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Modelling conjugation with stochastic differential equations

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Abstract

Conjugation is an important mechanism involved in the transfer of resistance between bacteria. In this article a stochastic differential equation based model consisting of a continuous time state equation and a discrete time measurement equation is introduced to model growth and conjugation of two *Enterococcus faecium* strains in a rich exhaustible media. The model contains a new expression for a substrate dependent conjugation rate. A Maximum Likelihood based method is used to estimate the model parameters. Different models including different noise structure for the system and observations are compared using a likelihood-ratio test and Akaike's information criterion. Experiments indicating conjugation on the agar plates selecting for transconjugants motivates the introduction of an extended model, for which conjugation on the agar plate is described in the measurement equation. This model is compared to the model without plate conjugation. The modelling approach described in this article can be applied generally when modelling dynamical systems.

24 *Key words:* SDE, horizontal gene transfer, resistance, CTSM, *E. faecium*,
25 mathematical modelling, Hidden Markov Model, likelihood-ratio test, AIC

26 **1. Introduction**

27 Development and spread of antimicrobial resistance in bacterial popu-
28 lations is of increasing concern, as it can lead to major difficulties for the
29 treatment of diseases. A first step in the direction of solving this prob-
30 lem is to gain a better understanding of the spread of resistance, and here
31 conjugation plays a major role. Conjugation is one of several mechanisms
32 of horizontal gene transfer by which plasmids coding for e.g. antimicrobial
33 resistance can be transferred between bacteria. The use of mathematical
34 modelling to describe the dynamics of plasmid spread and persistence was
35 first introduced by Levin et al. (1979) and since then many studies have
36 been made to improve the model framework and parameter estimation and
37 to incorporate more accurate expressions for the plasmid dynamics (Slater
38 et al., 2008). Most studies have used ordinary differential equations, ODE
39 (Levin et al., 1979; Freter et al., 1983; Clewlow et al., 1990; MacDonald et al.,
40 1992; Willms et al., 2006), or ordinary difference equations (Knudsen et al.,
41 1988; Sudarshana and Knudsen, 2006) as the modelling framework. This
42 approach can be discussed since a part of the complexity of conjugation,
43 e.g. dependence on the surrounding environment, is not included in these
44 models. A way of overcoming this problem is to use a stochastic modelling
45 approach where the randomness accounts for those processes not included in

46 the model. Some efforts have been made to include randomness in plasmid
47 models (Joyce et al., 2005; De Gelder et al., 2007; Ponciano et al., 2007).
48 Common for these studies is that they use discrete-time models, which is
49 a good approximation at low bacteria densities, but not at higher densities
50 where growth and conjugation is a continuous process. Joyce et al. (2005)
51 gives a first and second order moment representation of their model which
52 requires a derivation of the means and variances. Thus, it is not easy to
53 transfer the method to other models. In the study by Ponciano et al. (2007)
54 one of the model parameters is implemented as a normal distributed random
55 variable. This paper presents a generic modelling framework first described
56 by Kristensen et al. (2004a), which is based on stochastic differential equa-
57 tions, SDE. The SDE is connected to data through a state-space formulation
58 consisting of continuous-time state equations (the SDE) and discrete-time
59 observation equations. The applied state-space approach is in fact a con-
60 tinuous time Hidden Markov Model. The state-space formulation opens up
61 for strong statistical tools for estimating model parameters and for inference
62 concerning the best model (Kristensen et al., 2004a). State-space modelling
63 has also been used in previous plasmid studies (De Gelder et al., 2004, 2007;
64 Ponciano et al., 2007), but our approach differs by enabling continuous-time
65 state equations in combination with discrete-time observations. The state-
66 space model enables a simultaneous estimation of the growth and conjugation
67 parameters, whereby the bacterial growth is accounted for when estimating
68 the conjugation rate. This is an improvement from previous studies, where

69 the donor and recipient concentrations were introduced as a mean value of
70 two measurements taken over time (Knudsen et al., 1988; Sudarshana and
71 Knudsen, 2006) or the experiment was constructed such that bacterial growth
72 could be neglected (MacDonald et al., 1992).

73 In this article the SDE based state-space modelling framework is applied
74 to analyze data from an *in vitro* experiment for conjugation between two
75 *Enterococcus faecium* species growing in a batch exhaustible media. This
76 experiment was made in order to study the transferability of vancomycin
77 resistance in *E. faecium*. To describe the experimental system a conjugation
78 model of bacteria growing in a broth exhaustible media is introduced, which
79 is an expansion of previous models. Several authors (Levin and Stewart,
80 1980; Freter et al., 1983; Knudsen et al., 1988; Clewlow et al., 1990; Top
81 et al., 1992; Willms et al., 2006; Sudarshana and Knudsen, 2006) have mod-
82 elled conjugation events with the mass action model proposed by Levin et al.
83 (1979). The mass action model states that the appearance of transconjugants
84 is proportional to the product of the donor and recipient concentrations. We
85 will introduce a new expression for the conjugation rate in an exhaustible
86 media for which the proportionality constant of the mass action model is
87 substrate dependent. An inference study is made to reduce the SDE model
88 to its minimum form. A further extension of the model is made to treat ad-
89 equately a methodological problem: the finding that conjugation continues
90 on the agar plates selecting for transconjugants. In that case the observed
91 transconjugants are a combination of transconjugants stemming from the

92 conjugation process in the flask and conjugation occurring on the selective
93 plates. The models with and without conjugation on the selective plates are
94 compared in order to examine which model best describes data.

95 2. Experimental methods

96 A conjugation experiment was made in order to study the transferabil-
97 ity of vancomycin resistance in *Enterococcus faecium*. As recipient the *E.*
98 *faecium* reference strain BM4105RF was used. This strain is resistant to
99 rifampicin (MIC > 25 $\mu\text{g}/\text{ml}$) and fusidic acid (MIC > 25 $\mu\text{g}/\text{ml}$) due to
100 chromosomally located mutations. As donor, the *E. faecium* A17sv1 (Has-
101 man and Aarestrup, 2002) was used. This strain is resistant to erythromycin
102 (MIC > 16 $\mu\text{g}/\text{ml}$) and vancomycin (MIC > 32 $\mu\text{g}/\text{ml}$) due to the presence of
103 the *erm*(B) gene and *Tn*1546 transposon (carrying the *vanA*-gene cluster),
104 respectively, located on a conjugative plasmid.

