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Maximum Likelihood based comparison of the specific growth rates for *P. aeruginosa* and four mutator strains

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ABSTRACT

The specific growth rate for *P. aeruginosa* and four mutator strains *mutT*, *mutY*, *mutM* and *mutY–mutM* is estimated by a suggested Maximum Likelihood, ML, method which takes the autocorrelation of the observation into account. For each bacteria strain, six wells of optical density, OD, measurements are used for parameter estimation. The data is log-transformed such that a linear model can be applied. The transformation changes the variance structure, and hence an OD-dependent variance is implemented in the model. The autocorrelation in the data is demonstrated, and a correlation model with an exponentially decaying function of the time between observations is suggested. A model with a full covariance structure containing OD-dependent variance and an autocorrelation structure is compared to a model with variance only and with no variance or correlation implemented. It is shown that the model that best describes data is a model taking into account the full covariance structure. An inference study is made in order to determine whether the growth rate of the five bacteria strains is the same. After applying a likelihood-ratio test to models with a full covariance structure, it is concluded that the specific growth rate is the same for all bacteria strains. This study highlights the importance of carrying out an explorative examination of residuals in order to make a correct parametrization of a model including the covariance structure. The ML method is shown to be a strong tool as it enables estimation of covariance parameters along with the other model parameters and it makes way for strong statistical tools for inference studies.

1. Introduction

Proper estimation of growth parameters is essential in many areas, for instance in determining the effect of antimicrobial treatment (Dalgaard and Koutsoumanis, 2001) or when modelling growth of bacteria in food processing and storage (Juneja et al., 2007; Shama et al., 2005). Furthermore, it is very important to be able to tell whether the growth of different bacteria strains is identical. This can form the basis of *in vivo* or *in vitro* experiments, such as competition experiments (Montanari et al., 2007), where two or more bacteria are competing to survive and overtake the population. If the growth rates of bacteria strains are not identical in a normal unstressed environment, this will affect the result of a competition experiment carried out in a stressed environment, e.g. by adding antibiotics. Thus, it is very important to correctly determine whether the growth rates are identical.

Bacterial growth is typically classified by the maximum growth rate μ_{max} and the lag time (Baty and Delignette-Muller, 2004), when the growth rate is considered to be time dependent. Alternatively the growth is described by a Monod expression (Monod, 1949), which depends on the substrate content and contains the parameters μ_{max} and the OD value were half the maximum growth is reached, κ_{50}. The Monod model should be considered when not enough substrate is available to reach intolerable numbers of bacteria before the growth rate decreases due to substrate depletion (Zwietering et al., 1990).

The objective of the current study is to determine whether the growth of *P. aeruginosa* and four mutator strains *mutT*, *mutY*, *mutM* and *mutY–mutM* can be regarded as identical. For this study optical density, OD, measurements are available for each strain growing in LB media. The study is motivated by a competition experiment between *P. aeruginosa* and each of the four mutator strains, for which interpretations of the results rely on the growth rates being identical. Examination of identical growth rates is relevant, as mutator strains are often considered to have lower fitness and thereby growth rate due to a higher mutation rate and thus more deleterious mutations. The mutation rates of the bacteria considered are listed in Table 1. OD measurements are used in stead of CFU count, as this method demands less resources, and it is also the choice of measurement

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Table 1

Bacteria strain	Mutation rate per generation	Ref.
P. aeruginosa	$4.61 \cdot 10^{-9}$	a
mutT	$1.28 \cdot 10^{-7}$	a
mutY	$3.85 \cdot 10^{-8}$	a
mutM	$6.38 \cdot 10^{-9}$	a
mutY–mutM	$1.94 \cdot 10^{-7}$	b

a: Mandsberg et al. (submitted for publication); b: Calculated using the method described by Ma et al. (1992).

method for the competition experiment made. It has been argued (Augustin et al., 1999) that due to the detection limit of the OD measurements, the specific growth rate estimated for these OD values will be lower than the maximum specific growth. However, the specific growth rate is assumed to be a usable measure, for the purpose of determining whether the growth is the same for the five bacterial strains.

Recent studies (Baty and Delignette-Muller, 2004; Dalgaard and Koutsoumanis, 2001; Fujikawa et al., 2004; Juneja et al., 2007; Lindqvist, 2006; Shama et al., 2005) have compared the estimation of the growth rates and/or lag times obtained by different mathematical models. All of these studies use un-weighted least squares to estimate the parameters. This paper suggests estimating the model parameters using the Maximum Likelihood (ML) method. This method enables us to introduce a full model including a variance and autocorrelation structure for the observations and to determine the related parameters along with the growth parameters. The suggested ML method enables the use of strong statistical tools to compare models. As an example we apply the likelihood-ratio test to examine whether the growth of the five bacteria strains can be assumed to be identical. The study demonstrates the importance of including full information about variance and correlation structure in a growth model.

