A structured growth model for Hairy roots of beet root (*Beta vulgaris*)

Optimization of bioreactor cultivation parameters and secondary metabolite production using a model-theoretic approach

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Introduction and Aims:
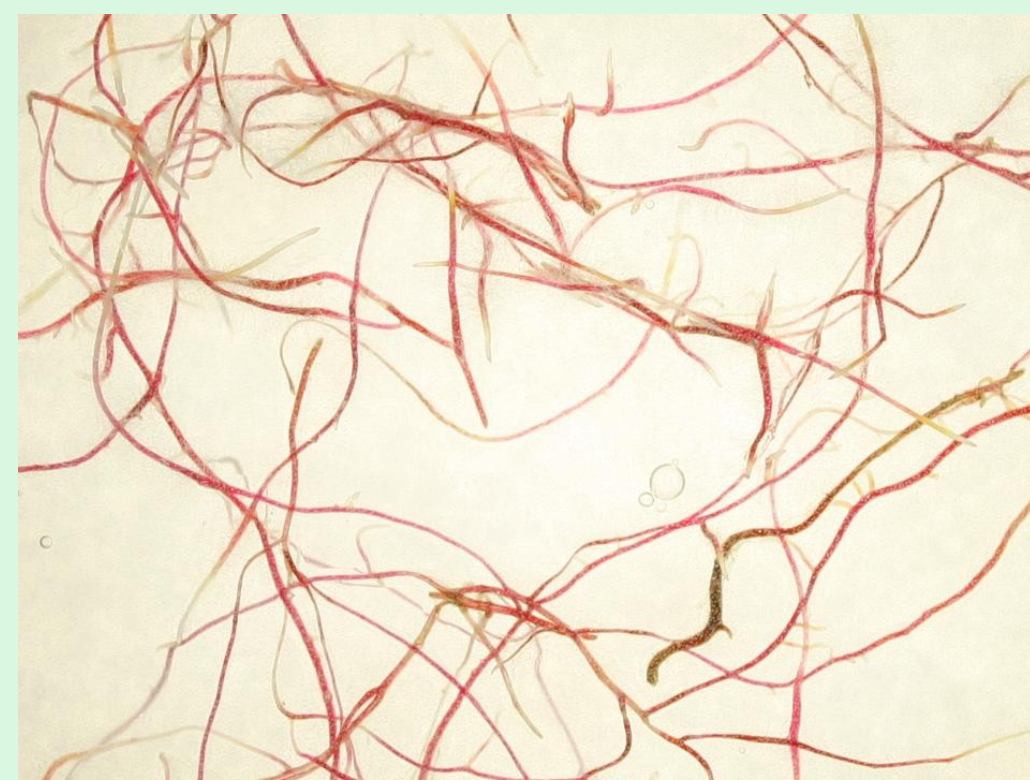

Fig. 1 *Beetroot* plant

Fig. 2 Hairy roots of *Beetroot*

Plant cells bare a wide range of nutritional-physiological and pharmaceutical relevant secondary metabolites. While usual suspended *in vitro* cultures need a constant level of different hormone concentrations, *Agrobacterium rhizogenes* induced Hairy roots can be cultivated in hormone free media. However the cultivation of these tissue cultures in bioreactors is difficult and several challenges exist. In general the growth of these tissue cultures on agar plates, in shaking flasks or bioreactors for industrial use has been heavily investigated experimentally but only limited theoretical descriptions of the growth process exist.

In order to model the growth morphology and the distribution of secondary metabolites beetroot (*Beta vulgaris*) was chosen as a model system. It produces the red dye *Betanin* which is used as a food color and is also responsible for the red color of the root network. Therefore it can be used to identify the distribution of *Betanin* optically. The presented structured growth model is an approach to simulate and visualize the growth of dense root networks in different environments.

While the model kinetics can be changed to adapt to other species, the gained knowledge can therefore be used by other scientists to improve their cultivation protocols and to simulate growth of their own cultures by amending the parameters of the model.

Materials and Methods:

The proposed model uses an individual-based matrix approach for growth simulations on agar plates. It consists of a 2-dimensional **organ matrix** con-taining a vector with information about each state of a cell (e.g. age, size, metabolite concen-trations) and a **nutrient matrix** which represents the composition of the nutrient media (e.g. carbon source, solved oxygen etc.).