105 The conjugation experiment was performed in liquid Brain-Heart Infusion
106 (BHI) media (Oxoid). Bacterial counting was performed on BHI agar plates
107 supplemented with the following antibiotics when appropriate: rifampicin 25
108 $\mu\text{g}/\text{ml}$, fusidic acid (25 $\mu\text{g}/\text{ml}$), erythromycin (16 $\mu\text{g}/\text{ml}$) and vancomycin
109 (32 $\mu\text{g}/\text{ml}$).

110 2.1. Conjugation experiment

111 Over night blood agar cultures of the two strains grown at 37°C were
112 inoculated in BHI media supplemented with the appropriate antibiotics as

113 described above. From these, 100 μl of each culture was transferred to fresh
114 tubes containing 10 ml preheated BHI media without antibiotics supple-
115 mentation. When these cultures reached late exponential growth (OD_{500} of
116 0.3-0.5), the number of cells in each culture was adjusted to the same amount
117 and 1.5 ml of each was transferred to 100 ml preheated (37°C) BHI media
118 in an 250 ml Erlenmeyer flask. The flask was placed in a shaking incubator
119 (125 rpm) at 37°C . Four 1 ml samples were taken immediately after the cells
120 were added ($t=0$) as well as 1.50, 2.80, 3.00, 3.40, 3.80, 4.00, 4.40, 4.80, 5.00,
121 5.50, 6.00, 6.30 and 7.00 hours after the cells were mixed. One of the four
122 1 ml samples was used to measure the OD_{500} of the culture. The remaining
123 three samples were diluted ten-fold until 10^{-7} . From here, 100 μl of each
124 dilution (where appropriate) was plated onto sets of plates containing BHI
125 agar supplemented with either of the following: 1) Recipient plates contain-
126 ing 25 $\mu\text{g}/\text{ml}$ rifampicin + 25 $\mu\text{g}/\text{ml}$ fusidic acid, 2) Donor plates contain-
127 ing 16 $\mu\text{g}/\text{ml}$ erythromycin + 32 $\mu\text{g}/\text{ml}$ vancomycin, and 3) transconjugant
128 plates containing 25 $\mu\text{g}/\text{ml}$ rifampicin + 25 $\mu\text{g}/\text{ml}$ fusidic acid +16 $\mu\text{g}/\text{ml}$
129 erythromycin + 32 $\mu\text{g}/\text{ml}$ vancomycin. All plates were incubated 48 hours
130 and the colony forming units, CFU, were counted. From these numbers the
131 bacterial concentration in the flask was calculated.

132 2.2. Calculating bacterial concentration

133 The concentrations of the different bacteria populations in the flask can
134 be determined from the plate count by a generalized linear model approach,

135 assuming that the CFU count for each plate is Poisson distributed with an
 136 offset corresponding to the given dilution. If, for any given observation time
 137 t_k , N_{jk} is the count number j with dilution n_j , then the expected CFU count
 138 $E(N_{jk})$ can be modelled with the generalized linear model

$$E(N_{jk}) = \lambda_{jk} = n_j \exp(\beta_k), \quad N_{jk} \sim \text{Poisson}(\lambda_{jk}) \quad (1)$$

$$\log(\lambda_{jk}) = \log(n_j) + \beta_k. \quad (2)$$

139 Fitting the model to data gives an estimate for the coefficient β_k and hereby
 140 an estimate of the bacterial concentration $Y_k = \exp(\beta_k)$ in the flask at time,
 141 t_k . The variance $\sigma_{Y,k}^2$ of the estimated concentration at time t_k can be deter-
 142 mined from the variance of β as

$$\sigma_{Y,k}^2 = \text{Var}[\exp(\beta_k)] = (\exp(\beta_k) \text{Var}[\beta])^2. \quad (3)$$

143 The concentrations Y_k calculated in this way are the observations to be used
 144 for the modelling procedure described in the remainder of this article. The
 145 command `glmfit` in `Matlab` is used for fitting the generalized linear model
 146 (see Appendix A).

147 3. Model formulation

148 The modelling framework used in this study is a continuous-discrete time
 149 state-space model consisting of a continuous time state equation (the SDE)
 150 and a discrete time observation equation. The model has the general form

151 (Kristensen et al., 2004b)

$$d\mathbf{X}_t = \mathbf{f}(\mathbf{X}_t, \mathbf{u}_t, t, \boldsymbol{\theta})dt + \boldsymbol{\sigma}(\mathbf{u}_t, t, \boldsymbol{\theta})d\boldsymbol{\omega}_t, \quad (4)$$

$$\mathbf{Y}_k = \mathbf{h}(\mathbf{X}_k, \mathbf{u}_k, t_k, \boldsymbol{\theta}) + \mathbf{e}_k, \quad (5)$$

152 where (4) is the SDE and (5) is the observation equation. \mathbf{X}_t is a n -
 153 dimensional vector of state variables and \mathbf{Y}_k is a l -dimensional vector of
 154 observations. The observations are obtained at discrete times t_k with the
 155 observation noise being $\mathbf{e}_k \in N(\mathbf{0}, \boldsymbol{\Sigma}_k)$. $\boldsymbol{\theta}$ is a vector of unknown parameters
 156 and \mathbf{u} is a vector of input variables, i.e. variables which can be observed and
 157 have an influence on the system dynamics. The functions \mathbf{f} and \mathbf{h} can be
 158 linear as well as nonlinear functions. $\{\boldsymbol{\omega}_t\}$ is a standard Wiener process rep-
 159 resenting sources of noise in the system. The first part on the right-hand side
 160 of the SDE (4) is called the drift term and the second part is called the dif-
 161 fusion term. The stochastic state-space model (4)-(5) has several advantages
 162 compared to deterministic models. For instance, the state-space approach
 163 separates the residual noise into system noise and observation noise, where
 164 the system noise is used to e.g. compensate for those biological processes
 165 not explicitly described by the model. The deterministic model often leads
 166 to autocorrelated residuals, which is not a problem with the SDE. Using the
 167 SDE based model also paves the way for strong statistical tools to estimate
 168 model parameters and make inferences.

