Rhizobacteria adhesion to starch granules

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INTRODUCTION

Biofertilizer based on rhizospheric beneficial micro-organisms have emerged as an alternative to chemical fertilizers to increase crop production (Bashan, 1998). Unfortunately, rhizobacteria are sensitive to storage conditions; it is therefore of interest to develop methods to protect cells against stress conditions.

However, cells immobilization into alginate beads has been disadvantageous, due to a lot of amount of water on beads composition. Use of alginate-starch beads, increase dry matter concentration and thus reduces the time and cost of drying process (Ivanova, 2006)

Starches are one of the most abundant biopolymers and are used in many forms because they offer a large variety of functionality (Forssell, et al. 2004). Starch has been used to protect bacteria in adverse environmental conditions, using the common mechanism of bacteria to adhesion to surface, is possible enhanced rhizobacteria survival using starch (O'Riordan et al., 2001)

The aims of the present study were to improve the adhesion of rhizobacteria by different type of granules-starch, which represents a first promising step towards prolonging cells viability during immobilization process in storage conditions.

MATERIAL AND METHODS

Micro-organisms and type of starch.
The micro-organisms and type of starch used in this study, are described in the next tables:

Micro-organisms
Azospirillum brasilense Sp245
Azospirillum brasilense Sp7
Bacillus pumilus C26
Enterobacter ludwigii BNM 0357
Paenibacillus polimyxa MXC5
Raoultella terrigena TFi08

Type of starch
Standard maize (native maize starch, Sigma, USA)
High maize (1043, high-amylose, National starch, UK)
Waxy maize (waxy corn starch; Sigma, St. Louis, USA)
Wheat (unmodified starch, Sigma, St. Louis, USA)
Potatoes (Native starch, Sigma, Steinheim, Germany)

Culture conditions.
The strains were grown in 30 ml of sterile YEP medium adjusted to pH 6.5 containing peptone casein (Biokar Diagnostic, Beauvais, France) 10 g l^{-1}, yeast extract (Biokar Diagnostic, Beauvais, France) 5 g l^{-1}, sodium chloride (Fisher Scientific, Leicestershire, UK) 5 g l^{-1}. Cultures were performed on a rotary shaker at 120 rev min^{-1} and 30°C to harvest cells on exponential or stationary growth phase.

Adhesion to starch granules.
Adhesion to starch granules was measured by a co-sedimentation assay following the method propose by Crittenden et al. (2001). Bacteria were first washed twice with 30 ml of 0.01 M of phosphate buffer pH 7.4 before their were re-suspended in the same phosphate buffer.

Five milliliters of bacterial suspension was thoroughly mixed in a test tube with an equal volume of a starch suspension (10 g l^{-1}) in a phosphate buffer.

The bacterium-starch mixture was let sedimented for 1 h at room temperature. Two milliliters samples of a test tube (1.5 * 16 cm) were then taken from 0.5 cm below the liquid surface, and the optical density at 540 nm (OD_{540}) was measured with a spectrophotometer (Unicam UV1). Phosphate buffer was used as a blank for all reading from the spectrophotometer.

The proportion of the adhered bacteria was calculated as follows:

$$\text{Adhered cells} = 1 - \frac{a - b}{c}$$

a is the OD_{540} containing bacteria-starch suspension,
b is the OD_{540} of a sample containing the starch but not bacteria and
c is the OD_{540} of a sample containing bacteria but not starch.

RESULTS AND DISCUSSION

Fig. 1 showed the capacity of cells adhesion to starch maize for each cells type. Adhesion of *A. brasilense* represented 35%. In contrast, *E. ludwigii* exhibited strong adhesion (65%) during cosedimentation assay. Cells adhesion was due, principally, to the exopolysaccharides production for the bacteria external membrane, produced principally in stationary growth phase.

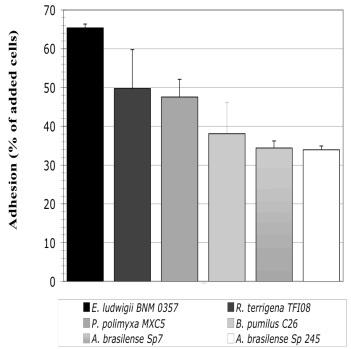

Figure 1. Adhesion of different type of rhizobacteria to standard maize starch

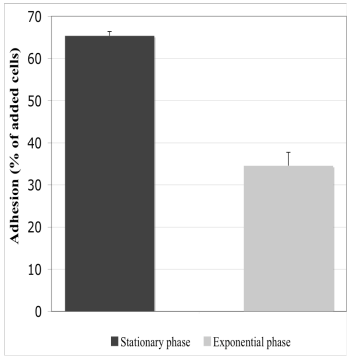

Figure 2. Effect of *E. ludwigii* growth phase on adhesion to standard maize starch

The effect of bacterial growth phase on adhesion was carried out with highly adherent strain *E. ludwigii* (Fig. 2). Cells in stationary phase were capable to adhere more than 60%. In contrast, cells in exponential growth phase reached only 35% of cells adhesion.