In dense root networks growth can be determined spatially at three different parts of the organ complex (see Fig. 4):
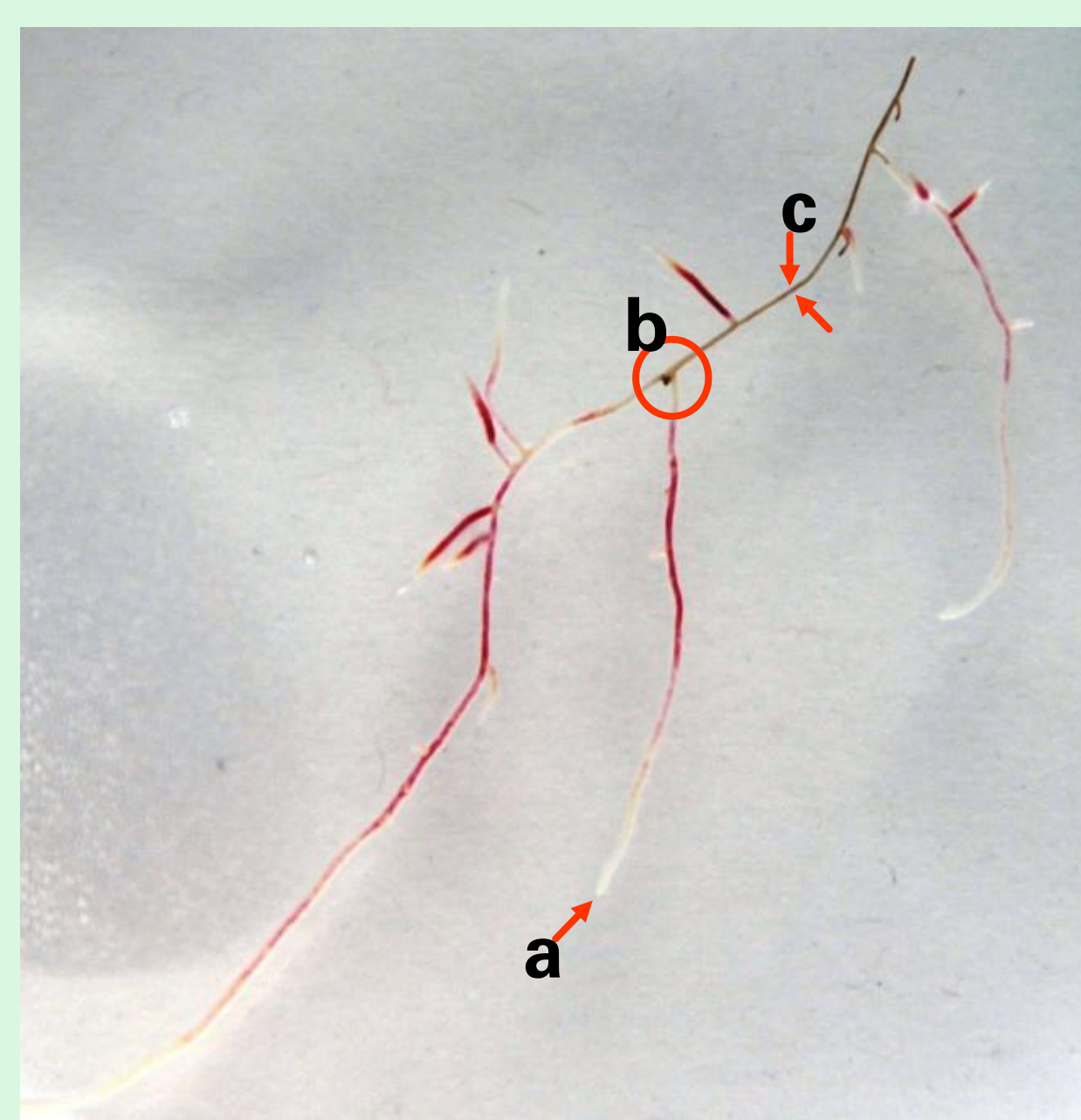- tip movement (a: elon-gation)
- branching (b)

Fig. 4 growth processes in Hairy roots

- overall biomass growth (c: secondary thickening)