169 3.1. The drift term - Model for conjugation

170 In this study we apply the state-space modelling framework on a model
 171 for conjugation. The first step of the modelling procedure is to formulate
 172 the drift part of the SDE based on microbiological knowledge of the sys-
 173 tem. A sketch of the conjugation dynamics is given by the flow diagram
 174 in Figure 1, where D is the donor, R the recipient, and T the transconju-
 gant concentrations. The flow diagram shows how recipients can be turned

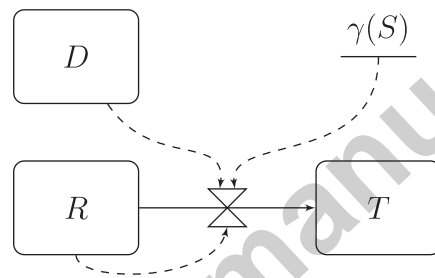


Figure 1: Flow diagram showing the structure of the conjugation models. The donor D can by encounter of a recipient R transfer a plasmid coding for resistance to the recipient. The recipient hereby is turned into a transconjugant T , which expresses the same antibiotic resistances as both the donor and the recipient.

175

176 into transconjugants with a rate dependent on the concentration of donor
 177 and recipient. In earlier studies (MacDonald et al., 1992; Knudsen et al.,
 178 1988; Sudarshana and Knudsen, 2006) the substrate concentration and/or
 179 donor and recipient concentrations were kept constant in the experiments
 180 and were thus held constant in the model. In order to better approximate
 181 *in vivo* situations, these constraints were not applied to our *E. faecium* con-
 182 jugation experiment, and thus all three populations as well as the substrate
 183 content must be included as state variables in the model. The drift term

184 corresponding to the model shown in Figure 1 is

$$\mathbf{f}(\mathbf{X}_t, \boldsymbol{\theta}) = \begin{bmatrix} \mu_D(S)D \\ \mu_R(S)R - \gamma(S)DR \\ \mu_T(S)T + \gamma(S)DR \\ -\eta(\mu_D(S)D + \mu_R(S)R + \mu_T(S)T) \end{bmatrix} \quad (6)$$

185 where $\mu_i(S)$ is the growth rate for the bacteria population i (D , R or T), and
 186 $\gamma(S)$ is the conjugation rate. $\mathbf{X} = [D, R, T, S]$ are the model state variables
 187 and $\boldsymbol{\theta} = [v_D, v_R, v_T, \kappa_D, \kappa_R, \kappa_T, \gamma_{\max}, \kappa_c, \eta]$ are the parameters for the model.
 188 The substrate, S , is simulated as a normalized variable with an initial value
 189 of 1. The amount of substrate in the solution decreases as it is utilized for
 190 bacterial growth. The parameter η is the amount of normalized substrate
 191 used for each cell division.
 192 Bacterial growth continues until the substrate is exhausted, and the station-
 193 ary state has been reached. The growth rate can be modelled with the well
 194 known Monod relation (Monod, 1949)

$$\mu_i(S) = \frac{v_i S}{\kappa_i + S}, S \in [0, 1], \quad (7)$$

195 where S is the substrate concentration, v_i is the maximum growth rate and
 196 κ_i is the substrate concentration when the growth rate is half of its maximum
 197 value. Both parameters are specific for a given bacteria population i .
 198 The conjugation event depends on the probability of an encounter between

199 donor and recipient. In the original mass action model the conjugation rate
200 γ was introduced as a constant parameter. Several authors (Knudsen et al.,
201 1988; Andrup et al., 1998; Andrup and Andersen, 1999; Ponciano et al., 2007)
202 have discussed this assumption. Levin et al. (1979) showed that the mass
203 action model presents a good estimation of the transconjugant population
204 during exponential growth and under chemostatic conditions, but the model
205 fails to describe the occurrences of transconjugants during the lag phase
206 and at the onset of stationary phase. MacDonald et al. (1992) suggested
207 that the conjugation rate depends on the substrate content and stated that
208 conjugation can not occur without the presence of nutrition. However, to
209 our knowledge this substrate dependence has not before been implemented
210 in a mathematical model for conjugation. We suggest a nonlinear substrate
211 dependent expression to model the conjugation rate

$$\gamma(S) = \frac{\gamma_{\max} S}{\kappa_c + S}, S \in [0, 1]. \quad (8)$$

212 This expression is similar to the Michaelis-Menten equation for enzyme ki-
213 netics and to the Monod relation. This expression is chosen as it forces the
214 conjugation rate to reach a maximum value γ_{\max} when the substrate con-
215 centration is abundant, and it turns to zero as the substrate is depleted.
216 Depending on the value of κ_c the conjugation rate will decrease concurrent
217 with or after the decrease in the bacterial growth rate.
218 Some assumptions are applied to keep the conjugation model simple. (i)

219 Transconjugants can function as donors transferring a plasmid to a recipient,
220 but this is not described by the model. It is assumed reasonable to omit it for
221 the *E. faecium* data set used in this study, as the concentration of transcon-
222 jugants is very low compared to the donor concentration, and therefore does
223 not contribute significantly to conjugation. (ii) The delay on 10-15 min that
224 has been found (Andrup et al., 1998) between two conjugation events for
225 the same donor is assumed to be insignificant. During the experiment there
226 is always a large number of donors not involved in a conjugation event and
227 thus ready to start the conjugation by encounter of a recipient. Therefore
228 the delay is disregarded. (iii) It is assumed that the maximum growth rate
229 of the transconjugants is either the same as the maximum growth rate for the
230 recipient or smaller due to a fitness cost of the plasmid, i.e. $v_T = v_R(1 - \alpha)$,
231 where $\alpha \in [0, 1[$. An inference study will be made to test if $\alpha = 0$.

