

## Pathogen Inhibition by strains in Winclove 345

### Aim of the experiment

The aim of the experiment is to show whether probiotic strains in Winclove 345 and the product are able to inhibit the growth of (potentially) pathogenic micro-organisms *in vitro*.

### Background information

To establish the inhibiting capacity of probiotic strains/products against pathogenic bacteria, an adapted version of the Well Diffusion Assay, described by Hechard *et al.* (1990)<sup>1</sup> is used.

A nutritious medium is poured into a petri-dish (figure 4a). In this agar medium a potentially pathogenic organism is grown. When the medium is dry, little holes (wells) are made in the solid medium. In these holes a small amount of the probiotic strain/product is put and the petri-dishes are incubated. In case of inhibition of the pathogen, a clear zone will appear around the holes (figure 4b). The larger this zone, the better is the capacity of the probiotic strain to inhibit the pathogenic organism.

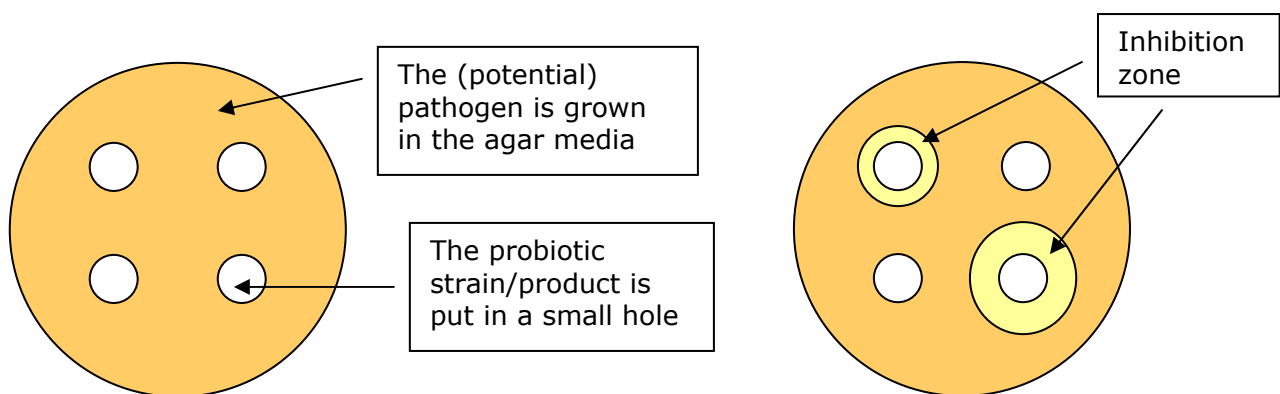


Figure 4a. Petri-dish before incubation

Figure 4b. Petri-dish after incubation

<sup>1</sup> Hechard Y, Dherbomez M, Canatiempo Y, Lettelier F. Antagonism of lactic acid bacteria from goat's milk against pathogenic strains assessed by the 'sandwich method'. Lett Appl Microbiol 1990;11;185-8.

### Method

#### Pathogen inhibition on solid medium

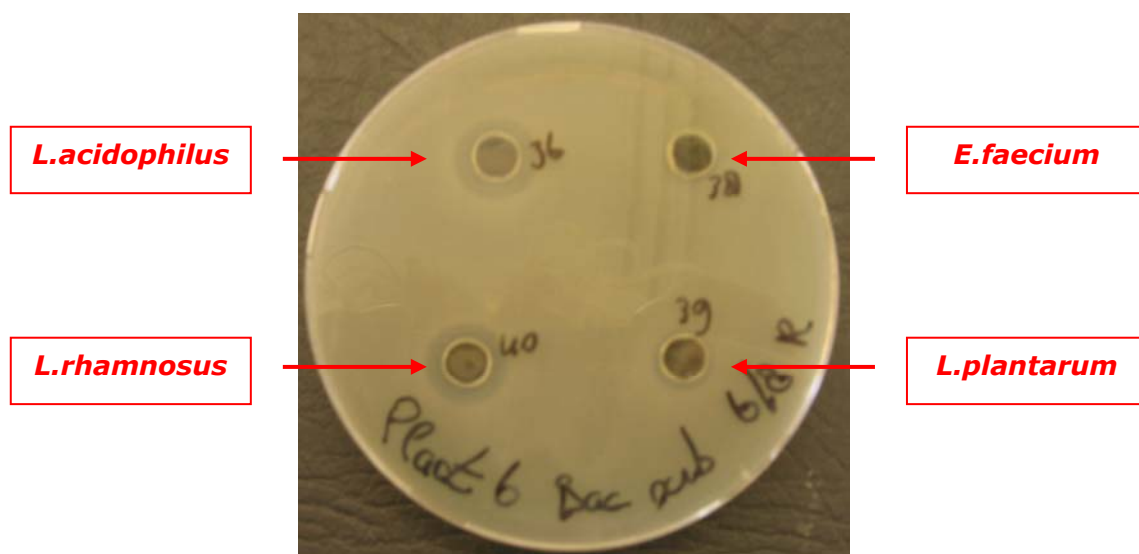
We have tested the inhibition of the Winclove 345 strains against several pathogenic strains, which are listed in the table below. The results are shown in inhibition factors, presenting the intersection ( $\emptyset$ ) of the clear zone. The higher the number, the higher the inhibition of the pathogen. The minimal inhibition factor is 0 (no inhibition), the maximum inhibition factor is 3 (high inhibition). The empty cells means that the analysis has not be performed.

#### Inhibition test in liquid medium and toxin inhibition

Both the indicator strain (*C.difficile*) as the probiotic strain were grown at the same time, in the same tube. After incubation the concentration of the *C.difficile* culture was assessed and compared to the *C.difficile* strain that was grown alone. The toxin production was measured using ELISA.