For the estimation of μ an exponential model is considered, which means that a linear model is fitted to the log-transformed OD curve where the slope is steepest (Zwietering et al., 1990). More advanced models have been proposed (for a recent review see Li et al., 2007) to fit the growth curve, defined as the logarithm of the number of bacteria as a function of time. These models contain both lag phase and μ_{max}, so it is not necessary to subjectively decide the interval for the exponential growth. However these models are limited to sigmoidal growth curves, which do not describe well the growth of *P. aeruginosa* in LB media. Also the Monod model is not appropriate for describing growth on rich media (Kovárová-Kovar and Egli, 1998). Moreover, for the purpose of introducing a weighted estimation of the growth parameters, it is desirable and sufficient to keep the model as simple as possible. Therefore we consider the exponential model for growth.

2. Materials and methods

2.1. Growth measurements

OD measurements are obtained for growth in LB media for *P. aeruginosa*, PAO1, and four different mutator strains; *mutT*, *mutY*, *mutM* and *mutY–mutM*. A description of the individual mutator strains is given in Mandsberg et al. (submitted for publication). The double mutant *mutY–mutM* is constructed by a method, not yet published, which is modified in accordance with Quenee et al. (2005). The bacteria are grown over night in LB media, after which they are adjusted to an OD600 on 0.03 and subsequently diluted to 10^{-4}. Each bacterium strain is transferred to six microtiter wells with 280 µl to each well. Measurements are made with a sampling interval of 5 min in a bioscreen (Labsystem C (Bie og Berntsen)) at 37 °C under continuous shaking. The measurements are shown in Fig. 1.

The specific growth rate occurs during exponential growth, and can be found by estimating the slope of the log-transformed data, where the slope is steepest. The interval for estimation of the specific growth rate shown in Fig. 2 is chosen by graphical inspection. The interval comprises 11 observations.

Several authors (Chorin et al., 1997; Augustin et al., 1999; Baty et al., 2002) have discussed the relation between OD and CFU measurements and the influence of the measurement method on the estimated growth parameters. For this study the relation between OD and the actual bacterial concentration has been examined experimentally, and the relation between OD and concentration is found to be linear in the examined interval. Since the relation is linear a transformation of the data from OD to CFU will not influence the estimate of the specific growth rate, and therefore the following study is continued with the OD values.

2.2. Model

The OD measurements are initially transformed by

$$\begin{aligned} B_{bij} &= \mathrm{OD}_{bij} - M_j \\ Y_{bij} &= \log(B_{bij}), \end{aligned} \tag{1}$$

where OD_{bij} is the measured OD value for bacteria strain b ($b = 1,2,\ldots,S$), repetition i ($i = 1,2,\ldots,R$) at time t_j ($j = 1,2,\ldots,T$). M_j is the mean of OD values for ten wells with media without bacteria, and thus B_{bij} is the OD contribution due to growth, corrected for the media. In this study $S = 5$, $R = 6$ and $T = 11$.

The linear relation seen in Fig. 2 for the log-transformed data is modelled by a general linear model of the form

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\theta} + \epsilon, \quad \text{where} \quad \mathbf{Y} \in N(\mathbf{X}\boldsymbol{\theta}, \sigma^2 \boldsymbol{\Sigma}), \tag{2}$$

where $\mathbf{X}$ is the design matrix and $\boldsymbol{\theta}$ is a set of parameters $[\boldsymbol{\alpha}, \boldsymbol{\mu}]$ with α being the intercept and $\boldsymbol{\mu}$ the slope, i.e. the specific growth rate. $\mathbf{Y}$ is a vector of length SRT containing all the observations in the estimation interval. To reduce the correlation between the estimated values for $\boldsymbol{\alpha}$ and $\boldsymbol{\mu}$ the time series are translated such that they starts at $t_1 = 0$. The values of $\boldsymbol{\alpha}$ is thus the $\mathbf{Y}$ values at the beginning of the estimation interval.

Two models are introduced with the purpose of determining whether all bacteria strains can be assumed to have the same specific growth: Model 1 where the growth rate is different for each bacteria strain, and Model 0 where the growth rate is the same for all repetitions of the experiment. For both models the intercept is

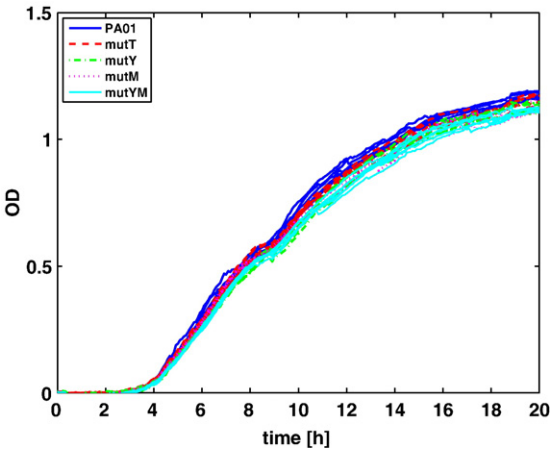

Fig. 1. The OD measurements for the five different bacteria, corrected for the OD of the media.

