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“Behavior of Escherichia coli in a heterogeneous gelatin-dextran mixture”

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Behavior of *Escherichia coli* in a heterogeneous gelatin-dextran mixture

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Running headline:

Preferential growth in a gelatin-dextran mixture

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ABSTRACT

In a gelatin-dextran mixture, changing the (relative and/or absolute) concentration of the components, leads to different microstructures. Confocal laser scanning microscopy illustrated that the nature of the microstructure determines the location and morphology of *E. coli* colonies. Observations indicate that bacterial growth preferentially occurs in the dextran phase, regardless of the microstructure.

BODY

It is generally accepted that food structure has an influence on bacterial growth (1). In the past decades, a myriad of research has been conducted on growth of bacteria in solid food (model) systems (2, 3, 4, 5, 6 and 7). These studies used only one gelling agent, resulting in a homogeneous microstructure. In reality, food products are most often heterogeneous as different phases are present (e.g., fat, water, proteins, ...). In addition, most studies are macroscopic, focusing on the overall population whereas bacterial growth occurs at the level of the individual bacteria. In this study, the effect of a heterogeneous microstructure on the growth of *Escherichia coli* JM-109 DE3 is investigated by means of a non-destructive technique, i.e., confocal laser scanning microscopy (CLSM). This work is a first step in the process of unraveling the mechanism of bacterial growth in heterogeneous microstructures, i.e., microstructures made up out of different phases (8).

To do so, a loop of an *E. coli* JM-109 DE3 pRSETb-Venus stock culture, kindly provided by the Department of Chemistry, KU Leuven, was transferred in an Erlenmeyer containing 20mL of Lysogeny Broth medium enriched with 20 μ L of ampicillin and placed for 7h at 37°C to obtain the preculture. Media were prepared by mixing 0.185g brain heart infusion (BHI) (Oxoid, UK) and 0.146g NaCl (Analar NORMAPUR, VWR, Belgium) with different ratios of gelatin (Gelatin from Bovine skin, type B, Sigma, USA) and dextran (Dextran from *Leuconostoc* spp. Mr ~ 500 000, Sigma, Denmark) in glass tubes with screw cap (See Table 1 for the composition of the different mixtures). After adding 5mL of distilled water, samples were placed in a water bath (GR 150 S12; Grant, UK) at 70°C for 12min. In a next step, 10 μ L of a 0.01% Rhodamine B (R953, Aldrich, Germany) and 5 μ L of ampicillin were added and the mixture was filter sterilized by

pushing it through a 0.2 μ m filter (Filtropur S 0.2, SARSTEDT, Germany) with the aid of a syringe (10mL Norm-Ject, Henke Sass Wolf, Germany). Samples were inoculated with 10 μ L of preculture. Well chambers (Chambered Borosilicate Coverglass System, Lab-Tek, Nunc, USA) were filled with 300 μ L of inoculated medium and allowed to solidify at room temperature. After approximately 40h, images were taken with a commercial laser scanning microscope (FV 1000, Olympus). The fluorescent probe Rhodamine B and the fluorescent protein Venus were used to visualize respectively the gelatin phase (see, e.g., 9) and the bacterial cells. The associated excitation wavelengths were 561 and 488 nm, respectively, emission maxima were at 625 and 528 nm. The emission ranges recorded were 655-755 nm and 505-555 nm. A 60x oil immersion objective was used with a numerical aperture of 1.35. Digital image files were acquired in .tif format and in 512x512 pixel resolution. This results in an image resolution of 0.414 μ m per pixel. Experiments are performed twice whereas multiple images were taken from each mixture. The images in Figure 1 are the images most representative for the observed phenomena.

Influence of the ratio gelatin: dextran on the microstructure. Visualizations of the microstructures listed in Table 1 are shown in Figure 1 (gelatin is shown in green). Starting from a 1:1 ratio of gelatin and dextran (Mixture 1, Figure 1 a), increasing the concentration of gelatin leads to the formation of a heterogeneous structure. Whereas the 1:1 ratio shows a uniform mixture, dextran spheres are formed in a gelatin matrix when the gelatin concentration is increased (Figure 1 c and d). Doubling the concentration of both gelling agents to a 2:2 ratio (Mixture 5), leads to phase inversion, i.e., the formation of gelatin spheres in a dextran matrix (Figure 1 e). Increasing the gelatin concentration further, induces bigger spheres and even parts in

the micro-structure where dextran spheres emerge in a gelatin matrix (Figure 1 f and g) similarly to the first mixtures. Whereas the 2:2 ratio with added salt (Mixture 5) consists of a dextran matrix with gelatin spheres of more or less the same size (Figure 1 e), leaving out the salt leads to a combination of few big droplets with a large amount of smaller droplets (Figure 1 h).

Preferential growth of *E. coli* in a gelatin-dextran mixture. Figure 1 also presents the *E. coli* colonies (red) in the gelatin-dextran mixture. Some diffusion of Rhodamine B, used for the staining of the gelatin phase, into the bacterial cells is observed. As a result, bacterial cells can also appear as green or yellow rods in the microstructure.

Depending on the microstructure, colonies evolve differently. In the microstructures of Mixtures 1-4, colonies form spheres (Figure 1 a-d). As the gelatin concentration increases and a heterogeneous structure arises, colonial growth occurs in the dextran phase. When the dextran concentration (Figure 1 e-h) is doubled, phase inversion occurs and colonies no longer grow as regular demarcated spheres. Instead, the colonies appear as diffuse strings permeating the dextran phase while avoiding the gelatin phase. In mixtures where both microstructures occur (gelatin in a dextran matrix and dextran in a gelatin matrix, e.g., Figure 1 f and g), bacteria grow only in the dextran phase, regardless the microstructure.

