced a marked stimulatory effect. This stimulation was true for all groups except the ad-4 mutants which are also adenine specific. The ad-8 mutants are blocked in the adenine pathway between inosine monophosphate and adenosine monophosphate succinate and accumulate hypoxanthine which can feed all adenine mutants prior to this step. The same type of procedure might prove to be useful in other biochemical pathways to determine whether a given mutant accumulates a compound that can be used to feed mutants blocked earlier in the sequence. (These experiments were done while the author was at Yale University, New Haven, Connecticut, U.S.A., Edited by M. Case).

Prakash, V. Random ascospore isolation. A perithecum growing on the surface of an agar slant may discharge its ripe ascospores quite freely and these can be seen as a black powder on the inside of the tube; whereas, when the perithecum grows below the surface inside the medium, it is unable to eject the ascospores which remain in dark masses after the perithecum disintegrates. The ascospores are ejected through a protruding neck of a perithecum after acquiring a definite state of maturity. Spore shedding occurs after eight to seventeen days, depending
upon the crosses and on the type of reproductive medium used. In certain crosses, they may not be noticed by even about the 22nd day. For purposes of random ascospore analysis, these ejected ascospores are generally isolated without any regard to selection which may be operating at the time of differential discharge exhibited by perithecia. It may be pointed out that certain workers suggest the isolation of ejected ascospores at least eighteen days after perithecium formation, but it is probable that the risk of selection due to 'differential ripening' of asci connected with 'differential discharge' cannot be avoided, even as late as this particular time lag. Perithecia have been found which do not evacuate their full contents altogether by a certain fixed time and some of them appear to do it by degrees. There are others which retain a part of their contents till the time of their disintegration.

Before the method for random ascospore isolation can be described, it may be necessary to have a brief insight into a perithecium. It may also be necessary to explain the outline of the mechanism involved in the 'differential discharge' of ascospores in relationship, particularly, to 'differential maturity' among asci.

A perithecium generally contains a cluster of asci. The cluster exhibits a pattern of its own, as to the arrangement of asci within a perithecium. Some of the asci are situated nearer the perithecial neck than the others and some of them are relatively more involved, as to different degrees of overlapping and crowding within the main body of a perithecium, than the others.

It is often observable that during the course of perithecial ripening, asci show different degrees of maturity. Some asci mature earlier than the others. Even within an ascus, some member ascospores are found at different levels of maturity. Genotype constitution of an ascus, along with other developmental factors, may well be responsible for 'differential maturity'. It may be noted that not only the position of different mature asci varies as to their relative distance from the perithecial neck (the place of discharge) but also the mature asci are found quite indiscriminately attached along with a number of un-matured ones within a perithecium. There appears to be thus a sort of nonlocalization and nonaggregation among the maturing asci.

As a perithecium, on reaching a right stage of maturity, generally starts shedding its ripe ascospores through its protruding neck, it is quite conceivable that those asci near the neck would be more favorably placed as to the shedding of their ascospores than the ones which are distantly situated from the neck. This is why it is not uncommon to find a number of mature asci within a perithecium that has stopped discharging its spores. It may be that, due to their genotypic constitution, certain asci gain such a position within a perithecium that renders them incapable of participating in ejecting their spores, even having reached the right type of maturity. Any sample isolated from the ejected spores may, therefore, preclude any chances of inclusion of genotypes from such asci and this may eventually lead to biased results.

Method: The collection of ascospores is undertaken directly from the fully matured perithecia, instead of the ascospores which are already ejected. This is achieved by running a pilot cross and noting the approximate time for shedding of the ascospores. Fully ripened perithecia are then removed from a cross which is made a day or two later than the pilot one and where no shedding seems to have been involved. A perithecium is judged to be suitable when it sheds spores immediately when placed in a drop of sterile water. All ascospores are isolated irrespective of their color in a microscopic field.

Prakash, V. Spore isolation in order (Tetrad Dissection).

The following sequence has been found effective during the dissection of asci:

a) Treatment for softening of the perithecial wall
b) Pre and post-treatment washing of perithecia
c) Use of 12% agar blocks
d) Use of sharply pointed tungsten needle for isolation of spores
e) Transferring of ascospores directly to the tubes, instead of first carrying to \( \times \times 0.5 \) mm agar blocks.