232 3.2. The diffusion term

233 Depending on the system which is modelled it can be adequate to im-
234 plement additive system noise, i.e. noise independent on the state variables,
235 or multiplicative system noise, i.e. where the noise depends on the state
236 variables. The choice of the system noise depends on assumption about the
237 system modelled. Tier and Floyd (1981) have described how different as-
238 sumption of a biological process can lead to either demographic stochasticity
239 (where the variance is proportional to the state variable) or environmen-
240 tal stochasticity (where the variance is proportional to the state variable

241 squared). In our system the noise is implemented as multiplicative (environ-
 242 mental) noise, as the random fluctuations affect the whole population and
 243 not only the growth process. For instance the mass action model has shown
 244 to be good during exponential growth but not during lag-phase and station-
 245 ary phase. Therefore multiplicative noise can be implemented to account
 246 for those processes not well described by the model. The method used for
 247 evaluating the likelihood function (Kristensen et al., 2004b) requires that the
 248 diffusion term is independent of the state variables. Therefore, instead of the
 249 state variables D , R and T , the input vectors \mathbf{u}_D , \mathbf{u}_R and \mathbf{u}_T , which contains
 250 observations for the three states, are inserted as a scaling for the standard
 251 deviation. The noise term is

$$\sigma_c^m = \begin{bmatrix} \sigma_D \mathbf{u}_D & 0 & 0 & 0 \\ 0 & \sigma_R \mathbf{u}_R & 0 & 0 \\ 0 & 0 & \sigma_T \mathbf{u}_T & 0 \\ -\eta \sigma_D \mathbf{u}_D & -\eta \sigma_R \mathbf{u}_R & -\eta \sigma_T \mathbf{u}_T & \sigma_S \end{bmatrix}, \quad (9)$$

252 where an increment in donor, recipient or transconjugant concentration lower
 253 the substrate content in order to keep the mass balance, and σ_S is introduced
 254 in order to ensure stability and is fixed to a small value.

255 An additive noise term σ_c^a is also introduced to compare with the multiplica-
 256 tive noise. This is done, as it is not sure that the data set is sufficient to
 257 make a good prediction of the system noise, and therefore a simplification of

258 the noise structure can be an advantage. The additive noise term is

$$\boldsymbol{\sigma}_c^a = \begin{bmatrix} \sigma_D & 0 & 0 & 0 \\ 0 & \sigma_R & 0 & 0 \\ 0 & 0 & \sigma_T & 0 \\ -\eta\sigma_D & -\eta\sigma_R & -\eta\sigma_T & \sigma_S \end{bmatrix}. \quad (10)$$

259 3.3. Observation equation

260 The observation equation relates observations to the state variables. In
 261 that way the state variables do not need to be measured directly and it
 262 is not necessary to have observations related to all state variables. In this
 263 study three of the state variables (donor, recipient and transconjugant) are
 264 observed. The concentration for the donor, recipient and transconjugant has
 265 been calculated from the CFU count as described in Section 2.2. We will
 266 refer to this as the observed concentration. The observation equation, when
 267 only conjugation in the broth media is considered, is

$$\begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \end{bmatrix}_k = \begin{bmatrix} D \\ R \\ T \end{bmatrix}_k + \mathbf{e}_k, \quad \mathbf{e}_k \in N(\mathbf{0}, \boldsymbol{\Sigma}_k). \quad (11)$$

268 The model with this observation equation is called the **Broth model**.

269 In addition to plasmid transfer in the broth, we have found that conjugation
 270 can occur on the transconjugant plates. This is a surprising result as antibi-

271 otics on the agar plate are traditionally considered to stop the conjugation
 272 process. However we have performed several experiments (data not shown),
 273 which have revealed that conjugation does also occur on the transconjugant
 274 plates. As the experiment is made to measure conjugation in the broth me-
 275 dia we wish to separate conjugation on the plate from that in the flask. The
 276 following set of ODE's is suggested to model conjugation on the plate for
 277 each observation time k

$$\frac{dD_{pk}}{dt} = -\lambda D_{pk} , \quad (12)$$

$$\frac{dR_{pk}}{dt} = -\lambda R_{pk} - \gamma_p D_{pk} R_{pk} , \quad (13)$$

$$\frac{dT_{pk}}{dt} = \gamma_p D_{pk} R_{pk} . \quad (14)$$

278 The initial values for the concentration of donor, D_{pk} , and recipient, R_{pk} ,
 279 on the plate correspond to the concentrations in the flask at the time for
 280 the observation. Due to antibiotics on the plate, the donors and recipients
 281 die with a death rate λ and even though substrate is present in abundant
 282 amounts it is assumed that the donor and recipient bacteria can not grow.
 283 The recipients which receives a plasmid on the agar plate (and thus become
 284 a transconjugants) become resistant to all the antibiotics present and can
 285 therefore grow and form colonies on the plate. The plate conjugation rate γ_p
 286 is assumed to be independent off the substrate content as the substrate will
 287 not be depleted before the conjugation process stops.
 288 The plate conjugation is modelled by finding the analytical solution for the

289 ODE and inserting the solution in the observation equation for transconju-
 290 gants Eq. (11). As the number of recipients receiving a plasmid on the plate
 291 is small, the last term in Eq. (13) can be disregarded. The equation to be
 292 solved is thus

$$\frac{dT_{pk}}{dt} = \gamma_p D_{pk}(t) R_{pk}(t) , \quad (15)$$

293 where

$$D_{pk}(t) = D_{k0} \exp(-\lambda t) , \quad (16)$$

$$R_{pk}(t) = R_{k0} \exp(-\lambda t) . \quad (17)$$

294 The solution is

$$T_{pk} = \frac{\gamma_p}{2\lambda} D_{k0} R_{k0} (1 - \exp(-2\lambda t)) , \quad (18)$$

295 which for $t \rightarrow \infty$ is

$$T_{pk} = \frac{\gamma_p}{2\lambda} D_{k0} R_{k0} . \quad (19)$$

296 The limit for $t \rightarrow \infty$ can be considered since the CFU count is made after
 297 12-24 hours, whereafter conjugation on the plate is very unlikely to occur
 298 due to death of the donor and recipients. Estimating both γ_p and λ would
 299 be an over-parametrization of the model. Therefore γ_p/λ is replaced by the

