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Host-vector systems in eukaryote microorganisms

Abstract
Editors note: Host-vector systems in eukaryote microorganisms

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Editors note: The National Institutes of Health has recently approved the use of *Neurospora crassa* and of *Saccharomyces cerevisiae* for use as HVI systems for the cloning of recombinant DNA. The following represents the pertinent sections as approved and published in the Federal Register (Fed. Reg. 44, No 71: 21730-21736, April 11, 1979).

Definition of a eukaryote microorganism which is an HVI system (moderate level of containment). Such on organism is (1) nonpathogenic (2) not adapted to normal escape routes (air, people, sewage) or is specially modified to minimize such escape and (3) shows little or no genetic exchange with other organisms encountered in escape route. Laboratory strains of *Neurospora crassa* when modified as specified in this report, and laboratory strains of *Saccharomyces cerevisiae*, both of which do not have the biological association with man characteristic of the enteric bacteria, clearly fulfill two criteria of HVI systems.

*Saccharomyces cerevisiae* as an HVI system. This yeast is nonpathogenic to man, animals and plants. It is not normally found in sewage or in the intestinal tract and has no aero dispersal mechanism. Of all strains of Group 1 *Saccharomyces* (the group to which the laboratory strain of *S. cerevisiae* belongs) encountered at random in nature, it is estimated that less than 5% are capable of mating with any given haploid strain of *S. cerevisiae* (H.J. Phoff, personal communication) and under natural conditions no other species of yeast will mate with the laboratory strain. Furthermore, no natural vectors are known that might effect interspecific transfer of genetic material. Because of these properties, laboratory strains of *S. cerevisiae* require no special modification to serve as HVI systems.

*Neurospora crassa* as on HVI system. *N. crassa* is nonpathogenic to man, animals and plants. It is an obligate aerobe and is not found in sewers or in the intestinal tract. It is only found sporadically in temperate regions. *N. crassa* is only capable of hybridization and gene exchange with the three most closely related species *N. tetrasperma*, *N. sitophila* and *N. intermedia*. Crosses with other *Neurospora* species or with fungi of other genera have been unsuccessful. Numerous vegetative incompatibility genes in *N. crassa* strains found in nature block transfer of genetic material from strain to strain by vegetative fusion. No vectors are known that might effect interspecific or intergeneric transfer of genetic material.

Since wild type conidia can be dispersed aerially, the HVI designation shall apply only to the following strains in which either survival away from a special substrate or a special containment is greatly compromised.

(i) **ink** (inositolless) strains 37102, 37401, 46316, 46802, 64001 and 89601. These strains require concentrations of the order of 5 micrograms/ml inositol for maximum growth. At 0.5 microgram/ml growth and sporulation are poor. On substrates without inositol the mutants rapidly die (inositollor death). Mutant 46802 is non-reverting, the other mutants revert at frequencies of less than 10⁻⁷.

(ii) **esp-1** (UCLA37) and **esp-2** (FS590, UCLA101) (conidial reparation). These mutants form adherent macroconidia that cannot fall free, even with vigorous topping. No reversions have been reported since the discovery of the mutants in 1974.

(iii) **ear** (UCLA191) (easily wettable). Macroconidia become wet and go into water suspension instantly (wild type is hydrophobic). Macroconidia appear sticky, do not fall free of mycelium even with vigorous topping. No reversions have been reported since the discovery of the mutants in 1974.

Wild type *Neurospora crassa* as an HVI system. Because of the fact that *Neurospora* has never been implicated in adverse effects for man, animals or plants and has no close association with man, animals or plants in nature, and because *N. crassa* in interspecific combination is incapable of forming heterokaryons, wild type *N. crassa* may be permitted to be used in experiments requiring HVI provided these are carried out at physical containment one level higher than required for HVI. However, if P3 physical containment is specified for HVI, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment special care must be exercised to prevent aerial dispersal of macroconidia.

**Disabled Saccharomyces cerevisiae** as an HVI system. Since laboratory strains of *S. cerevisiae* intrinsically more than satisfy HVI criteria, and since the ability of laboratory strains to establish themselves in the wild is low, the main route of escape of a cloned segment to the environment would be through mating of the host to a more robust wild type yeast. Therefore, a sufficient basis for construction of an HVI 2 *S. cerevisiae* would be a drastic reduction in the frequency of mating and, therefore, transmission of a cloned segment over the HVI level by the introduction of sterility mutations. Even without such mutations, on exceedingly low frequency of mating would be expected under dilute natural conditions where mating pheromones would not be in concentrations necessary for the initiation of mating.

**Equivalence of lower eukaryote HVI systems with those of E. coli.** Except for the cloning of complete genomes of eukaryote viruses, the *S. cerevisiae* and *N. crassa* HVI systems and *S. cerevisiae* HVI systems are to be equivalent to EK1 and EK2, respectively; no special action by the RAC is needed for experiments requiring these levels of containment. Experiments involving com-
Complete genomes of class I eukaryote viruses will require P3-HV1 or P2-HV2 containment. Other eukaryote viruses are to be handled on a case-by-case basis.

