



MICROBIOLOGICAL IMAGE

Veil-like pellicle development by *Azospirillum brasilense* in semisolid Nfb medium



Desarrollo de película típica en medio Nfb semisólido por *Azospirillum brasilense*

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Azospirillum brasilense is a vastly studied plant growth-promoting rhizobacterium, which produces direct and indirect beneficial effects on several crops⁴. Historically, the culture of this bacterium has been performed using the semisolid Nfb medium³, which is a nitrogen-free medium². Although other diazotrophs can grow in this culture medium², *A. brasilense* can be isolated and identified by a typical growth after 120 h of incubation³. Other authors have exhaustively described the Nfb medium composition and growth of many diazotrophs in semisolid media¹. Here, we show pictures of seven stages during the development of *A. brasilense* in the course of the incubation period.

A colony of *A. brasilense* was inoculated in a vial containing this culture medium. After 24 h of incubation at 30 °C, growth development can be observed by a halo formation around the colony as a ‘ghostly-balloon’ (Fig. 1A). The halo

grows in size (Fig. 1B) and opens (Fig. 1C) after 48 h of incubation, approximately. By aerotaxis, cells move to the specific place in the medium (Fig. 1D) where the rate of oxygen diffusion and bacterial respiration allows the nitrogenase enzyme to fix N₂ without the irreversible inhibitory effect of oxygen². Then, bacteria continue growing and form a typical sub-superficial whitish ‘veil-like’ pellicle after five days of incubation (Fig. 1E). As growth occurs, the alkalization of the culture medium can be observed by a change in color from green (Fig. 1F) to blue (Fig. 1G) due to the bromothymol blue redox dye. This culturing technique can also be used for other purposes, such as the inoculation of serial dilutions (Fig. 1F and G) to perform the estimation of the most probable number of this plant beneficial bacterium in commercial inoculants or the count of diazotrophs from environmental samples.

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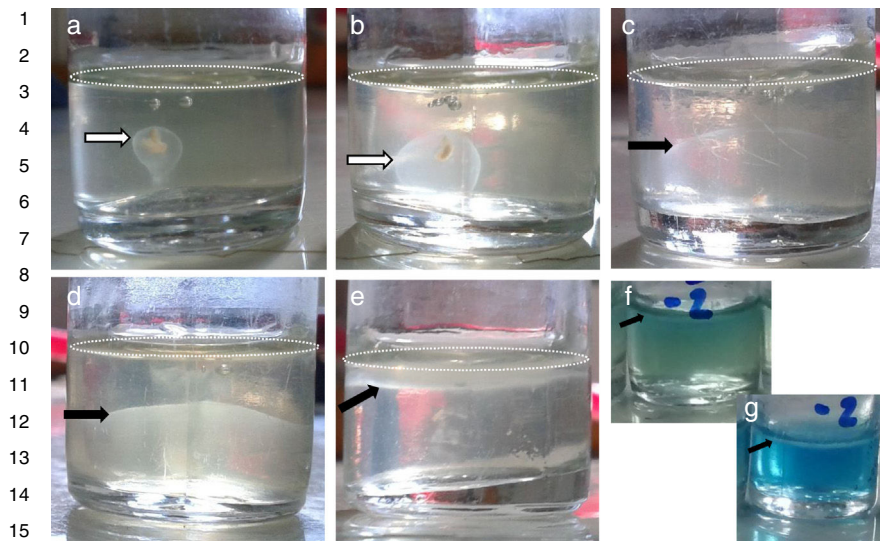


Figure 1 Growth development by *Azospirillum brasilense* in vials with semisolid Nfb medium during the incubation period (a, b, c, d and e). Growth starts with the formation of a halo (white arrows) around the colony. Typical growth as a 'veil-like' pellicle (black arrows) under the medium surface (dotted circles) is formed. After six (f) and seven (g) days of incubation, it can be observed the change of color indicating the alkalization of the culture medium. Vials showed in a to e were inoculated with one colony of *A. brasilense*. Vials showed in f and g were inoculated with 50 μ l of the 10^{-2} dilution of an inoculant sample.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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