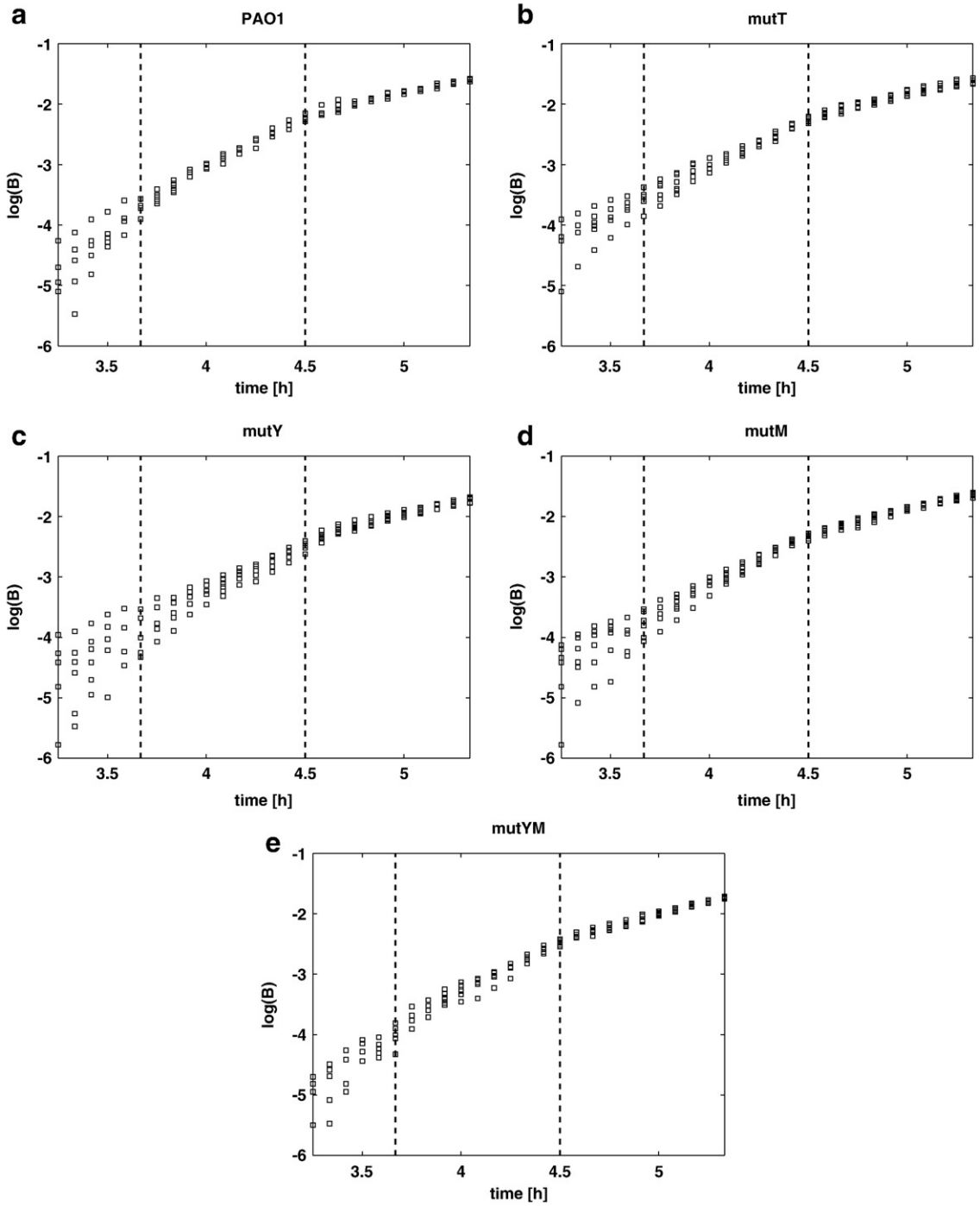

Fig. 2. The log-transformed OD measurements, corrected for media content, for the five different bacteria strains. The estimation interval for the maximal growth rate is marked by the vertical dotted lines.

different for all bacteria in order to account for the small difference in initial OD and media concentration. The following notation will be used for the vector of all time points $\mathbf{t}=[t_1, t_2, \ldots, t_T]^T$, and $\mathbf{T}$ is a column vector with R repetitions of the vector $\mathbf{t}$. A column vector of length T containing only ones is written in short-hand notation as $\boldsymbol{e}=[1,1,\ldots,1]^T$ and a matrix comprising R repetitions of $\boldsymbol{e}$ is defined as

$$\boldsymbol{E}=\begin{bmatrix}\boldsymbol{e} & 0 & \ldots & 0\\ 0 & \boldsymbol{e} & & \vdots\\ \vdots & & \ddots & 0\\ 0 & \ldots & 0 & \boldsymbol{e}\end{bmatrix}.$$