For **model parameterization** all three growth processes have to experimentally investigated. A picture recognition software is used to determine mean tip movement, identification of branching points and mean overall biomass accumulation. All three growth processes have statistical values and variances and are therefore subject to investigation on a broad scale. Identified segments are numbered and their characteristic parameters are summed up in an machine-readable format (see Fig. 5).
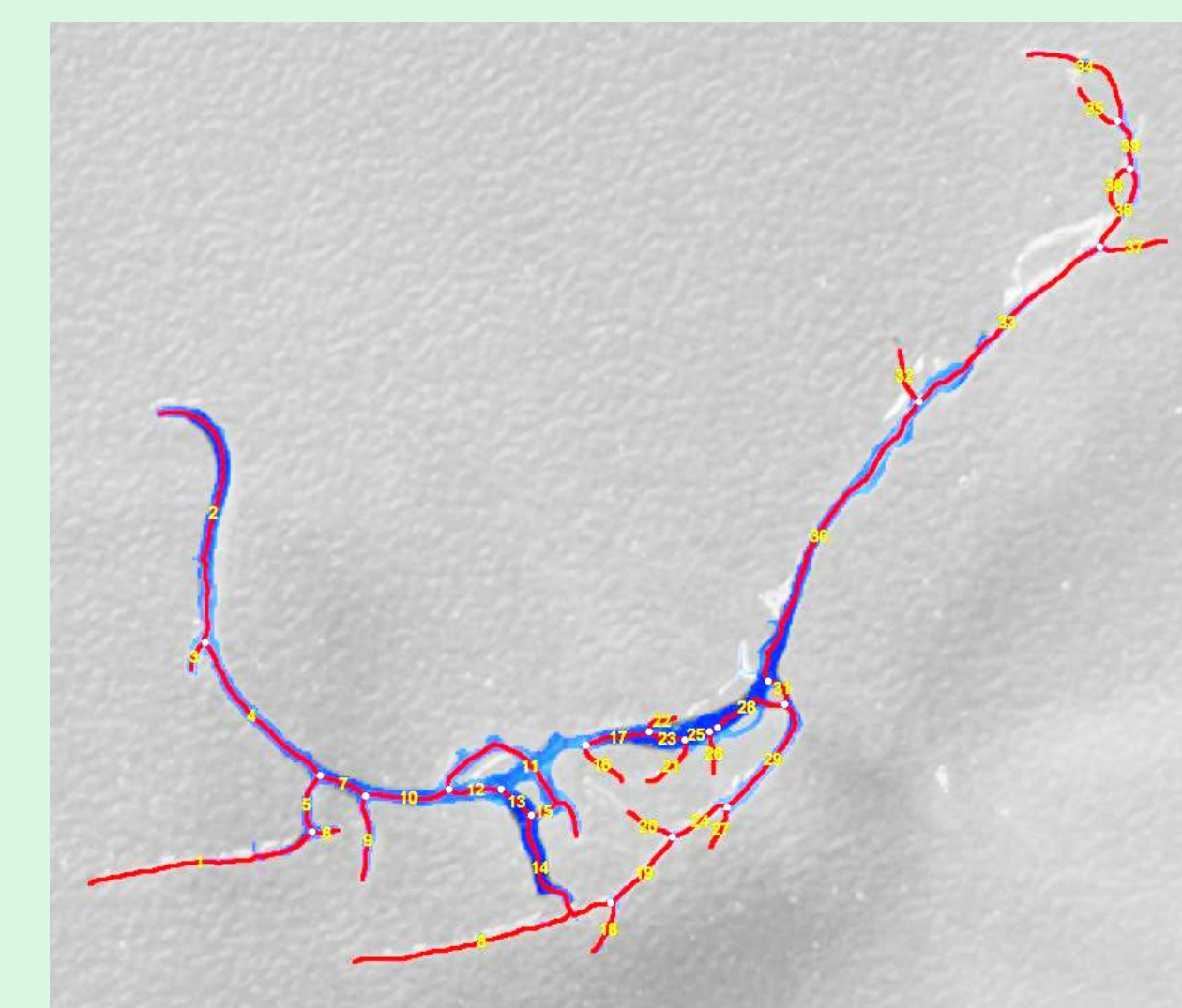

