Computer analysis of N. crassa growth curves.

C. Scharf

B. L. Seidel

Follow this and additional works at: http://newprairiepress.org/fgr

Recommended Citation


This Regular Paper is brought to you for free and open access by New Prairie Press. It has been accepted for inclusion in Fungal Genetics Reports by an authorized administrator of New Prairie Press. For more information, please contact cads@k-state.edu.
Computer analysis of N. crassa growth curves.

Abstract
Computer analysis of N. crassa growth curves.

Creative Commons License
This work is licensed under a Creative Commons Attribution-Share Alike 4.0 License.

This regular paper is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol33/iss1/14
Rather than prepare tubes in duplicate or triplicate for individual tests, only a single race tube was used for each strain, and rates were determined using several different strains to represent each species. This could not be done for the homothallic species where only a single isolate was available.

N. crassa, N. intermedia, N. sitophila, and N. tetrasperma differ little in rate of linear growth. An apparent exception is the yellow ecotype of N. intermedia (represented by P60, P201) which is found characteristically on nonburned substrates in the Eastern hemisphere.

In contrast, the five homothallic species grow at only half the speed of the heterothallic, or less. The slowest species, N. terricola, from soil in Wisconsin, is also set off from the others morphologically by having rounded ascospores with a single germ pore. Strain D301 from Dominica, West Indies, has been diagnosed as a variety of N. galapagosensis (H.L. Huang, personal communication). All the homothallic strains are devoid of conidia.

Representative strains of the new species N. discreta are also distinctly slower than the other heterothallic species. The Kirbyville isolates from Texas are slowest. P390 (Florida) and P755 (Guatemala) are somewhat faster.

Our growth-rate determinations based on single tubes are certainly not definitive. However, these results agree well with the more extensive data of other workers for species tested previously. Our 25° C rates compare with those of Ryan et al. (1943) as follows: 3.7-4.0 vs 3.7-4.2 mm/h for N. crassa; 3.8-4.0 vs 4.1-4.2 mm/h for N. sitophila. For N. intermedia, our 4.0-4.2 mm/h compares with about 4.2 mm/h of Griffiths et al. (personal communication). Ryan et al. calculated that a difference of less than 0.4 mm/h between two single race tubes is probably not significant.

---

Department of Biological Sciences, Stanford University, Stanford, California 94305

Scharf, C. and B.L. Seidel

The determination of the best fit line to a growth curve has been difficult because of its sigmoidal nature. A number of equations have been developed which attempt to describe growth curves. These include the logistic, Gompertz, von Bertalanffy, and Richardson equations (Ricklefs, 1967, Ecology 48:978-983; Richardson, 1984, Bull. Southern Calif. Acad. Sci. 83:101-115). Several statistical packages are available which allow the use of these equations for computer analysis of the data, including the SAS system; BMDP (Bio-Medical Data Processing), and Systat (IBM). All packages can be run on an IBM microcomputer, but require a hard disk drive. We describe here the use of the SAS system for the analysis of N. crassa growth curve data.

The SAS system is an all-purpose system designed for data analysis and is available through SAS Institute, Inc. Box 8000; Cary, NC 27511-8000 (919-467-8000). The software is compatible with IBM 370/30XX/43XX; Digital Corporation VAX 11/7XX; and Data General ECLIPSE series to name a few. We used the Data General System available through the Academic Computer Center at SUNY-Plattsburgh.

The particular procedure used was PROC NLIN and is written as shown in Table I. This is a least-squares procedure for estimating parameters for non-linear models. Data is entered into the program using the DATA statement. This is accomplished by retrieving data stored in a separate file or by typing the data directly into the program as was done here. PROC NLIN invokes the SAS procedure. BEST = 10 requests that the residual sums of squares for only the best iterations are printed. PARMS sets the starting values for the parameters. In this case, BO = asymptote and is set at 50 mg dry weight; B1 = growth rate constant: the program will iterate from a value of 0 to 0.99 in increments of 0.05; B2 = inflection point: the program will iterate from a value of 15 to 30 in increments of 1.0. The MODEL statement is the equation for the logistic curve written in SAS nomenclature. The remaining statements involve instructions for output of the analysis. Since no method was chosen, the default method DUD was used as the iterative procedure. Several other methods of iteration are possible. The above procedures and statements can be modified for use with the Gompertz, von Bertalanffy, and Richardson equations. As a result, one can determine the equation which best describes the data.
FIGURE 1 - Growth of Wild-Type *N. crassa* at 30°C (•) and 25°C (X). Lines are drawn through the values predicted by computer analysis. Bars indicate the 95% confidence intervals for the asymptote and inflection points.