### Example pathogen inhibition on solid medium

In the photograph below, the ability of four different probiotic strains to inhibit *Bacillus subtilis* is shown. In this case *Lactobacillus acidophilus* and *Lactobacillus rhamnosus* show the highest inhibition against *Bacillus subtilis*. *L.plantarum* shows a small inhibition zone and *E.faecium* does not inhibit this pathogen.



Example of Petri-dish with inhibition zones

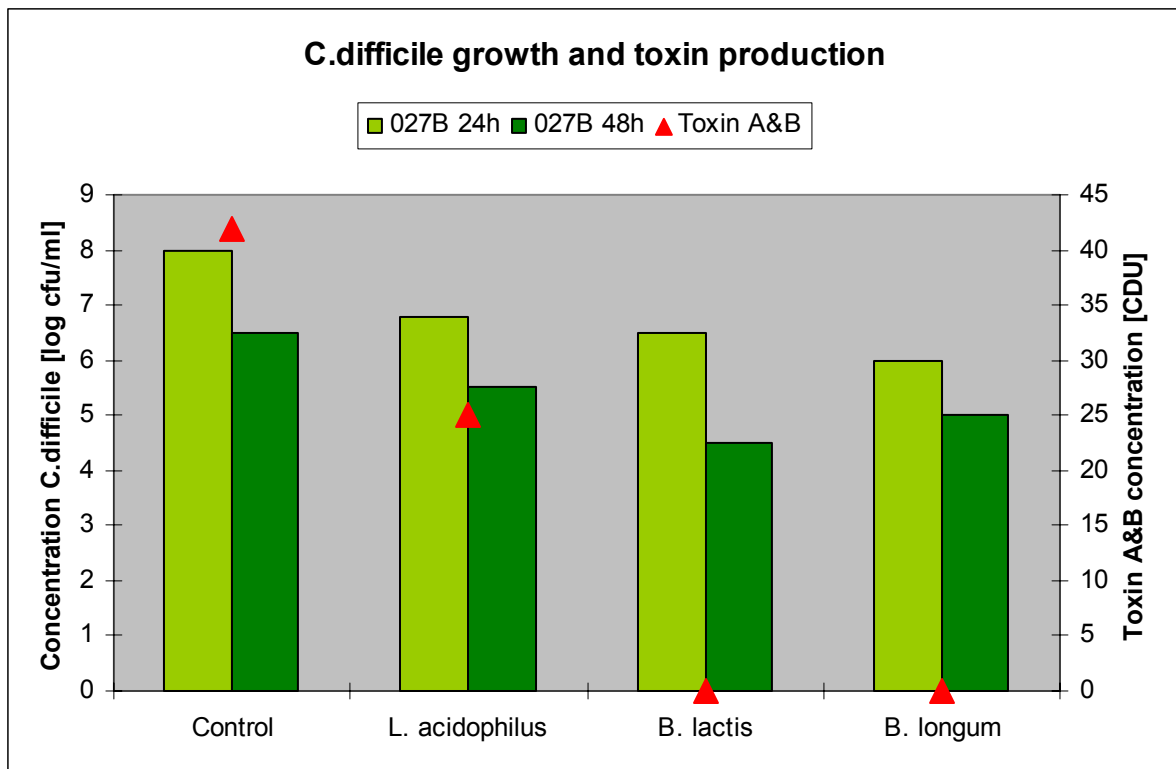
### Results

The results regarding inhibition of pathogens by strains in Winlove 345 are presented in the table 1. The empty cells mean that the analysis has not been performed. The number in the table are the 'inhibition factors'; the higher the number, the higher the inhibition of the pathogen. The minimal inhibition factor is 0 (no inhibition), the maximum inhibition factor is 3 (high inhibition). In general, the lactobacilli are better inhibitors than bifidobacteria.

The results regarding inhibition of *C. difficile* in liquid medium and toxin productions inhibition show that after 48 hours in the presence of several strains in Winlove 345, no toxin A&B could be detected anymore. This means that either *C.difficile* 027B did not produce toxins in the presence of the probiotic strain or the toxin was still produced but it was eliminated by the probiotic strain. The strains that are missing from the figure were not tested for toxin production inhibition. Nevertheless, all six strains tested completely inhibit toxin production of *C. difficile* 027B.

|                       | <i>E.coli</i> | <i>E.faecalis</i> | <i>B.subtilis</i> | <i>Cl.perfringens</i> |
|-----------------------|---------------|-------------------|-------------------|-----------------------|
| <i>L. acidophilus</i> | 2             | 2                 | 1                 | 1                     |
| <i>L. casei</i>       | 3             | 3                 | 2                 | 0                     |
| <i>L. salivarius</i>  | 3             | 2                 | 3                 | 1                     |
| <i>Lc. lactis</i>     | 1             | 0                 | 1                 | 0                     |
| <i>B. infantis</i>    | 1             | 0                 | 0                 | 0                     |
| <i>B. lactis</i>      | 0             | 0                 | 0                 | 0                     |
| <i>B. longum</i>      | 0             | 0                 | 0                 | 0                     |

**Table 1:** Pathogen inhibition on solid medium by strains in Winclove 345



**Figure 1:** *C. difficile* 027B inhibition in liquid medium and inhibition of toxin production by several strains in Winclove 345

### Conclusions

Most of the individual strains are capable of inhibiting the growth of several pathogenic bacteria *in vitro*. The full product often shows a better inhibition pattern than the individual strains. It is known that lactobacilli are usually better inhibitors than bifidobacteria. A very interesting outcome is that 2 bifidobacteria fully reduce the production of toxins by *Cl.difficile* 027B.