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Genetics of the Ergot Fungus *Claviceps purpurea*

Part 2: Exchange of Genetic Material via Meiotic Recombination

P. Tudzynski and K. Esser
Lehrstuhl für Allgemeine Botanik, Ruhr-Universität, Bochum (Federal Republik of Germany)

H. Gröschel
Boehringer Ingelheim KG, Drogenpflanzenlabor, Ingelheim (Federal Republik of Germany)

Summary. With the purpose of improving alkaloid production in *Claviceps purpurea,* concerted breeding studies were conducted in order to investigate the exchange of genetic material by meiotic recombination.

1) From two homokaryotic strains differing in the amount of ergocristin produced, 52 biochemical variants were obtained including both auxotrophs and fungicide resistants, 38 of which showed virulence.

2) After host passage, sclerotia formation and ascospore production, 9 stable mutants were obtained (1 auxotrophic and 8 resistant).

3) Several cross infections between the mutant auxotrophic for lysine and different resistant mutants were established and the offspring of one cross was evaluated in detail for both segregation of the marker genes and quantitation of alkaloid production.

4) The marker genes segregated in a Mendelian pattern, as was expected for a two factor cross: 50% parental types and 50% recombination types. This shows that both marker genes are unlinked.

5) Independent of the segregation pattern of the marker genes a "segregation" of quantitative alkaloid production was found. All recombinant strains did not only exceed the level of the low producing parent but some of them showed a higher alkaloid yield than the parent with the higher production rate.

The general implication of these data is twofold: under controlled conditions it is possible to perform meiotic recombination with a self-fertile parasitic fungus and it is possible to use this technique for strain improvement.

Key words: *Claviceps purpurea* – Mutants – Recombination – Alkaloid production

Introduction

The biotechnological exploitation of *Claviceps purpurea* requires a strain improvement program not only with respect to the increase and maintenance of alkaloid production but also for the breeding of strains which predominantly produce specific alkaloids. This necessitates a genetic manipulation within the scope of concerted breeding. It was shown in the first paper of this series that the breeding system responsible for the sexual cycle of *Claviceps purpurea* is characterized by monoecism and selfcompatibility and that it is further possible to isolate and germinate single ascospores from wild strains. The segregation pattern indicated that the production of specific alkaloids is correlated with distinct genotypes (Esser and Tudzynski 1978).

In following this approach it is now necessary to work out procedures to overcome the problem which always occurs when two strains of a selfcompatible fungus are mated: to prove that a cross, i.e. karyogamy and meiosis between the mating partners, really takes place. The application of the classical method, use of marker genes in both mating strains, is not so easy to perform with this parasitic fungus.

In some other laboratories variants were obtained after UV or chemical treatment, e.g. morphological variants (Strnadova 1964a; Strnadova and Kybal 1976), auxotrophs (Strnadova 1964b, 1967), and altered alkaloid content, either qualitative (Kobel and Sanglier 1973), or quantitative (Kobel et al. 1962). Some of these variant symptoms were also transmitted after infection to the sclerotia, but as far as we know no true mutants have yet been found to represent stable ascospore lines.

In this paper the production of auxotrophic and resistant variants is described which yielded stable ascospore lines. After matings recombinants were found for both the marker genes and alkaloid production.

Materials and Methods

Strains: Variants were obtained from several ascospore lines of strain 7 and 8 described in Esser and Tudzynski (1978).

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Culture media: Complete medium according to Esser and Tudzynski (1978); complete-sorbose-medium: 5 g/l sorbose, reduced sucrose content: 5 g/l; minimal-sorbose-medium: 5 g glucose 5 g sorbose, Ammoniumcitrate 17.6 g, KCl 2.0 g, KH_2PO_4 0.5 g, $MgSO_4 \times 7\ H_2O$ 0.15 g, $ZnSO_4 \times 7\ H_2O$ 9 mg, agar 22 g, distilled water 1,000 ml, pH 4.7.

Chemicals: Benomyl (Du Pont): 50% active substance; Cercobin M (BASF): 70% active substance (methyl thiophanate); Bavistin (BASF): 50% active substance (carbendazim).