Table 1 - Program for Data Analysis

```plaintext
DATA A
  INPUT X Y @@;
  CARDS;
  13.0  08.0  13.0  08.7  17.0  09.0  etc.

PROC NLIN      BEST = 10;
  PARMS        BO   = 50.0
                B1  = 0 to 0.99 by 0.05
                B2  = 15 to 30 by 1.0;
  MODEL Y = BO/(1 + EXP (-B1*(X-B2)));
  OUTPUT OUT = B    P = YHAT  R = YRESID;

PROC PLOT DATA = B;
  PLOT  Y*X = 'A' YHAT *X = 'P'/OVERLAY VPOS = 25;
  PLOT  YRESID * X/VREF = 0  VPOS = 25;
```

Table 2 - Summary of Computed Variables for Growth at 30°C (A) and 25°C (B). All values are rounded off to two decimal places.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>asymptote A</td>
<td>53.69</td>
<td>1.65</td>
<td>50.41</td>
<td>56.97</td>
</tr>
<tr>
<td>growth rate A</td>
<td>0.12</td>
<td>0.01</td>
<td>0.10</td>
<td>0.14</td>
</tr>
<tr>
<td>constant inflection</td>
<td>34.25</td>
<td>0.77</td>
<td>32.72</td>
<td>35.78</td>
</tr>
<tr>
<td>asymptote B</td>
<td>26.94</td>
<td>0.70</td>
<td>25.51</td>
<td>28.37</td>
</tr>
<tr>
<td>growth rate B</td>
<td>0.15</td>
<td>0.01</td>
<td>0.12</td>
<td>0.17</td>
</tr>
<tr>
<td>constant inflection</td>
<td>31.37</td>
<td>0.70</td>
<td>29.93</td>
<td>32.80</td>
</tr>
</tbody>
</table>
This program calculates predicted values from the equation which describes the best fit line through the actual data (Figure 1). In addition, the output includes a table of values for the asymptote, growth rate constant, and inflection point (Table 2). Note that for growth at 30°C the asymptote is estimated to be 53.69 mg dry wt with a standard error of 1.65. The 95% confidence interval falls between values of 50.41 and 56.97 mg dry weight. For growth at 25°C, the asymptote is 26.94 with a standard error of 0.70 and a 95% confidence interval of 25.51 to 28.37. The inflection points can be compared in a similar manner.

One advantage of using this method of analysis is that it allows a comparison of different growth curves. Due to the absence of overlap between the values for the 95% confidence intervals for both asymptote and inflection point, we can conclude that the curves are significantly different. The program also provides an analysis of variance from which the coefficient of determination (r^2) can be calculated. In this case, r^2 for the growth curve at 30°C was 98.9%, while r^2 for the curve at 25°C was 99.2%. It can therefore be concluded that the logistic equation accurately describes the data. Furthermore, once the equation has been defined, additional techniques for comparison among growth curves can be used (Ricklefs, 1967).

Schechtman, M.  
Several methods for preparing DNA from Neurospora have been reported and are in current use (Metzenberg and Baisch 1981 Neurospora News 28:20-21; Stevens and Metzenberg 1982 Neurospora News 29:27-28, Feher and Schablik 1983 Neurospora News 30:14; Vollmer and Davis 1985 Neurospora News 32:16-17). These procedures, while yielding usable DNA, in our hands all have certain drawbacks: either they are not easily scaled up or yield DNA that is refractory to digestion by certain restriction enzymes. We have combined steps from several of these methods and from a procedure for purification of Aspergillus DNA (Yelton et al. 1984 PNAS 81:1470-1474). The following protocol yields a substantial quantity (200-300 ug) of fairly pure, easily cuttable DNA when either logarithmically growing hyphae or a post-logarithmic pad is used. The DNA obtained is greater than 50 kb in length. Given enough mortars and pestles, a dozen samples can be conveniently processed in one day.

1. Inoculate a 40 ml culture (Vogel's medium) with 10^6 conidia/ml. Grow 40-48 h at 25°C with shaking.
2. Filter the mycelia on a Buchner funnel and wash with H2O.
3. Transfer the mycelial pad to a pre-chilled (-70°C) mortar, add liquid N2, and grind to a very fine powder.
4. Transfer the powder to a 50 ml polypropylene tube and add 15 ml 5 mM EDTA pH 8.5 - 0.2% SDS, and 15 ul diethylpyrocarbonate (DEPC). Shake vigorously only long enough to dislodge the frozen material, then continue to invert slowly for one minute.
5. Incubate at 70% for 15 min.
6. Chill on ice at least 10 min. (If multiple samples are to be processed on the same day, tubes can be left on ice for up to 1 h in order to synchronize all the samples.)
7. Add 0.95 ml 8M potassium acetate pH 4.3. Incubate on ice 1 h.
8. Centrifuge at 14,000 rpm for 15 min, at 4°C.
9. Transfer the supernatant to a new tube. Add 15 ml isopropanol and mix gently. A clot should immediately form.
10. Rinse pellet with 70% ethanol. Drain well and air dry for 10 min.
11. Resuspend pellet in 4 ml 1 mM EDTA pH 8.0. Add 2 ml High Salt Buffer (Metzenberg and Baisch, op. cit.) plus 15 ul (boiled) 10 mg/ml RNase A. Incubate at 37°C for 30 min.
12. Add 180 ul 100 mM spermine-4HCl. Mix gently, then incubate on ice 20 min. Pipet off the liquid from the clotted DNA.
13. Rinse the pellet 3x on ice, for 30 min. each, with 2 ml cold spermine wash buffer (75% ethanol, 10 mM Mg acetate, 0.3 M Na acetate, pH 6.0), pipetting off rinse each time.
14. Rinse briefly with 70% ethanol. Drain well.
15. Air dry 10 min, then add 1 ml 10 mM Tris-Cl pH 8.0, 1 mM EDTA, 0.1 M NaCl. Allow to resuspend overnight at 4°C.
16. Reprecipitate the DNA with 2 ml ethanol at room temperature. A clot will immediately form. Pipet off the supernatant, rinse pellet with 70% ethanol, air dry 10 min., and resuspend in 1 ml TE (10 mM Tris-Cl, 1 mM EDTA, pH 8.0)