# ADHESION OF RUMEN CELLULOLYTIC BACTERIA TO CELLULOSE: A DIFFERENT MECHANISM FOR *BACTEROIDES SUCCINOGENES* S85 AND FOR *RUMINOCCOCUS FLAVEFACIENS* 007?

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## Introduction

The adhesion of cellulolytic bacteria to cellulose which brings the bacteria in close contact to its specific substrate and prevents the waste of celluloytic enzymes is a central step in cellulolysis and the degradation of plant cell walls in the rumen. Numerous studies of electron microscopy have focused on the two main species of cellulolytic bacteria in the rumen, *Bacteroides succinogenes* and *Ruminoccocus flavefaciens*. They have been essentially descriptive and quantitative (Cheng, 1983/84). The structures responsible for adhesion of these two species are yet poorly known. Our objective has been to study the effect, in vitro, of various physicochemical factors susceptible to influence adhesion of these bacteria to cellulose in order to reach in term a better understanding of the mechanisms involved.

## Materials and Methods

*B. succinogenes* S85 and *R. flavefaciens* 007 have been grown on cellobiose using liquid media as previously described (Roger et al., 1988) and under a gas phase of $CO_2$. Cultures were harvested in late exponential growth phase by centrifugation (10 min, 3500 g). The pellet was resuspended in a medium of similar composition, without cellobiose but containing the factor the effect of which we want to study. The percentage of bacterial cells capable of adhesion to cellulose was determined according to the method of Minato and Suto (1978). A minimum of four repetitions of the adhesion test was performed for each factor.

The microcristalline cellulose Avicel was chosen as reference and added to the growth medium at a concentration of 0.2%. We have studied the influence of pH, sodium ions deprivation, the nature of the gas phase ($CO_2$ or $N_2$) as well as the effect of certain metabolic inhibitors on the adhesion of *B. succinogenes* and *R. flavefaciens*.

## Results and Discussion

The maximum of adherent cells of *B. succinogenes* was observed for pH values between 6 and 7. Beyond these two values, the percentage of adhering bacteria sharply dropped whereas the adhesion of *R. flavefaciens* was unchanged within a pH range between 4 and 7.5 (figure 1). Adhesion in this latter species, as well in *R. albus* (Morris, 1988) is unaffected by low pH values.

The absence of sodium ions almost completly inhibited the adhesion of *B. succionogenes* under a gas phase of 100 % $N_2$ whereas under a gas phase of $CO_2$ (supply on hydrogen ions by dissolution of $CO_2$ in the medium) the decrease in percentage of adhering bacteria was comparatively minor (table 1). Under a nitrogen gas phase, a supply of lithium ions partially restaured attachment. The adhesion of *B. succinogenes* was also inhibited almost completly by N-N' Dicyclohexylcarbodiimide (DCCD) which is an inhibitor of membrane ATPases. On the contrary, inhibitors of electron transport chains (Antimycin A, Hydroxy-quinoline-N-Oxyde, Sodium Azide) and the protonophores (2, 4 Dinitrophenol and Tetrachlorosalicylanilide) used at concentrations which normally inhibit growth of *B. succinogenes* (Franklund and Glass, 1987) did not modify the percentage of adhering cells of the species. The adhesion of *R. flavefaciens* was only weakly affected by each of these factors.

The inhibition of attachment due to the presence of ionophores was much greater with *B. succinogenes* than with *R. flavefaciens*. Added at a concentration of 0.02 mM, the inhibition effect was greater for Lasalocid than Monensine (table