Editors note: A description of six criterion which must be satisfied in order that a yeast strain can be certified as a HV2 host is not reproduced here. (The primary consideration is the use of sterility mutations which essentially preclude mating of the proposed HV2 strain with all other yeast strains.) It is of interest that the Recombinant DNA Advisory Committee (RAC) is now considering whether to approve four mutant strains of Saccharomyces cerevisiae as HV2 hosts; also under consideration are a number of potential vectors, all derivatives of the plasmid pBR322 (some also contain sequences derived from the yeast 2-micron plasmid or from yeast chromosomal DNA). These vectors are designed to permit cloning in both suitable E. coli K12 and yeast hosts. For additional information please consult the Office of Recombinant DNA Activities, the National Institutes of Health. The article by Perkins and Björkman in this issue of the Newsletter describes the Neurospora mutants which have been approved for cloning.

REFERENCES


NEW MUTANTS AND STOCKS

Chalmers, J.H. and P. St. Lawrence.

\[ \text{cpl-1: A Neurospora mutant sensitive to chloramphenicol.} \]

Wild type Neurospora is relatively resistant to most of the antibiotics and inhibitors which have been used to select mitochondrial mutants in yeast and other organisms (Thayer, 1969, Neurospora News 15: 21; Chalmers, 1974, Neurospora News 21: 20; Al-Saqur, 1975, Neurospora News 22: 6). This resistance may be due either to permeability barriers or to an alternate terminal oxidase which bypasses most of the mitochondrial electron transport chain (Lambowitz and Slayman, 1971, J. Bact. 108: 1087). We have selected mutants which lack this alternate pathway and are therefore hypersensitive to antimycin A even on fermentable media (Chalmers, 1974, Genetics 78: 543; Edwards et al., 1976, in "Genetics and Biogenesis of Chloroplasts and Mitochondria", Th. Bucher, et al., eds. North Holland Press) by starvation for inositol in the presence of low levels of the drug. One such mutant, ANTAS6, was found also to be inhibited by chloramphenicol. Because other antimycin A sensitive mutants are not appreciably more sensitive to chloramphenicol than is wild type, the mutant was renamed cpl-1.

The cpl-1 mutant was induced by U.V. light in a sporulating strain (Perkins, 1971, Neurospora News 18: 12) which also carried inl (JH319) and trp-3 (d120). Inositol starvation was continued for 5 days at 30°C in Vogel's Medium N, supplemented with 200 \( \mu \text{g/ml} \) of L-trypthophan and 0.25 \( \mu \text{g/ml} \) of antimycin A. Surviving conidia were plated on Vogel's medium without the drug, but with the addition of inositol (150 \( \mu \text{g/ml} \)) and tryptophan. Colonies obtained after 2-3 days of growth were replica plated on medium containing 0.3 \( \mu \text{g/ml} \) of antimycin A. Putative mutants were crossed to an Oak Ridge wild type to restore mycelial morphology.

The cpl-1 mutant is inhibited by about 1 \( \mu \text{g/ml} \) of antimycin A and by less than 0.5 \( \mu \text{g/ml} \) of chloramphenicol (wild type is resistant to 4 \( \mu \text{g/ml} \) chloramphenicol). The cytochrome spectrum of the mutant resembles that of wild type when both are grown on minimal medium without drugs. Although other antimycin A sensitive mutants lock the cyanide-bonded oxidase-insensitive alternate oxidase, cpl-1 retains it and will express the oxidase when incubated with chloramphenicol (2 \( \mu \text{g/ml} \) for a few hours.

To test whether the observed sensitivity was due to an alteration in cytosolic ribosomes, the incorporation of \(^3\text{H}-\text{leucine} was studied with and without 1 or 2 \( \mu \text{g/ml} \) of chloramphenicol or 100 \( \mu \text{g/ml} \) of cycloheximide (Hawley and Greenawalt, 1970, J. Biol. Chem. 248: 3574). Over a 30 minute period, no significant differences between cpl-1 and wild type were seen.

The nature of the cpl-1 mutation is unknown. It has been mapped to linkage Group VI and displays 42% recombination with trp-2 and 24% with yia-1. Two known modifiers of permeability mod-5 (Barrett and St. Lawrence, 1969, Neurospora News 15: 15) and mts (Catcheside, 1978, Neurospora News 25: 17) also map to linkage Group VI, but allelism tests have not been performed. The mutant readily reverts with both U.V. and "nitrosoquinidine". We have examined a number of such revertants, induced in both monocaryons and heterocaryons, for non-Mendelian inheritance, but to date only nuclear mutations have been observed; this may be a function of the genetic background. cpl-1 should be a useful mutant for studies on mitochondrial biosynthesis. (Supported in part by Training Grant T32-GM00367.)

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