300 parameter γ'_p . Inserting the result into Eq. (11) leads to the observation
 301 equation for the so-called **Broth-plate model**

$$\begin{bmatrix} Y_1 \\ Y_2 \\ Y_3 \end{bmatrix}_k = \begin{bmatrix} D \\ R \\ T + \frac{\gamma'_p}{2}DR \end{bmatrix}_k + \mathbf{e}_k, \mathbf{e}_k \in N(\mathbf{0}, \Sigma). \quad (20)$$

302 3.4. Observation noise

303 Three different covariance matrices for the observation equation are im-
 304 plemented and compared

305 Additive:

$$\Sigma_k^a = \begin{bmatrix} s_1^2 & 0 & 0 \\ 0 & s_2^2 & 0 \\ 0 & 0 & s_3^2 \end{bmatrix} \quad (21)$$

306 Proportional:

$$\Sigma_k^p = \begin{bmatrix} s_4^2 \sigma_{D,k}^2 & 0 & 0 \\ 0 & s_5^2 \sigma_{R,k}^2 & 0 \\ 0 & 0 & s_6^2 \sigma_{T,k}^2 \end{bmatrix} \quad (22)$$

307 Additive+Proportional:

$$\Sigma_k^{a,p} = \begin{bmatrix} s_1^2 + s_4^2 \sigma_{D,k}^2 & 0 & 0 \\ 0 & s_2^2 + s_5^2 \sigma_{R,k}^2 & 0 \\ 0 & 0 & s_3^2 + s_6^2 \sigma_{T,k}^2 \end{bmatrix} \quad (23)$$

308 Hence, the observations Y_1 , Y_2 and Y_3 are assumed to be uncorrelated in all
309 cases. The variances $\sigma_{R,k}^2$, $\sigma_{R,k}^2$ and $\sigma_{T,k}^2$ are estimates from Eq. (3) at time t_k .
310 The most simple noise form is the additive noise, but it is only reasonable, if
311 the variance of the observations is independent of the observations. If this is
312 not the case one alternative is to transform the data to stabilize the variance.
313 In this article instead a proportional noise term is suggested, which include
314 the estimated variance of the observations. Additionally a covariance matrix
315 is considered with both additive and proportional noise.

316 3.5. *Statistical methods*

317 The modelling procedure consist of several steps of parameter estimations
318 and goodness of fit statistics. First the Broth model and Broth-plate models
319 are reduced separately, i.e. the likelihood function is optimized for differ-
320 ent implementation of the system noise, observation noise and drift term.
321 The best fit for the Broth and Broth-plate models are found applying good-
322 ness of fit statistics based on a ML approach. The inference study is made
323 using a likelihood-ratio test and Akaike Information Criterion, AIC. After
324 reducing the Broth and Broth-plate models they are compared, again us-
325 ing a likelihood-ratio test and AIC. Following the parameter estimation the
326 models are simulated in `Matlab`, by implementing a numerical Euler method
327 as described by Higham (2001).

328 *3.5.1. Parameter estimation*

329 A ML estimation method is used to determine the SDE model parameters.
 330 The parameters are found as those maximizing the likelihood function

$$\mathcal{L}(\boldsymbol{\theta}; \mathcal{Y}_N) = \left(\prod_{k=1}^N p(\mathbf{Y}_k | \mathcal{Y}_{k-1}, \boldsymbol{\theta}) \right) p(\mathbf{Y}_0 | \boldsymbol{\theta}) \quad (24)$$

331 for the sequence of observations $\mathcal{Y}_N = [\mathbf{Y}_N, \mathbf{Y}_{N-1}, \dots, \mathbf{Y}_1, \mathbf{Y}_0]$. The con-
 332 ditional probability densities p are approximated by gaussian densities mo-
 333 tivated by the fact that the SDE (4) is driven by a Wiener process having
 334 gaussian increments, i.e.

$$p(\mathbf{Y}_k | \mathcal{Y}_{k-1}, \boldsymbol{\theta}) = \frac{\exp\left(-\frac{1}{2} \boldsymbol{\epsilon}_k^T (\boldsymbol{\Sigma}_{k|k-1}^{yy})^{-1} \boldsymbol{\epsilon}_k\right)}{\sqrt{\det\left(\boldsymbol{\Sigma}_{k|k-1}^{yy}\right)} (\sqrt{2\pi})^l} \quad (25)$$

335 where

$$\boldsymbol{\Sigma}_{k|k-1}^{yy} = \text{Var}[\mathbf{Y}_k | \mathcal{Y}_{k-1}, \boldsymbol{\theta}] , \quad (26)$$

$$\boldsymbol{\epsilon}_k = \mathbf{Y}_k - \hat{\mathbf{Y}}_{k|k-1} , \text{ and} \quad (27)$$

$$\hat{\mathbf{Y}}_{k|k-1} = \text{E}[\mathbf{Y} | \mathcal{Y}_{k-1}, \boldsymbol{\theta}] . \quad (28)$$

336 The conditional mean $\hat{\mathbf{Y}}_{k|k-1}$ and covariance $\boldsymbol{\Sigma}_{k|k-1}^{yy}$ in the likelihood function
 337 (24) and (25) can be estimated recursively by means of the Extended Kalman
 338 Filter (Kristensen et al., 2004b). In this study we use the software CTSM, from
 339 which the parameter estimation, correlation of the parameter estimates and
 340 the log-likelihood values are obtained. CTSM can be downloaded from the

341 webpage: <http://www2.imm.dtu.dk/~ctsm/>, from where a user manual is
 342 also available. CTSM is easy to use as it has a built in graphical user interface.
 343 Recently an implementation of the method has also been made in R (Klim
 344 et al., 2009).

345 3.5.2. Goodness of fit statistics

346 Nested models are compared with a likelihood ratio test statistics given
 347 by

$$-2 \log \Lambda = 2 (\ell(\boldsymbol{\theta}) - \ell(\boldsymbol{\theta}_0)) , \quad (29)$$

348 where the test statistics $-2 \log \Lambda$ is asymptotically χ^2 distributed with de-
 349 grees of freedom equal to the difference in dimensions between the two mod-
 350 els. $\ell(\boldsymbol{\theta}) = \log(\mathcal{L}(\boldsymbol{\theta}; \mathcal{Y}_N))$ and $\ell(\boldsymbol{\theta}_0) = \log(\mathcal{L}(\boldsymbol{\theta}_0; \mathcal{Y}_N))$ are the log-likelihood
 351 values of the model and the submodel, respectively. The inference study is
 352 also performed with AIC, which is given by

$$AIC = -2\ell(\boldsymbol{\theta}) + 2k , \quad (30)$$

353 where k is the number of parameters in the model and $\ell(\boldsymbol{\theta})$ is the log-
 354 likelihood value of the model. When comparing models the preferred model
 355 is the one with the lowest AIC value.