Production of Variants

a) Auxotrophic variants: Mycelia were grown on solid complete-medium (20 d, 26 °C); conidia were harvested in 5 ml of Ringer solution and adjusted to a concentration of about 10^6 conidia/ml. After UV-irradiation to achieve a survival rate of about 1% (3600 erg/cm² × sec; 12–15 min), conidia were plated on complete-sorbose-medium. After 5–6 d filter discs were subsequently placed on the plates, and after 10–25 h incubation at 26 °C, these were transferred to minimal-sorbose-medium.

Colonies not growing after transfer to the minimal-medium were tested by auxanography for their auxotrophy.

b) Resistant variants: Conidia were harvested and irradiated as described above and plated on solid complete medium containing fungicides in toxic concentrations that were not tolerated by the untreated controls: benomyl 10 mg/l, cercobin 200 mg/l, bavistin 1 mg/l.

Performance of crosses: Single plants of *Secale cereale* were infected with balanced mixtures of conidia from axenic cultures of differently marked strains.

Growth conditions, isolation of mycelia and ascospores, parasitic culture and determination of alkaloid content were conducted as described in Esser and Tudzynski (1978).

Results and Discussion

1 Production of Mutants

In using the above-quoted optimized technique, a number of auxotrophic and resistant variants was obtained (Table 1). These were used for further experiments.

From Table 1 we may deduce the following:

1) It is relatively easy to obtain a number of variants after mutagenic treatment of conidia. Most of these strains have also retained their virulence. This confirms the findings of other authors, as mentioned above.

2) However, during the host passage these characters are often lost. This is particularly true for the auxotrophs, of which only one mutant was obtained. Because of the small numbers obtained due to the low germination rate of the sclerotia, it is not possible to determine whether this depends on back mutation or inviability of the mutated genome under parasitic conditions.

In this context it seems worthwhile to mention, that in all cases we obtained in the offspring of a sclerotium some ascospore lines which did not have the marker gene, probably due to some genetic instability in the axenic or parasitic culture. This, however, demonstrates the usefulness of these characters as marker genes.

Table 1. Compilation and analysis of auxotrophic and resistant variants of *Claviceps purpurea*. The variants were obtained after mutagenic treatment and subsequent germination of conidiospores. Auxotrophs were tested by auxanography, resistants by incorporation of the substance concerned into the medium as indicated. After infection of host plants the resulting sclerotia were germinated and from each sclerotium 10–40 ascospores were isolated and assayed for the postulated characters

Character	Tested in parasitic culture		Number of variants from which stable ascospore lines were obtained
	Total number	Number of infectious lines (sclerotia formed)	
Auxotrophs			
histidine	11	5	–
lysine	3	2	1
glutamine	1	1	–
methionine	3	3	–
arginine	1	–	–
Resistant lines			
benomyl (10 mg/l)	10	8	3
cercobin (200 mg/l)	22	18	5
bavistin (1 mg/l)	1	1	–

In conclusion: One may say that although it is rather difficult and time-consuming to obtain stable haploid mutants it is certainly possible. Fungicide-resistant mutants obviously have some advantages in practice. They are easily selectable, more stable in parasitic culture and, if grown in the field, the cereals may be treated with the fungicides to avoid contamination with other fungi which especially occur in rainy seasons.

2 Recombination of Genetic Material

The main problem to be solved in performing crosses with *Claviceps purpurea* is its self-fertility. For cross-fertilization heterokaryon formation is needed.

The common method used under laboratory conditions to force heterokaryons between auxotrophs or resistant cultures failed. Due to the absence of selective pressure under parasitic conditions the heterokaryons became unstable. Even in the few successful infections reported no recombination of the characters involved has been found (Strnadova 1968). After several ineffectual attempts we succeeded in infecting the host plants with a mixture of conidiospores of the genetically marked strains. The three mutants were crossed in all possible pairwise combinations. In each case we obtained scle-

rotia, but again, as in the sexual reproduction of the variants, a great number of sclerotia did not germinate. The data of a typical cross are summarized in Table 2.

The data of Table 2 show:

1) Recombinants were found not only in the offspring of the two sclerotia from one single plant, but also in the different heads of a single sclerotium. This demonstrates that fertilization has taken place after the mixed infection and proves that the cross is not a single event. In this context it is of minor interest, whether recombination was brought about by heterokaryosis or by cross-fertilization at the origin of the various perithecia.