Fig. 5 recognized root segments

The mentioned growth processes can be structured further. At first the type of growth must be identified (a, b or c). For case a kinetics and directions of growth (angle τ) are used while in case of b it is
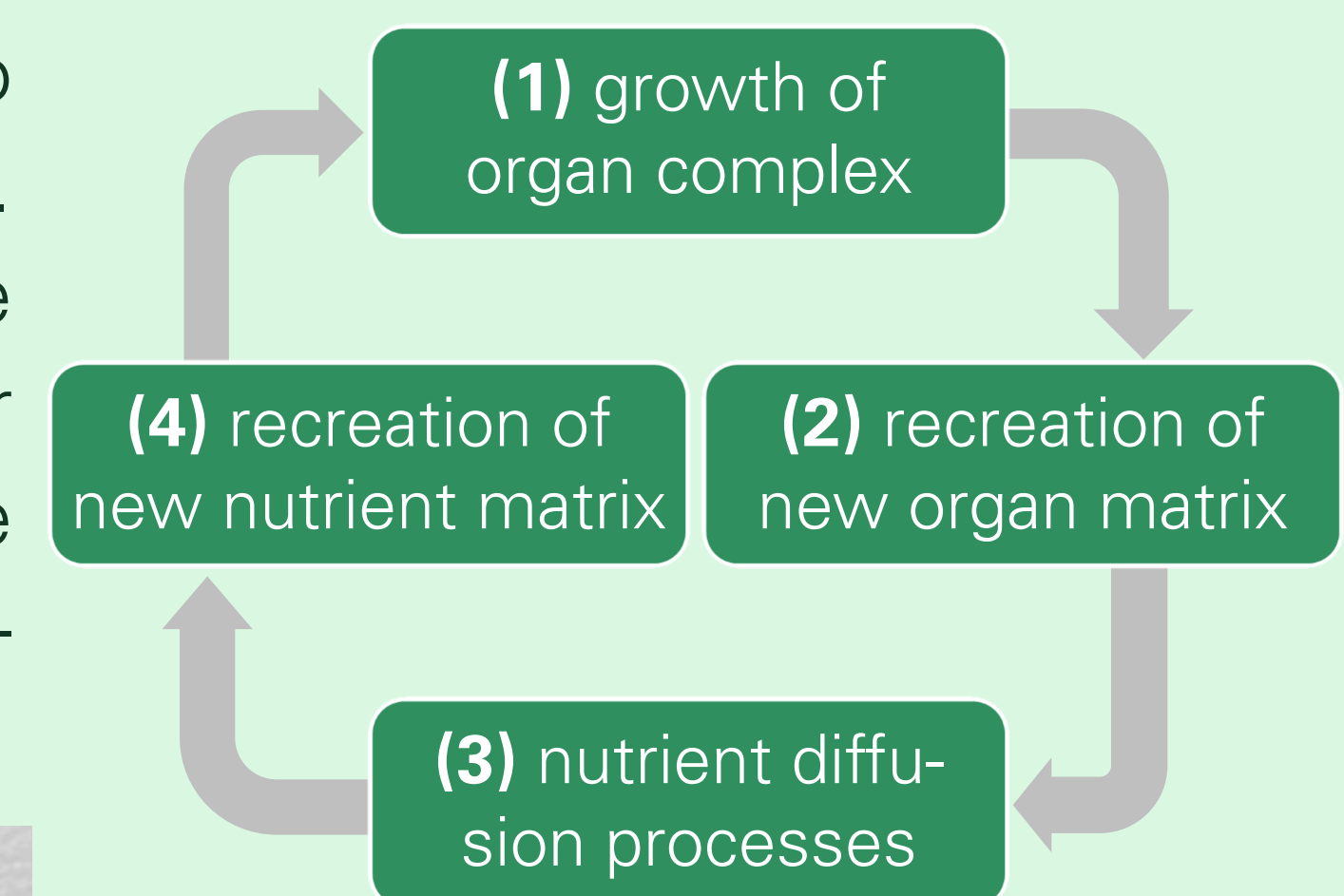

(1) growth of organ complex

(4) recreation of new nutrient matrix → **(2)** recreation of new organ matrix

(3) nutrient diffu-sion processes

Fig. 6 schematics of recursive algorithm

stochastically determined how often cells form a new branch. Secondary thickening (case c) For the simulation of all underlying growth processes a recursive algorithm which only uses the former state of organ and nutrient matrix is recalculated for a given number of defined time steps. For each time step the implemented differential equations (ODE's and PDE's) for model kinetics and diffusion processes are solved numerically.