356 **4. Results and discussion**

357 The conjugation experiment continued for 7 h, during which samples were
 358 taken from the broth mixture approximately every 20 min and plated on se-
 359 lective agar plates. The CFUs were counted, and the bacterial concentrations
 360 in the broth were determined by a generalized linear model approach. The
 experimental results can be seen in Figure 2.

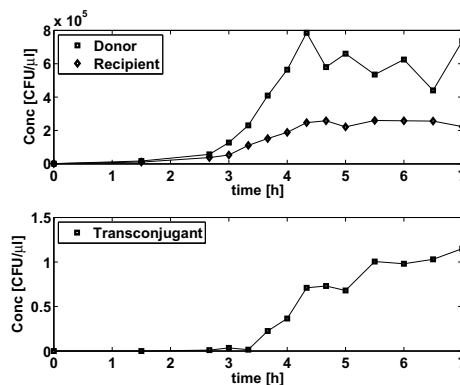


Figure 2: Data from the conjugation experiment with *E. faecium* growing in broth culture.

361

362 *4.1. Inference study*

363 Log-likelihood values for the Broth model and the Broth-plate model
 364 tested with respect to the state noise, the observation noise, and finally the
 365 drift term is summarized in Table 1. When estimating the model parameters
 366 only biological plausible parameter intervals are considered. The multiplica-
 367 tive system noise term (9) is tested against additive system noise (10), and
 368 it is found that there is no significant difference. We therefore continue the
 369 study for both the Broth and Broth-plate model with additive system noise.

Table 1: Log-likelihood values, p-values and AIC for different versions of the Broth model and Broth-plate model. The nested models listed below each other are tested with the likelihood-ratio test and the p-value for the comparison is listed in the line of the smallest of the two models tested. The proportional and additive observation noise structures are both tested against the proportional+additive noise term.

	Broth model			Broth-plate model		
	$\ell(\hat{\theta})$	p-value	AIC	$\ell(\hat{\theta})$	p-value	AIC
<u>System noise:</u>						
Multiplicative	-299.04	-	638.08	-298.05	-	634.09
Additive	-299.19	-	634.38	-298.05	-	634.09
Additive ($\sigma = \sigma_D = \sigma_R = \sigma_T$)	-299.19	0.9999	630.38	-298.05	0.9999	630.09
<u>Observation noise:</u>						
Proportional+additive	-299.19	-	630.38	-298.05	-	630.09
Proportional	-300.52	0.4467	627.04	-299.47	0.4162	626.94
Additive	-312.67	0.0006	651.34	-311.43	0.0007	650.86
<u>Drift term:</u>						
$\alpha = 0$	-300.52	0.9969	625.04	-299.47	0.9695	624.94
$\kappa_T = \kappa_R$	-300.59	0.7014	623.19	-299.53	0.7232	623.06

370 It would be interesting to investigate the system noise structure with a ML
371 estimation based on the particle filter (Ionides et al., 2006), for which the
372 multiplicative noise can be implemented directly dependent on the state vari-
373 able. We will leave this for a future study. The additive system noise is well
374 modelled with $\sigma = \sigma_D = \sigma_R = \sigma_T$ for both the Broth and Broth-plate mod-
375 els.
376 Both the proportional observation noise and the additive observation noise
377 can be tested against the proportional+additive observation noise using a
378 likelihood-ratio test. The model with additive observation noise is seen to
379 perform significantly worse than the proportional+additive noise model (p-
380 value = 0.0006 and p-value = 0.0007), whereas data is well modelled with

381 proportional observation noise (p-value = 0.4467 and p-value = 0.4162). All
382 the observation noise covariance matrices can be compared using AIC, from
383 which it is also found that data is best modelled with proportional obser-
384 vation noise. This result is as expected, as the variance of the observations
385 increases for higher CFU counts, which is best captured by proportional
386 noise.

387 With this noise structure the two hypothesis $\alpha = 0$ and $\kappa_T = \kappa_R$ are tested.
388 We fail to reject both hypothesis as the p-values are high (between 0.7014
389 and 0.9969).

390 The best description of data for each of the models is thus a model with ad-
391 ditive system noise where the standard deviation is the same for D , R and T .
392 The observation noise should be modelled as proportional noise and growth
393 for the transconjugants and recipients can be modelled with the same set of
394 parameters.

395 The reduced Broth and Broth-plate models can be compared with the likelihood-
396 ratio test, as they are nested models with the Broth-plate model containing
397 one additional parameter (γ'_p) compared to the Broth model. The test statis-
398 tics for this test is 2.12, which gives a p-value of 0.1451. This is not significant
399 on ordinary level and thus the smaller Broth model should be chosen. How-
400 ever, this conclusion is based on very few datapoints, and it might change if
401 more data was available.

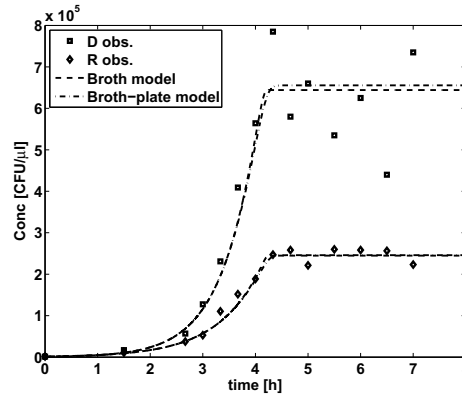


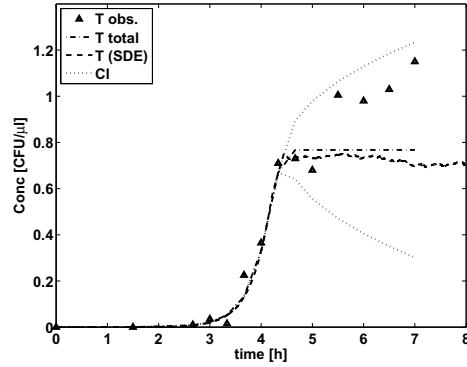
Figure 3: The donor and recipient concentration over time as simulated by the Broth and Broth-plate models plotted together with data values.