2.2.1. Model 1

$$\mathrm{Y}_{bij}^{(1)}=\alpha_{bi}+\mu_b t_j+\epsilon_{bij}, \tag{3}$$

or in matrix formulation

$$\mathbf{Y}^{(1)}=\mathbf{X}_1\boldsymbol{\theta}_1+\epsilon, \quad \text{where} \tag{4}$$

$$\mathrm{X}_1=\begin{bmatrix}\boldsymbol{E} & 0 & \ldots & 0 & \boldsymbol{T} & 0 & \ldots & 0\\ 0 & \boldsymbol{E} & & \vdots & 0 & \boldsymbol{T} & & \vdots\\ \vdots & & \ddots & 0 & \vdots & & \ddots & 0\\ 0 & \ldots & 0 & \boldsymbol{E} & 0 & \ldots & 0 & \boldsymbol{T}\end{bmatrix},$$

$$\boldsymbol{\theta}_1=[\alpha_{11},\ldots,\alpha_{1R},\alpha_{21},\ldots,\alpha_{SR},\mu_1,\ldots,\mu_S]^T$$

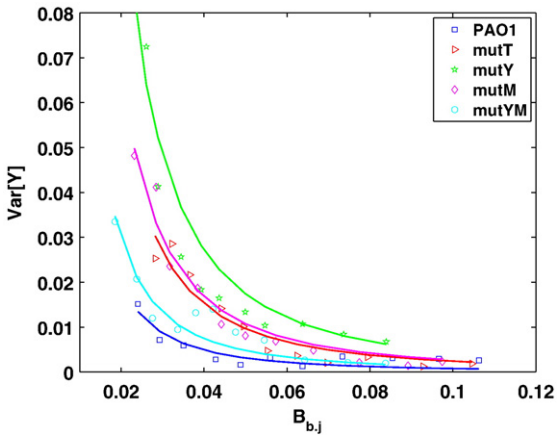

Fig. 3. The variance of **Y** against $B_{b,j}$ as calculated from data (symbols) plotted together with $\sigma^2(1/B_{b,j})^2$ (lines), where $B_{b,j}$ is the mean value for each time point and bacteria strain and σ^2 is estimated by least squares regression.

2.2.2. Model 0

$$\mathbf{Y}_{bij}^{(0)} = \alpha_{bi} + \mu t_j + \epsilon_{bij}, \tag{5}$$

or in matrix formulation

$$\mathbf{Y}^{(0)} = \boldsymbol{X}_0\boldsymbol{\theta}_0 + \epsilon, \quad \text{where}$$

$$\mathbf{X}_0 = \begin{bmatrix} \boldsymbol{e} & 0 & \dots & 0 & \mathbf{t} \\ 0 & \boldsymbol{e} & & \vdots & \mathbf{t} \\ \vdots & & \ddots & 0 & \vdots \\ 0 & \dots & 0 & \boldsymbol{e} & \mathbf{t} \end{bmatrix}, \tag{6}$$

$$\boldsymbol{\theta}_0 = [\alpha_{11}, \alpha_{12}, \dots, \alpha_{1R}, \alpha_{21}, \dots, \alpha_{SR}, \mu]^T$$

When introducing Model 1 it is assumed that the specific growth rate for each repetition within a bacterium strain is the same. This is biologically plausible as the bacteria and media mixture in each of the six wells come from the same batch culture. A third model has been considered where the specific growth rate for each repetition is different, but the analysis indicates that there are too few data points for each curve to give a good estimation of the model parameters. Therefore the rest of this study continues with Model 0 and Model 1.

2.3. Maximum Likelihood estimation

The model parameters are estimated by maximizing the log-likelihood function. The likelihood function is equal to the joint probability density of the data, $p(\mathbf{Y}|\boldsymbol{\theta})$

$$L(\boldsymbol{\theta}|\mathbf{Y}) = p(\mathbf{Y}|\boldsymbol{\theta}). \tag{7}$$

As the data can be assumed to be normally distributed, the probability density for **Y** is

$$p(\mathbf{Y}|\boldsymbol{\theta}) = \frac{1}{\sqrt{(2\pi\sigma^2)^N \det(\boldsymbol{\Sigma})}} \cdot \exp\left(-\frac{1}{2\sigma^2}(\mathbf{Y}-\mathbf{X}\boldsymbol{\theta})^T\boldsymbol{\Sigma}^{-1}(\mathbf{Y}-\mathbf{X}\boldsymbol{\theta})\right), \tag{8}$$

where N is the total number of observations. The log-likelihood function for normally distributed data is thus

$$\log(L(\boldsymbol{\theta}|\mathbf{Y})) = -\frac{1}{2}N\log(\sigma^2) - \frac{1}{2}\log(\det(\boldsymbol{\Sigma})) - \frac{1}{2\sigma^2}(\mathbf{Y}-\mathbf{X}\boldsymbol{\theta})^T\boldsymbol{\Sigma}^{-1}(\mathbf{Y}-\mathbf{X}\boldsymbol{\theta}) \tag{9}$$

plus a constant term $(-\frac{1}{2}\log(2\pi))$, which for simplicity is ignored, since it does not depend on the parameters.

In order to parameterize $\boldsymbol{\Sigma}$, an examination of the variance and autocorrelation structure of the data is needed. Assuming that the variance of B is Var[B], the variance of the log-transformed data can be determined from

$$\mathrm{Var}[f(x)] = \mathrm{Var}[x]f'(x)^2 \tag{10}$$

i.e.,

$$\mathrm{Var}[\mathbf{Y}] = \mathrm{Var}[B]\left(\frac{1}{B}\right)^2 = \sigma^2\left(\frac{1}{B}\right)^2. \tag{11}$$

Indeed the variance depends on the inverse of the square of B, as seen in Fig. 3. The figure shows the variance of **Y** as calculated from six repetitions within each bacteria strain at each time plotted, together with a fit of the theoretical expressions in Eq.(11) to the inverse square mean of B.