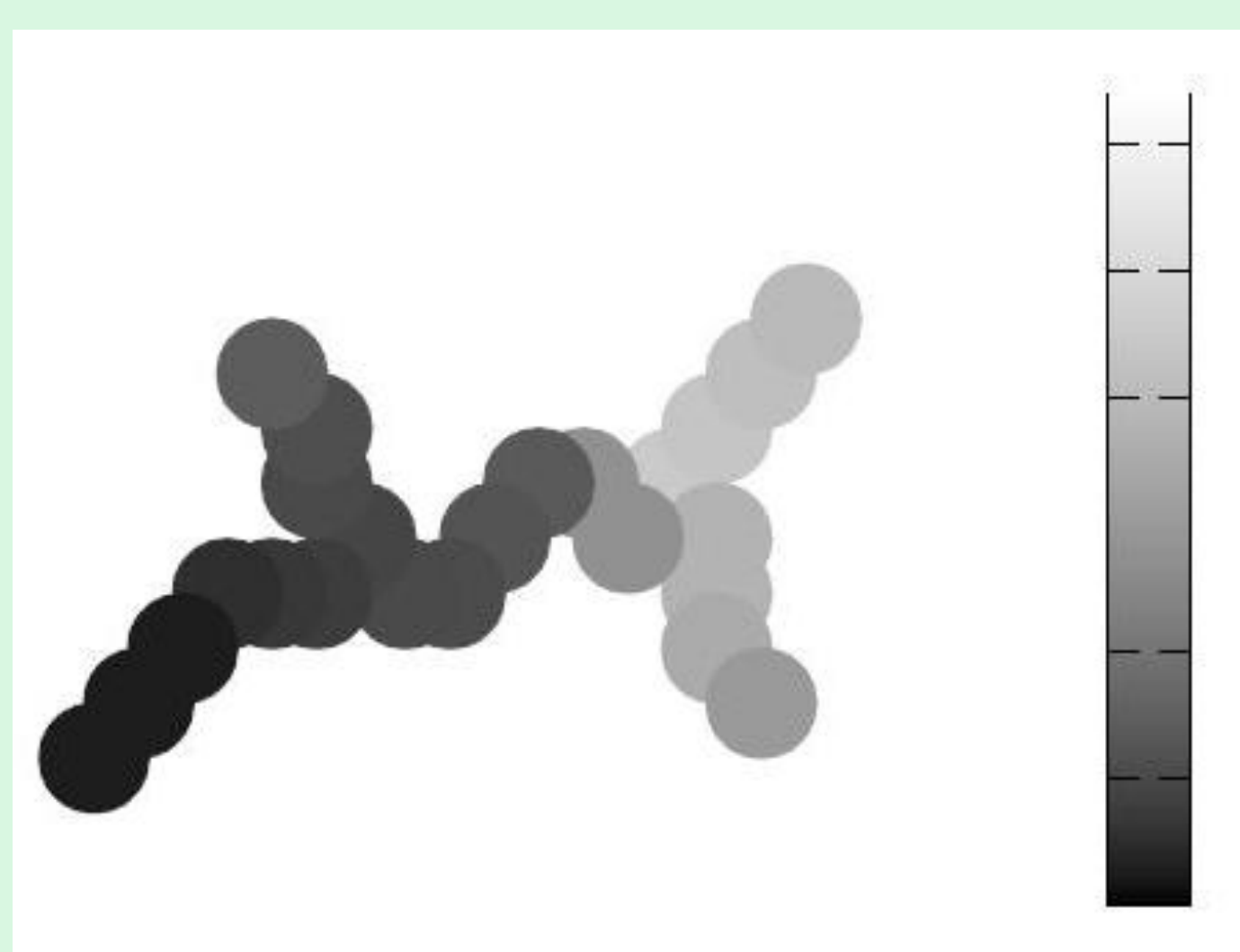### Results and future prospects:

Fig. 6 simulation results for Hairy root growth

A model grid for the simulation of Hairy root growth was established and the three main forms of growth in dense root net-works identified and structured into the growth process algorithm. System-atical investigations to quan-tify the distributions of the growth parameters have been conducted. For comparable simulation results information about morphology and distribution of secondary metabolites was taken from automatically analyzed images of root networks during the cultivation process. Therefore a customized solution for pic-ture recognition was used. Results shown in Figure 6 are a very limited simulation of growth. The grey scale represents the concentration of secondary metabolite. Each dot represents a distinct amount of cell which form the root network.

References:

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