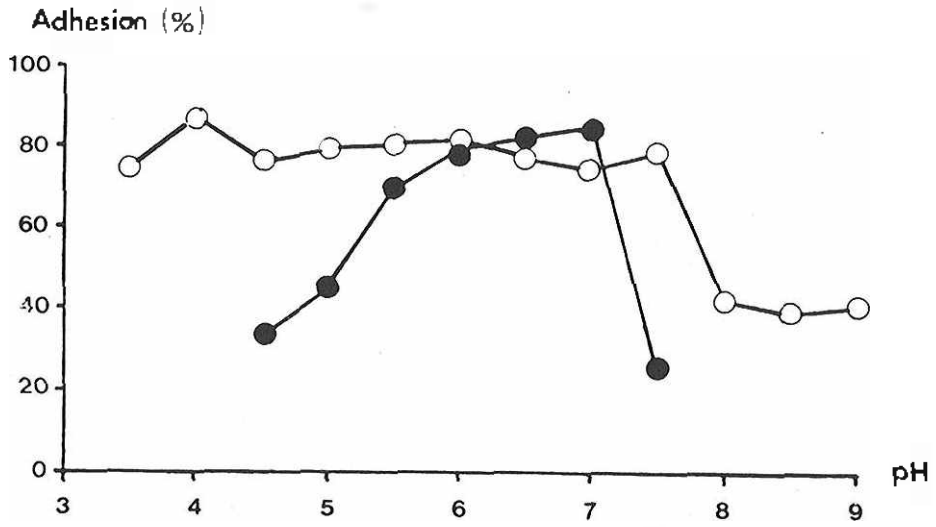


Figure 1. Effect of pH on the adhesion to cellulose of *B. succinogenes* ( ● ) and *R. flavefaciens* ( ○ ) (% of adherent cells from a culture at the end of the exponential phase).

TABLE 1. EFFECT OF SODIUM AND METABOLIC INHIBITORS ON THE ADHESION OF *B. SUCCINOGENES* AND *R. FLAVEFACIENS* TO CELLULOSE.

| Factor | | Adherent cells (% of control) | |
|---|---|---|---|
| | | B. succinogenes | R. flavefaciens |
| Metal ionophores | | | |
| Lasalocid | 0.02 mM | −94%±4% | −18%±14% |
| Monensine | 0.02 mM | −30%±6% | −15%±6% |
| Proton ionophores | | | |
| 2.4DNP | 1.6 mM | −6%±3% | No effect |
| TCS | 0.02 mM | 5%±4% | No effect |
| Membrane ATPases inhibitor | | | |
| DCCD | 0.2 mM | −90%±6% | 6%±1% |
| Electron transport inhibitors | | | |
| Antimycine A | 0.1 mM | 12%+10% | −13%±9% |
| HOQNO1 | 0.1 mM | −10%±4% | −10%±4% |
| Na N3 | 40 mM | No effect | No effect |
| Deprivation in sodium ions | | | |
| Under 100% $N_2$ | | −60%±7% | −9%±5% |
| Under 100% $CO_2$ | | 10%+6% | −11%±5% |
| $N_2$ + 50 mM Lithium | | −52%±6% | −11%±5% |
| $N_2$ + Sodium ions | | −16%±3% | −12%±7% |

1).

Function of membrane ATPases thus seems to be required for adhesion of *B. succinogenes*. The effect of deprivation in monovalent cations can be explained by the fact that membrane ATPase of *B. succinogenes* are sodium dependant and that sodium can be replaced by lithium (Forano, unpublished data). Mechanism of action of hydrogen ions has yet to be established. The two ionophores tested catalyse an exchange between sodium ions and hydrogen ions which pertubes the membrane ATPases activity; this could explain their effect.

*R. flavefaciens* and *B. succinogenes* which show very different responses to the factors studied, most probably rely on different mechanisms for adhesion. We have indeed observed in a previous study (Roger et al., 1988) that the adhesion of *R. flavefaciens* to cellulose is immediate and sensitive at the deprivation in divalent cations ($Mg^{++}$ and $Ca^{++}$) whereas that of *B. succinogenes* requires 25 minutes of contact with cellulose to be maximum. The attachment of *B. succinogenes* was inhibited by low temperatures, glucose and cellobiose in high concentration (5%) and the presence of oxygen unlike that of *R. flavefaciens*.

These results suggest that *R. flavefaciens* attaches to cellulose by ionic interactions between cellulose and its abundant glycocalyx observed in electron microscopy (Cheng, 1983/84). Mechanisms of adhesion of *B. succinogenes* seem to rely on energetic processes and the bound between bacteria and cellulose could involved bacterial cellulases themselves.

(**Key Words:** Adhesion, Cellulolytic Bacteria, Cellulose)

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