A significant autocorrelation was found in earlier studies (López et al., 2004), and should be included in the model to give a full parameterization of the data. In order to determine the correlation structure of the residuals ε_{bij}, a ML estimate of Model 0 and Model 1 with $\boldsymbol{\Sigma}=\boldsymbol{I}$ is initially examined by plotting the autocorrelation function in Fig. 4. This plot indicates that the noise sequence is indeed correlated in time. For simplicity we suggest the following exponentially decaying function of the time between two observations

$$\mathrm{Corr}[\epsilon_j, \epsilon_k] = \rho^{|j-k|} \tag{12}$$

to describe the autocorrelation (Madsen and Thyregod, 1988). This parametrization of the autocorrelation can be chosen since the observations are equidistant. The parameter ρ now corresponds to the lag 1 correlation, i.e. the correlation between two consecutive observations.

Three different structures of the covariance matrix $\boldsymbol{\Sigma}$ are examined in order to compare the models and determine the $\boldsymbol{\Sigma}$ that best describes data. The most simple is the identity matrix

$$I: \boldsymbol{\Sigma} = \boldsymbol{I}, \tag{13}$$

for which the ML estimation corresponds to performing a least squares estimate of the model parameters. On the basis of the

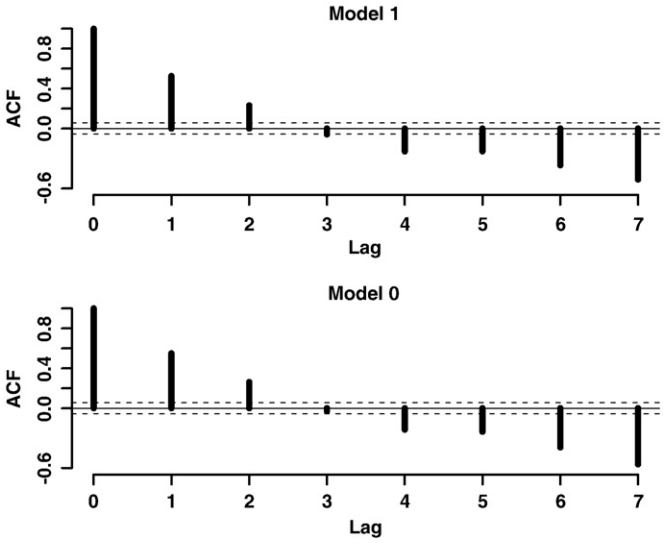

Fig. 4. The autocorrelation function, ACF, for the residuals of Model 0 and Model 1 with $\boldsymbol{\Sigma}$ being the identity matrix fitted to the measured OD values.

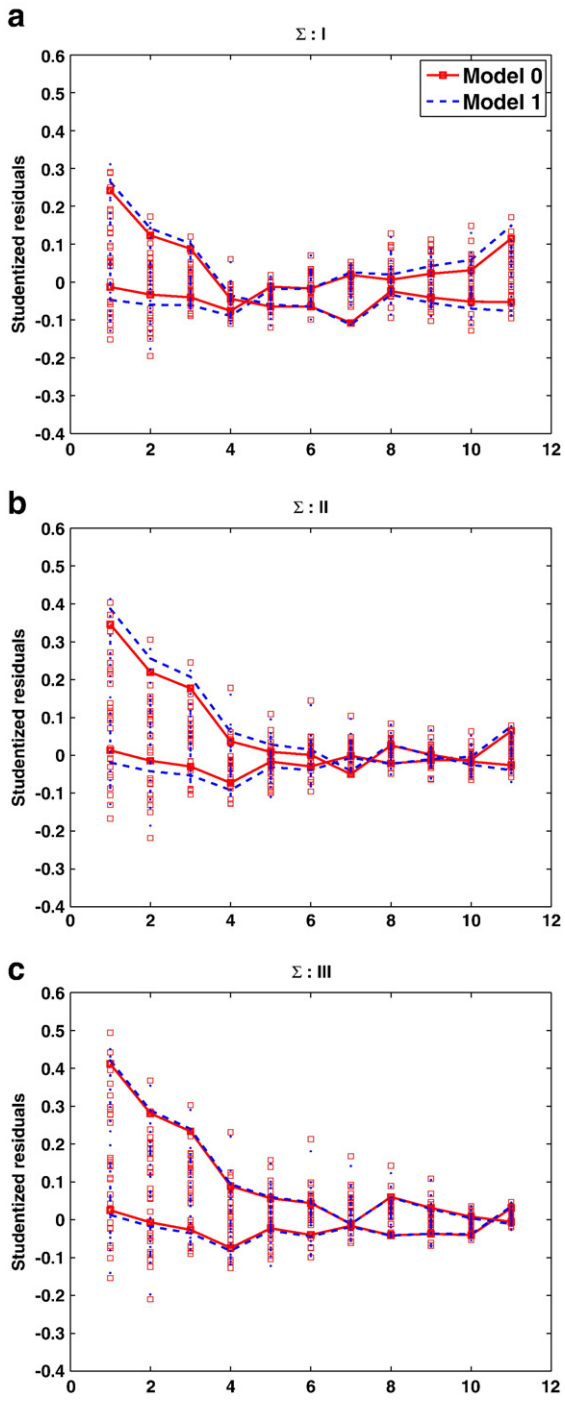

Fig. 5. Residuals for the ML estimation of Model 0 and Model 1. Two curves are shown for PA01 and mutY, respectively, to illustrate the correlation structure in the data.

