COMPARATIVE ANALYSIS OF TWO ATTACHMENT VARIANTS OF BUTYRIVIBRIO FIBRISOLVENS

Djarot Sasongko Hami Seno

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Contents

Summary

The attachment of *Butyrivibrio fibrisolvens* to surfaces was studied. *B. fibrisolvens* strain E14, sticky (S) and loose (L) that had been reported previously (Nili and Brooker 1995) were used as models. In preliminary studies, the two cell types were compared; studies included physical and growth characteristics in defined, solid or liquid medium containing various carbon sources, the presence of compounds that may induce or inhibit attachment, and their phenotypic stability. Extracellular protein, chromosomal DNA, plasmid and 16S rDNA profiles of the two variants were examined. Compared to the non-adhering L cells, the adhering S cells were shinier, spherical, more intensely pigmented (yellow), more firmly attached to the agar surface and could only be removed with scraping. After longer incubation, the cells were released from the agar but the colonies tended to stick together, and only became separable when further incubated. In contrast, the L cells were non spherical, loosely attached to the agar and separable at all stages of growth. In liquid medium, the S cells tended to clump during the early stages of growth, and be dispersed at later stages. The L cells were dispersed throughout the medium at all stages of growth. The phenotypes of the 2 variants were stable; both variants maintained their characteristics through multiple passages on solid and in liquid medium. The presence of molecules that induced attachment of S or inhibited attachment of L cells were not detected, but it was noted that S cells produced more extensive extracellular polymer than did L cells. The extracellular proteins, chromosomal DNA, endogenous plasmid, and 16S rDNA profiles of the two variants were identical, indicating that they were of the same species.

The effect of attachment on nutrient utilisation was studied by comparing the growth of the two variants in various carbon or nitrogen sources, as well as their xylanase, CMCase and proteolytic activities. Although not significant, the attachment of S cells seemed to have a slight effect on nutrient utilisation, compared to L cells.

The morphology of the variants were compared and examined by scanning electron microscopy (SEM). Extracellular polymer (EP) biosynthesis and attachment was studied using S and L samples prepared at various stages of growth. The effect of carbon source on morphology was studied using S and L samples prepared from cells grown in the presence of various carbon sources. The L cells seemed unable to spread EP to surfaces or to neighbouring cells, forming globular EP. Compared to other soluble carbon sources, cellobiose seemed to induce more globular (condensed) EP and less polymer spreading. It was also observed that EP might be secreted to the medium at stationary phase.

The level of EP production as well as its monosaccharide and fatty acid composition between S and L cells was compared. The cell associated, secreted or total EP was isolated using gradient centrifugation, cell free medium (Stack 1988) or plate (Berri and Rollings 1995) methods, respectively. The effect of carbon source on the level of EP as well as the monosaccharide and fatty acid composition was also studied. The S cells tended to produce more EP than the L cells; the maximum ratio of EP production between S and L cells was approximately 2:1. There were no differences in the monosaccharide and fatty acid compositions between S and L EP, but the proportions of components did

differ. This was most pronounced in lipid, especially C16:0 content, which was much higher in S than in L EP. Compared to other soluble carbon sources, less EP and reduced C16:0 was produced in cells grown on cellobiose. It was observed that the S and L EP behaved differently during phenol or water/methanol-chloroform extraction, which suggested property differences between their EP. The C16:0 may reflect the presence of lipoteichoic acid (LTA), and there were indications that there may be an association between LTA and extracellular polysaccharide (EPS) in S but not in L EP.

An attempt was made to isolate attachment gene(s). S chromosomal DNA was electroporated into competent *E. coli* or *B. fibrisolvens* E14 L cells using pBHerm plasmid as a vector. No sticky *E. coli* transformants were observed, but sticky-like transformants were observed from L cells that were transformed with S DNA. These transformants were stable through 5 passages under selection pressure (10 g/ml of erythromycin), although some revertants were observed after the 3rd passage when the passage was carried out without selection. Total chromosomal DNA and 16 S rDNA profiles of the transformants were identical to the original variants, and together with hybridisation analysis suggested that the transformants and the variants were related. There were indications that chromosomal integration occurred within the sticky-like transformants, probably due to homology between the donor and the host DNA.

These studies have shown that *B. fibrisolvens* strain E14 S and L cells are closely related and that attachment of S cells is associated with characteristics and lipid

content of their EP. Genetic complementation studies suggest that a change in attachment phenotype can be brought about by chromosomal integration.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Djarot Sasongko Hami Seno and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Djarot Sasongko Hami Seno

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S = *B. fibrisolvens* E14 variant S

L = *B. fibrisolvens* E14 variant L

g = glucose

cb = cellobiose

x = xylan

xc = crystalline cellulose

suc = sucrose

m = maltose

st = starch

DF = defined medium

CMC = carboxymethyl cellulose

DFg, DFcb, DFx, DFxc, DFsuc, DFm, DFst indicate defined medium containing glucose, cellobiose, xylan, crystalline cellulose, sucrose, maltose or starch, respectively

DfgE = defined medium containing glucose and erythromycin 10 μg/ml

Sg, Scb, Sx, Sxc, Ssuc, Sm, Sst indicate sample or culture of S variant grown in DFg, DFcb, DFx, DFxc, DFsuc, DFm, or DFst, respectively. Similarly for Lg, Lcb, Lx, Lxc, Lsuc, Lm, Lst.

LB = Luria Bertani

BHI = Brain Heart Infusion

LTA = lipoteichoic acid

WTA = wall teichoic acid

EPS = extracellular polysaccharide

EP = extracellular polymer

DTT = Dithiothreitol

PMSF = Phenyl methyl sulfonyl fluoride