2) The low number of auxotrophs (*lys cer*$^+$and *lys cer*) may depend on an interference of the nutritional deficiency with the germination of ascospores.

3) Since the ratio of parental strains to recombinant stains is within statistical confidence limits (X^2 – test $p > 0.1$) a linkage between the two marker genes is rather improbable.

In conclusion: It is therewith shown that meiotic recombination may take place after infection of a host plant with heterogenic conidiospores. Therefore, it should be possible to recombine those genetic traits responsible for alkaloid characters.

3 Improvement of Alkaloid Content by Recombination of the Genetic Material

In order to prove meiotic segregation of marker genes as described above, a high producer (cercobin resistant) and a low producer (lysine auxotrophic) were expressly used. In order to prove whether the recombination of marker genes is correlated with a recombination of the yet unknown genetic traits responsible for alkaloid production 37 recombinants from the offspring of the cross described in Table 2 were selected for an alkaloid assay. This selection was necessary because of the rather laborious experimental procedure. 6 strains of the *cer*-parental genotype were used as controls. No strain of the *lys* parental genotype was assayed because those strains have a rather low virulence.

Under controlled conditions each strain was used to infect about 10 host plants. The sclerotia obtained from each lot were pooled and from one sclerotium mycelium was regenerated. In each case the original marker genes were retained after the host passage. This and the fact that the alkaloid test performed with the rest of the sclerotia (see Table 3) never showed any other main alkaloids but ergocristin proved that none of the host plants was contaminated.

From the data of Table 3 may be seen:

1) All recombinants tested show an alkaloid level higher than the low-producing lysine parent: 17 are on the level of the cercorbin parents, 17 are intermediate between the alkaloid content of the 2 parents and 3 even show a considerable increase in productivity.

2) Despite the fact that only 6 strains of the cercobin parent were tested, it is evident that also in this genotype the alkaloid content shows a quantitative variability.

3) It is thus obvious that in addition to the segregation pattern of the marker genes, a quantitative segregation of the alkaloid production has taken place as a consequence of meiotic processes. It becomes further

Table 2. Ascospore analysis of the offspring of a cross between an auxotrophic mutant for lysine (*lys*) and a mutant resistant to Cercobin (*cer*) of *Claviceps purpurea.* Two sclerotia were assayed

Sclerotium	Stroma head	Number of ascospores		Genotype of ascospores			
		Isolated	Germinated	Parental		Recombinant	
				Lys	Cer	Lys$^+$ cer$^+$ (wild)	Lys cer
A	1	102	51	4	22	17	8
B	2	70	37	2	16	12	7
	3	50	24	1	12	6	5
	4	171	72	4	43	21	4
Total		393	184	11	93	56	24

Table 3. Alkaloid analysis of descendants of a cross between a mutant auxotrophic for lysine and a mutant resistant to cercobin (Table 2). The alkaloid content (ergocristin) is given in arbitrary units using the parent *cer* as reference (100%); this attributes 48% to the parent *lys.* In order to classify the alkaloid content of the mutants 5 different classes were established: 2 corresponding to the contents of the parents, one lower than parent *lys*, one intermediate, one exceeding parent *cer*

Alkaloid content (% Ergocristin)	Number of descendants			
	Total	Parental Cer	Recombinants	
			Cer-lys	Wild
= Parent cer (90 – 110)	19	2	7	10
= Parent lys (43 – 53)	0	0	0	0
< Parent lys (< 43)	0	0	0	0
Intermediate (53 – 90)	21	4	5	12
> Parent cer (110 – 130)	3	0	1	2
	43	6	13	24

evident that with a high degree of probability the two segregation patterns are not correlated.

In conclusion: It is thus shown in principle that it is possible to improve the alkaloid production of ergot by concerted breeding. Naturally, crosses between high yield strains are expected to show an even greater improvement in alkaloid quantity. The next step would involve evaluation of additional crosses to decipher the qualitative correlation between genes and certain types of alkaloids.

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Dr. Paul Tudzynski
Prof. Dr. Karl Esser
Lehrstuhl für Allgemeine Botanik
Ruhr-Universität
D-4630 Bochum 1 (Federal Republic of Germany)

Dr. Hartmut Gröschel
Boehringer Ingelheim KG
Drogenpflanzenlabor
D-6507 Ingelheim (Federal Republic of Germany)