theoretical explanation and explorative examination of the data, it is clear that the variance depends on B as described in Eq. (11). Therefore the following variance structure for each bacterium b and each repetition i is suggested

$$\text{II} : \left\{\Sigma_{jj}^{bi}\right\} = \frac{1}{(B_{bij})^2}, \tag{14}$$

where B_{bij} is the OD measurement of repetition i at time k for bacteria b less the contribution from the media M_j.

Furthermore, in order to include the autocorrelation as well as the variance structure, we introduce the full $\boldsymbol{\Sigma}$ matrix. This is a block diagonal matrix with one block matrix for each repetition of the experiment, thus for each bacterium b and repetition i it is given by

$$\text{III} : \left\{\boldsymbol{\Sigma}_{jk}^{bi}\right\} = \frac{\rho^{|j-k|}}{B_{bij}B_{bik}} \tag{15}$$

where $\boldsymbol{\Sigma}_{jk}^{bi}$ is the jk element ($j=1, 2, \ldots, T$ and $k=1, 2, \ldots, T$) of the block matrix belonging to repetition i and bacteria b.

The total set of parameters to be estimated is thus σ, ρ and $\boldsymbol{\theta}$ where θ_{bij} contains the model parameters α_{bij} and μ_{bij}. In order to reduce the computation time for the estimation, only the parameters σ and ρ are estimated by non-linear optimization. With these parameters given, the remaining model parameters $\boldsymbol{\theta}$ are found by the ML optimization as

$$\hat{\boldsymbol{\theta}} = \left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\mathbf{X}\right)^{-1}\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\mathbf{Y}, \tag{16}$$

where $\boldsymbol{\Sigma}$ equals one of the expressions (13), (14) or (15). The resulting parameter estimation is equivalent to estimating all parameters simultaneously.

The variance of the estimated parameters and the correlation between the estimated parameters can be calculated from the inverse Hessian, where the Hessian matrix $\boldsymbol{H}$ is equal to the second order partial derivative of the log-likelihood function, $\ell = \log(L(\boldsymbol{\theta}|\mathbf{Y}))$ Eq. (9). Derivation of the Hessian matrix is found in Appendix A.

The models, the log-likelihood function and the algorithm for calculating the Hessian matrix are implemented in Matlab 7.3.0 (R2006b). The Matlab command fminsearch is used to determine the maximum of the log-likelihood function.

2.4. Likelihood-ratio test

The likelihood-ratio test is used for an inference study concerning the nested models (3) and (5). The hypothesis is that the specific growth rate for each bacteria population is identical, and it can thus be described by Model 0. This hypothesis is biological plausible if the possibility of mutations is low within the considered interval. The test statistic is

$$-2\log\left(\frac{L_0}{L_1}\right) = -2(\ell_0 - \ell_1), \tag{17}$$

which is asymptotically χ^2 distributed with degrees of freedom corresponding to the difference in number of parameters between the

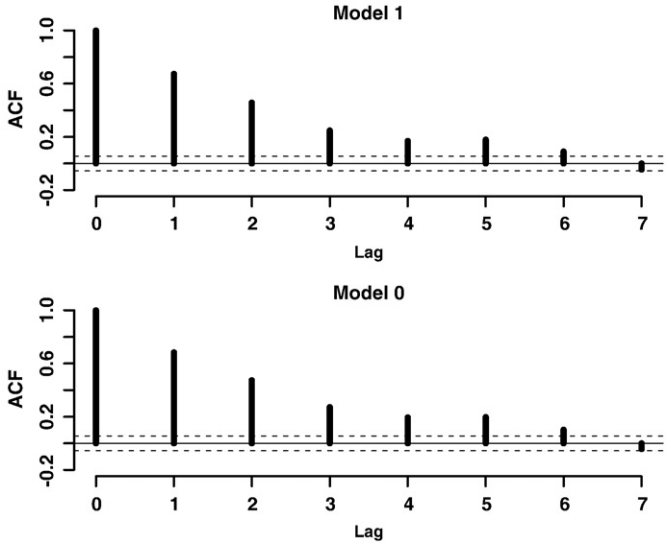

Fig. 6. Autocorrelation function for Model 0 and Model 1 with the full $\boldsymbol{\Sigma}$ matrix.