402 4.2. Parameters

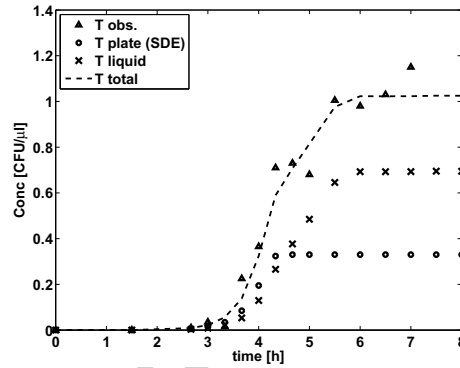
403 The result of the ML parameter estimation for the reduced Broth and
 404 Broth-plate models is shown in Table 2, and the results of simulations with
 405 these parameters are plotted in Figure 3 and 4.

406 The conjugation rate is estimated to $2.008 \cdot 10^{-12} (\text{CFU}/\mu\text{l})^{-1}\text{h}^{-1}$ for the
 407 Broth-plate model and $4.913 \cdot 10^{-12} (\text{CFU}/\mu\text{l})^{-1}\text{h}^{-1}$ for the Broth model.
 408 Thus, the choice of model influence the estimate of the conjugation rate.

409 The value of κ_c ($10^{-16} - 10^{-15}$) is low compared to the κ value for the growth
 410 of the bacteria ($10^{-3} - 10^{-2}$). This means that the mass action model γDR
 411 in this conjugation experiment gives a good description of conjugation until
 412 the substrate is depleted. As a consequence of the low κ_c value the model
 413 predicts conjugation to continue after the growth of the bacteria has reached
 414 stationary phase. By comparing the simulations of the donor and recipient
 415 concentrations Figure 3 with simulation of the transconjugant concentration



(a) Broth model



(b) Broth-plate model

Figure 4: The transconjugant concentration plotted together with simulations of the Broth and Broth-plate models. For the Broth model the mean of the simulated transconjugant concentration and its confidence interval, as estimated by CTSM, is shown. For each model one example of the SDE simulation made in `Matlab` is plotted.

416 Figure 4 we see that new transconjugants appear until approximately one
 417 hour after initiation of the stationary growth phase.

418 When calculating the bacterial concentration from the CFU count it was
 419 assumed that the data was Poisson distributed, and thus that the variance
 420 equals the mean. If this was indeed the case each of the parameters σ_4^2 , σ_5^2 ,
 421 and σ_6^2 should equal one. In this case σ_4^2 is around 14 and σ_5^2 and σ_6^2 are around

Table 2: The result of the ML parameter estimation for the Broth model and the Broth-plate model.

Parameter	Broth model (SD)		Broth-plate model (SD)	
v_D [h^{-1}]	1.678	(0.113)	1.787	(0.092)
$v_R = v_T$ [h^{-1}]	1.216	(0.028)	1.261	(0.035)
κ_D	0.052	(0.047)	0.110	(0.047)
$\kappa_R = \kappa_T$	0.0034	(0.0032)	0.0364	(0.0199)
η	$1.128 \cdot 10^{-6}$	$(0.079 \cdot 10^{-6})$	$1.112 \cdot 10^{-6}$	$(0.087 \cdot 10^{-6})$
κ_c	$4.201 \cdot 10^{-16}$	$(3.966 \cdot 10^{-16})$	$5.583 \cdot 10^{-15}$	$(8.811 \cdot 10^{-15})$
γ_{\max} [$(\text{CFU}/\mu\text{l})^{-1}\text{h}^{-1}$]	$4.913 \cdot 10^{-12}$	$(0.636 \cdot 10^{-12})$	$2.008 \cdot 10^{-12}$	$(0.699 \cdot 10^{-12})$
γ'_p [$(\text{CFU}/\mu\text{l})^{-1}$]	0	-	$4.096 \cdot 10^{-12}$	$(1.630 \cdot 10^{-12})$
σ	$4.010 \cdot 10^{-10}$	$(2.828 \cdot 10^{-10})$	$1.128 \cdot 10^{-11}$	$(2.488 \cdot 10^{-11})$
σ_4	13.815	(5.349)	14.862	(5.575)
σ_5	3.507	(1.212)	3.572	(1.304)
σ_6	3.150	(1.422)	3.184	(1.280)

3. This means that the data is over-dispersed, and this over-dispersion is ac-
counted for by σ_4^2 , σ_5^2 , and σ_6^2 . These parameters are in Poisson regression
also referred to as dispersion parameters. Several authors have found asym-
metric likelihood profiles (Dennis et al., 2006; Ionides et al., 2006; Ponciano
et al., 2007; King et al., 2008) in state-space models for dynamical biolog-
ical systems (e.g. bird growth, plasmid persistence and cholera pandemic).
In order to check the likelihood structure for our conjugation model, the
profile-likelihood is calculated for v_D and γ_{\max} . The profile likelihoods seen
in Figure 5 are calculated by optimizing the likelihood function for fixed val-
ues of the parameter of interest. The 95% confidence interval (CI) plotted
is the region of parameter values for which the profile log-likelihood value is
larger than $\ell(\boldsymbol{\theta})_{\max} - c/2$, where $\ell(\boldsymbol{\theta})_{\max}$ is the maximum log-likelihood value

434 and c is defined by $\text{Prob}[\chi^2(1) < c] = 0.95$. The profile likelihoods are indeed
435 asymmetric, which also in 3 out of 4 cases leads to asymmetric CI's. This is
436 as expected due to the small number of observations. Furthermore it is seen
437 that the profile likelihood CI is generally wider than the CI calculated by
438 CTSM. However, this difference is not very large and we therefore believe that
439 it has no or only a limited influence on the inference study performed. It
440 should be noted that the observation noise seems to increase when the profile
441 likelihood value is decreasing. This indicates that the observation noise (and
442 not as expected the system noise) explains the difference between observa-
443 tions and the model. This is due to the few observations, which makes it
444 difficult to adequately separate observation and system noise as discussed by
445 Dennis et al. (2006).