Fig. 3 presented *E. ludwigii* adhered about 50% to granular potato starch. The same strain adhered to wheat starch at much higher level (95%). There was a strong relation between *E. ludwigii* adsorption and starch granules surface area. Wheat starch had highest ratio average surface area in comparison to maize and potato starch. However, there was not a general association between ratio surface and starch adhesion for *R. terrigena* and *B. pumilus.*

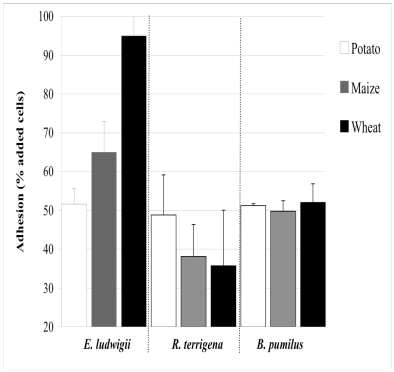

Figure 3. Rhizobacteria adhesion in different type of granule natives starch: potato (∅ 15-100 µm), maize (∅5-25 µm) and wheat (∅ 5-15 µm).

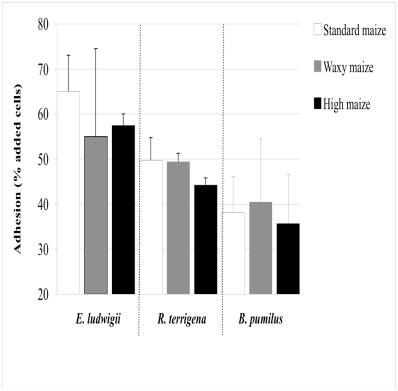

Figure 4. Rhizobacteria adhesion to maize starch granules, with different percentage of amylose: waxy maize (1%), standard maize (20-25%) and high maize (60-80%).

The effect of cells adhesion and percentage of amylose on starch granules was evaluated in Fig 4. The difference amount of amylose did not influence the degree of adhesion to the starch granules. *Bacillus pumilus* an amylololytic rhizobacteria did not present difference about cells adsorption. Nevertheless, Crittenden et al. (2001) observed a relation between bifidobacteria adhesion and starch utilization.

However, not important evidence was observed about the relationship between cells adsorption and use starch like as carbon source in this type of rhizobacteria. The high adhesion presented in *E. ludwigii* and *R. terrigena* maybe is due to the high external membrane protein produced for this type of rhizobacteria (Leone, et al., 2007).

The study also detected the cells adhesion on maize starch granules, that adsorption were visualized under scanning electron microscopy (SEM). SEM indicated visual evidence to *E. ludwigii* adsorption to granule starch surface (Fig. 6) in comparison to the smooth surface of control starch without cells (Fig. 5).

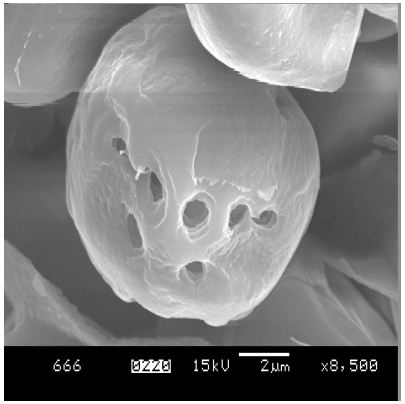

Figure 5. Scanning electron micrograph of standard maize starch granule pregelatinised, without cells.

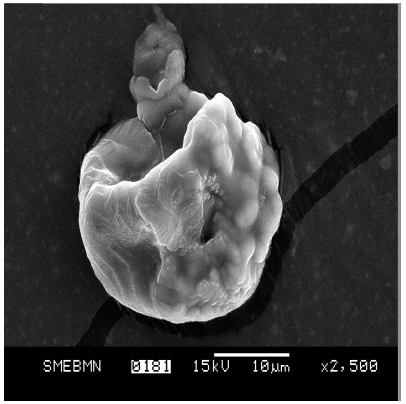

Figure 6. Scanning electron micrograph of standard maize starch granule, with *Enterobacter ludwigii* adsorbed.

CONCLUSIONS

The result of this study indicates the high percentage of adhesion of rhizobacteria to starch is not a typical characteristic of all strains. This starch adhesion depends of many factors, type of rhizobacteria strain, cells physiological growth phase and starch type However, the high adherent strain, *Enterobacter ludwigii* was not capable to adhere to similar degrees to starch granules from a variety of different plant sources, although the cells adsorption of the starch was dependent on the granule surface area. SEM presented visual evidence to *Enterobacter ludwigii* adsorption on surface maize starch granules.
Bacillus pumilus, the amylolytic strain used in this study, did not present difference in adhesion, using starch, with different percentage of amylose composition on granules (standard, waxy and high-maize). Further investigations will be conducted to elucidate cells viability of the adhered cells on surface granule starch.

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