Table 2
Estimates of the specific growth rate μ and doubling time T_d

	Growth rate [1/h] (SD)			T_d [min]
Σ form	I	II	III	III
PAO1	1.77 (0.032)	1.653 (0.023)	1.566 (0.045)	26.56
mutT	1.57 (0.032)	1.524 (0.023)	1.517 (0.039)	27.42
mutY	1.64 (0.032)	1.534 (0.028)	1.528 (0.049)	27.22
mutM	1.74 (0.032)	1.644 (0.025)	1.560 (0.047)	26.66
mutYM	1.74 (0.032)	1.657 (0.030)	1.621 (0.053)	25.66
All	1.69 (0.015)	1.600 (0.012)	1.546 (0.026)	26.90

two models tested. Here ℓ_0 is the log-likelihood value for Model 0, and ℓ_1 is the log-likelihood value for Model 1— both evaluated at their optimal value.

3. Results and discussion

The model parameters have successfully been estimated for Model 1 Eq. (3) and Model 0 Eq. (5) for each of the three proposed covariance matrices I Eq. (13), II Eq. (14) and III Eq. (15). Residuals from the estimation have been examined in Fig. 5 where the residuals are plotted as a function of index. In the first case, where no variance structure is introduced in the model, an unclear residual structure is seen. For the covariance matrices II and III, the variance of the residuals follows the estimated squared structure, which indicates that the variance has been implemented correctly. The correlation structure is examined by plotting the autocorrelation of ε for the full covariance structure in Fig. 6. An exponentially decaying correlation is observed, as initially assumed. Thus, there seems to be no unexplained variance or correlation structure when applying the suggested full covariance matrix.

The growth rates for each bacteria type estimated by Model 1 and Model 0, are listed in Table 2, for each of the three suggested covariance matrices I, II and III. The growth rate is generally estimated highest when $\mathbf{\Sigma}_I$ is used, and lowest when the full covariance matrix is applied. The difference in the estimated specific growth rates shows that it is very important to use the correct covariance matrix in order to obtain a correct estimation for the growth rates. In many studies the doubling time T_d is used instead of the growth rate. The doubling time and growth rate are related by $T_d = \log(2)/\mu$. The doubling time for the full model is given in Table 2 to assist comparison with other microbiological studies. It should be noted that the specific growth rate might be smaller than the maximum specific growth rate, as explained in the introduction. It would therefore be of interest for a future study to repeat the estimation with a CFU count experiment, in order to examine the difference between the two experimental methods.

The log-likelihood values for each model are given in Table 4. The models with $\mathbf{\Sigma}_{II}$ and $\mathbf{\Sigma}_{III}$ can be compared using the likelihood-ratio test, as these models are nested. Doing this for Model 1 gives a test statistic of 148.62, which using a χ^2 distribution with one degree of freedom gives a P value close to 0. The same result is obtained by Model 0. This means that $\mathbf{\Sigma}_{III}$ should be used instead of $\mathbf{\Sigma}_{II}$ and indicates further that the full covariance matrix is preferable to the identity matrix $\mathbf{\Sigma}_I$. This conclusion is further emphasized by the estimated value for ρ shown in Table 3, which clearly shows that there is autocorrelation in the data, and this should therefore be included in the model to give a correct estimate for μ. The same table shows the estimates for σ. As expected, the variance is higher when the identity matrix $\mathbf{\Sigma}_I$ is used, than when the increased variation for smaller B values is accounted for.

Table 3
Estimates of the standard deviation and correlation parameters σ and ρ

	Σ form	Model 1	Model 0
σ (SD)	I	0.0685 (0.0027)	0.0715 (0.0028)
	II	0.0026 (0.0001)	0.0027 (0.0001)
	III	0.0029 (0.0003)	0.0031 (0.0003)
ρ (SD)	III	0.7360 (0.0553)	0.7656 (0.0550)

Table 4
The results of the inference analysis

Σ form	$\log(L_1)$	$\log(L_0)$	P
I	719.69	705.74	$1.30 \cdot 10^{-5}$
II	799.94	786.18	$1.56 \cdot 10^{-5}$
III	874.25	872.89	0.607

The correlations between the estimated parameters are obtained from the inverse Hessian matrix. If the time series are not translated as described in Section 2 a very high correlation on up to 0.999 is seen between the intercept and the related maximal growth rate. By translating the time series, this correlation is reduced to 0.665 for Model 1 and 0.468 for Model 0, which is why the translated time series are used for the estimation. For the translated data none of the correlations of the estimated parameters are critical. The highest correlation is found to be between σ and ρ (0.901 for Model 1 and 0.922 for Model 0).

The results of the inference analysis for the two models are summarized in Table 4. The result is very dependent on the choice of $\mathbf{\Sigma}$. For $\mathbf{\Sigma}_I$ and $\mathbf{\Sigma}_{II}$ the specific growth rate for the bacteria strains cannot be regarded as the same. However, we have argued that the full covariance matrix $\mathbf{\Sigma}_{III}$ must be used for the model to describe the total variance and correlation structure of the data. For the full covariance matrix, it can be concluded from the inference study that the specific growth rate is the same for all bacteria strains.