446 In addition to the samples directly plated on the transconjugant plates, also
447 10 times concentrated samples were plated for the first six time points, where
448 transconjugant concentration in the flask was low. However, an expected 100
449 times increase in conjugation on the plate compared to the non-concentrated
450 sample was not observed. The reason for this is not clear. The sample is
451 centrifuged in order to make a concentrated sample, this might change the
452 ability for the donor and recipient to conjugate on the plate which could be
453 one explanation. Further experiments should be performed to support the
454 finding of conjugation under antibiotic pressure on the selective agar plate.

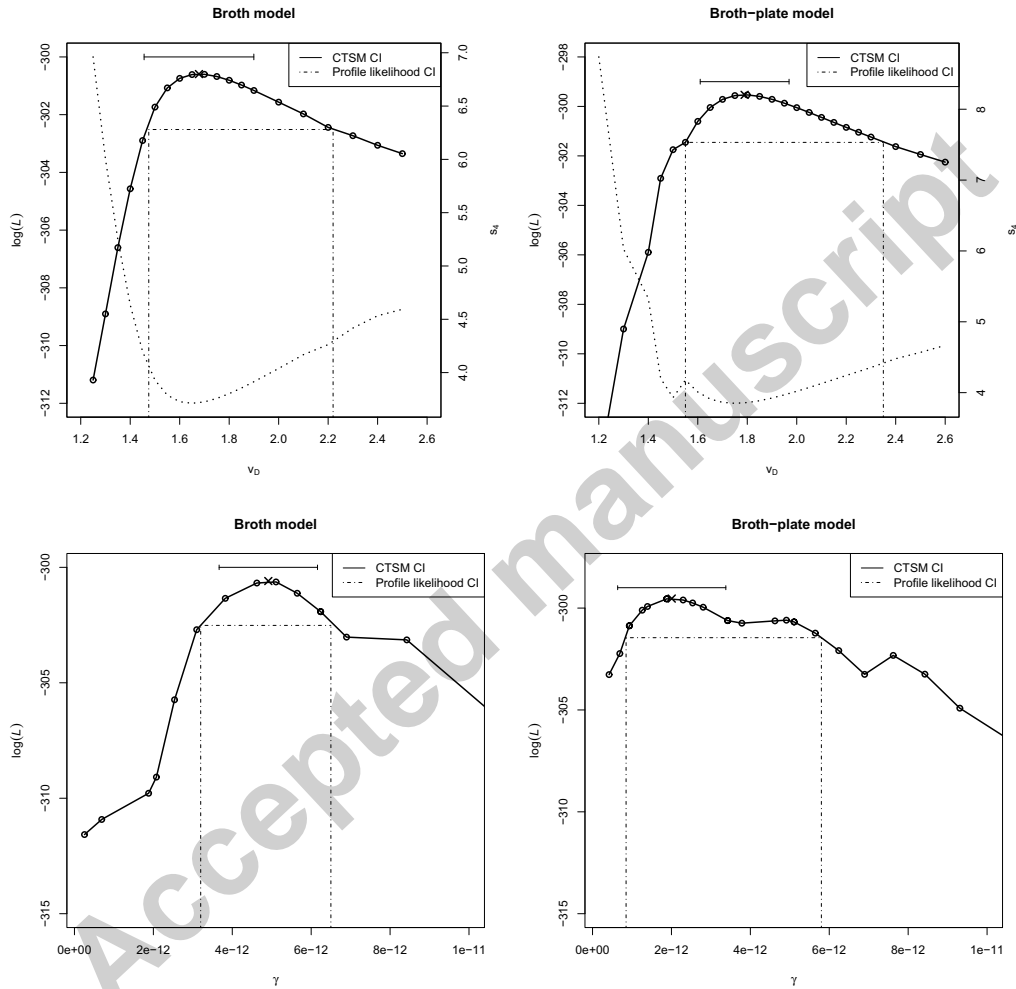


Figure 5: Profile likelihood (full line and circles) and approximate 95% confidence interval for v_D and γ_{\max} . The cross marks the estimated parameter value. The dotted lines in the top plots give the standard deviation s_4 .

455 5. Conclusions

456 The proposed SDE based state-space model is shown to successfully model
457 conjugation in a broth exhaustible media. The suggested substrate depen-
458 dent expression for the conjugation rate satisfactory model the conjugation
459 process, which stops as the substrate is depleted. The ML based framework
460 for estimating model parameters combined with likelihood-ratio tests and
461 AIC for inference studies provides strong tools for model improvements. It
462 is shown that the stochasticity of the observations is best modelled as pro-
463 portional noise.

464 The methodological problem of conjugation occurring on the transconjugant
465 plates motivates the development of the Broth-plate model, which includes
466 plate conjugation in the observation equation, whereby the plate conjuga-
467 tion can be separated from conjugation in the broth media. However, for the
468 given data the Broth-plate model does not perform significantly better than
469 the Broth-model.

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473 adaptation of antimicrobial resistance in bacterial populations".

474 **A. Generalized linear model estimation**

475 For any given observation time t_k , the concentration Y_k in the flask of a
 476 given bacteria population can be found from the following Matlab code:

```

477
478 % Create a vector N_k with the CFU counts at
479 % time t_k, e.g.
480     N_k = [94, 152, 8, 5, 1, 3];
481
482 % Create a vector n with the dilution for each
483 % of the observations N_k at time t_k, e.g.
484     n = [0.1, 0.1, 0.01, 0.01, 0.001, 0.001];
485
486     x0 = ones(length(N_k),1);
487     X = log(n);
488
489 % Estimating the parameter, beta, the
490 % deviance of the estimation, dev, and the
491 % statistics for the test (including
492 % standard deviation).
493
494     [beta,dev,stat] = glmfit(x0,N_k,...
495         'poisson','link','log',...
496         'offset',X,'constant','off');
497
498 % The concentration at time t_k is
499     Y_k = exp(beta);

```


500

501 `% The standard deviation at time t_k is`502 `sigma_Yk = Y_k*stat.se;`

503

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