Literature reports that several bacteria can metabolize dextran and use it as a substrate (10, 11), which could explain the preference of the bacteria for the dextran phase. However, for this specific strain, no references were found. Testing growth of *E. coli* in “poor” growth media with and without the addition of dextran, did not show any difference in growth curves, i.e., no growth occurred after 24h. If the bacteria would have been able to metabolize dextran, the addition of dextran to the poor medium would have to result in growth. As this does not occur, the experimental results indicate that dextran cannot be metabolized by *E. coli* JM-109 DE3.

97 In previous experiments, the authors were able to visualize *E. coli* micro-colonies in gelatin only
98 (not published). This means that gelatin does not form a microstructure that physically excludes
99 *E. coli* cells as is happening with acid-modified corn starch in gelatin gels (12). The presence of
100 the micro-colonies also proves that cell multiplication is not restricted by the presence of the
101 gelatin.

102 It can be concluded that the location and morphology of *E. coli* JM-109 DE3 colonies is
103 influenced by the microstructure in a gelatin-dextran mixture. Images taken with CLSM illustrate
104 that *E. coli* bacteria have a preferential phase for growth, i.e., the dextran phase. The preference
105 ascribed to dextran cannot be attributed to (i) an ability to metabolize dextran molecules, (ii)
106 exclusion of *E. coli* cells by the gelatin, or (iii) restriction of cell multiplication in the gelatin
107 phase. The preference of *E. coli* cells for the dextran phase is probably due to more favorable
108 physical properties in the dextran phase.

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Figures

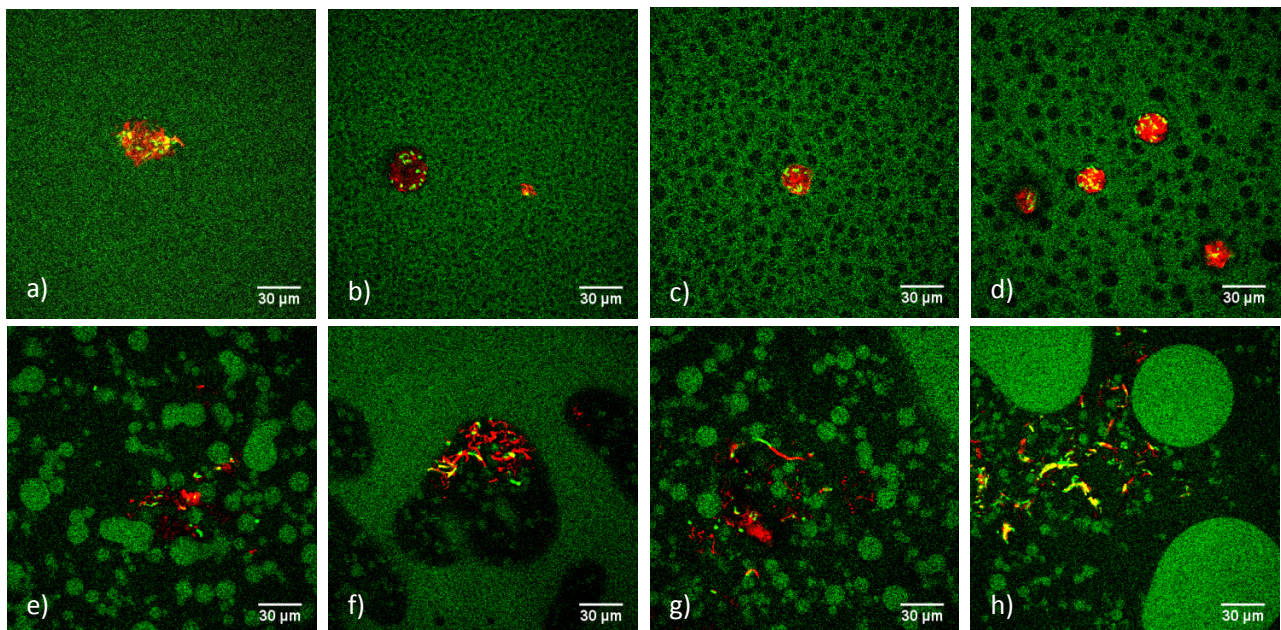


Figure 1: Growth of *E. coli* JM-109 DE3 (red) in gelatin (G, green)-dextran (D, black) mixtures:
a) 1G/1D, b) 2G/1D, c) 3G/1D, d) 4G/1D, e) 2G/2D, f) 3G/2D, g) 4G/2D, h) 2G/2D with no added salt.

Tables

Table 1: Composition of different mixtures.

	Gelatin (G)	Dextran (D)	Salt
Mixture 1 (1G/1D)	0.125g	0.125g	0.146g
Mixture 2 (2G/1D)	0.250g	0.125g	0.146g
Mixture 3 (3G/1D)	0.375g	0.125g	0.146g
Mixture 4 (4G/1D)	0.500g	0.125g	0.146g
Mixture 5 (2G/2D)	0.250g	0.250g	0.146g
Mixture 6 (3G/2D)	0.375g	0.250g	0.146g
Mixture 7 (4G/2D)	0.500g	0.250g	0.146g
Mixture 8 (2G/2D NS)	0.250g	0.250g	/