The different results for the three different $\mathbf{\Sigma}$ highlight the importance of including the correct covariance structure in the model. In this connection the ML estimation is preferable to least squares estimation, as the parameters for $\mathbf{\Sigma}$ can be estimated along with the other model parameters.

The model examined in this study is a linear model, but it can easily be replaced by a non-linear model (Madsen and Thyregod, 1988; Madsen, 2008). The disadvantage of introducing a non-linear model is that this significantly increases the complexity of the Hessian matrix, so that it might not be possible to calculate it analytically. However many computational tools are available for calculating the Hessian matrix numerically. Therefore, a continuation of this study could be to introduce a non-linear model which can describe the entire growth process. This would require the use of a substrate-dependent growth function as the growth enters the stationary phase due to substrate depletion.

4. Conclusions

The importance of including full variance and correlation structure in a model for bacterial growth has been shown. The estimation of model parameters is dependent on the parametrization of the covariance matrix, and disregarding the variance and correlation structure can therefore have consequences for the results of a study.

In this study the objective was to estimate the specific growth rate for PAO1 and four mutator strains. An explorative analysis of the OD measurements showed a strong correlation in time. The correlation was successfully described by an exponentially decaying function of the time between observations. Additionally, a variance structure for the log-transformed observations was implemented. A ML approach to estimating the model parameters is used. As an example of the strong statistical tools available with the ML method, we use the likelihood-ratio test to determine whether the growth rates of the five

bacteria strains can be assumed to be identical. From the test it can be concluded that the specific growth rate is indeed the same.

Acknowledgements

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Appendix A. Derivation of the Hessian matrix

For the full model containing the covariance matrix (15) the Hessian is

$$\boldsymbol{H} = \begin{bmatrix} \ell_{\sigma,\sigma} & \ell_{\sigma,\rho} & \ell_{\sigma,\theta} \\ \ell_{\rho,\sigma} & \ell_{\rho,\rho} & \ell_{\rho,\theta} \\ \ell_{\theta,\sigma} & \ell_{\theta,\rho} & \ell_{\theta,\theta} \end{bmatrix}, \tag{A.1}$$

where $\ell_{p1,p2}$ is the second order derivative of ℓ with respect to the parameters p_1 and p_2. Before continuing some short-hand notation is introduced

$$g(\rho) = \left(\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\theta}}\right)^T \boldsymbol{\Sigma}(\rho)^{-1} \left(\mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\theta}}\right), \tag{A.2}$$

and

$$\epsilon = \mathbf{Y} - \mathbf{X}\hat{\boldsymbol{\theta}} \tag{A.3}$$

The first order partial derivatives for ℓ are

$$\ell_\theta = \frac{1}{\sigma^2}\left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\epsilon\right) \tag{A.4}$$

$$\ell_\sigma = -\frac{N}{\sigma} + \frac{1}{\sigma^3} g(\rho) \tag{A.5}$$

$$\ell_\rho = -\frac{1}{2} Tr\left[\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho + \frac{1}{2\sigma^2}\left(\epsilon^T\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1}\epsilon\right)\right] \tag{A.6}$$

where

$$\boldsymbol{\Sigma}_\rho(jk) = \frac{\partial \boldsymbol{\Sigma}_{jk}^{bi}}{\partial\rho} = |j-k| \frac{\rho^{|j-k|-1}}{B_{bij}B_{bik}} \tag{A.7}$$

The second order partial derivatives of ℓ are

$$\ell_{\theta,\theta} = -\frac{1}{\sigma^2}\left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\mathbf{X}\right) \tag{A.8}$$

$$\ell_{\sigma,\sigma} = \frac{N}{\sigma^2} - \frac{3}{\sigma^4} g(\rho) \tag{A.9}$$

$$\ell_{\rho,\rho} = -\frac{1}{2} Tr\left[-\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho + \boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_{\rho,\rho}\right] + \frac{1}{2\sigma^2}\left[\epsilon^T\left[-2\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1} + \boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_{\rho,\rho}\boldsymbol{\Sigma}^{-1}\right]\epsilon\right] \tag{A.10}$$

$$\ell_{\theta,\theta} = -\frac{1}{\sigma^2}\left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\mathbf{X}\right) \tag{A.11}$$

$$\ell_{\sigma,\rho} = -\frac{1}{\sigma^3}\left[\epsilon^T\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1}\epsilon\right] \tag{A.12}$$

$$\ell_{\theta,\sigma} = -\frac{2}{\sigma^3}\left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\epsilon\right) \tag{A.13}$$

$$\ell_{\theta,\rho} = -\frac{1}{\sigma^2}\left(\mathbf{X}^T\boldsymbol{\Sigma}^{-1}\boldsymbol{\Sigma}_\rho\boldsymbol{\Sigma}^{-1}\epsilon\right) \tag{A.14}$$

where

$$\boldsymbol{\Sigma}_{\rho,\rho}(jk) = \frac{\partial^2 \boldsymbol{\Sigma}_{jk}^{bi}}{\partial\rho^2} = |j-k|(|j-k|-1)\frac{\rho^{|j-k|-2}}{B_{bij}B_{bik}} \tag{A.15}$$

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