ROLE OF MOTILITY AND CHEMOTAXIS IN SOLVENT PRODUCTION BY *CLOSTRIDIUM ACETOBUTYLICUM*

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To those who love me and whom I love,
especially my husband Ian and
my parents, Luding and Turing.

ABSTRACT

The motility of Clostridium acetobutylicum P262 and its relationship to solventogenesis were investigated. Motility was monitored in a typical batch fermentation process using sulphuric acid casein whey permeate as substrate. The motile behaviour of C. acetobutylicum was characterized by "runs" wherein the cells were observed to swim in a long, smooth line, then the cells "tumbled" by thrashing around for a few seconds before running again. The "runs" were particularly associated with the early phase of sugar utilization and acid production, while "tumbles" were associated with the onset of solventogenesis. During solvent production, the cells tumbled more frequently and the runs progressively became shorter and slower. The proportion of cells in the culture which exhibited motility increased to almost 100% up to 13h after inoculation, but decreased considerably after this time.

Assays for positive chemotaxis (chemoattraction) and negative chemotaxis (chemorepulsion) were performed to identify the chemoeffectors of C. acetobutylicum. Motile cells of C. acetobutylicum were observed to migrate towards glucose, galactose, and lactose. These sugars were identified as attractants. Acetate and butyrate elicited a dual response. Cells were repelled from the dissociated form and attracted towards the undissociated form above a minimum threshold concentration. Chemoattraction to butyric acid was observed at a threshold concentration of 9×10^{-2} M which is similar to the concentration of undissociated butyric acid inside the cell (1.3×10^{-2} M) at which solventogenesis is reported to be initiated, suggesting that the intracellular butyric acid concentration is the likely switch for

solventogenesis to commence. The solvents acetone, butanol and ethanol were identified as repellents.

The behavioural response of C. acetobutylicum towards the sugars, acids and solvents demonstrates that the motility observed during fermentation is a chemotactic response. Chemotaxis appears to provide survival advantage to C. acetobutylicum.

A non-motile mutant was isolated by mutagenesis using ethyl methane sulfonate. This mutant was morphologically indistinguishable from the motile parent strain, such that it possesses flagella in typical number and shape as those of the parent, and is capable of producing clostridial forms and endospores. This type of mutant is a paralyzed mutant and the mutation may be a defect in any of the genes that code for flagellar rotation.

The non-motile mutant was capable of solvent production suggesting that motility is not a regulatory mechanism for the switch to solvent production, but merely a behavioural chemotactic response. However, the maximum butanol concentration achieved, the initial rate of butanol production, the yield, and the sugar utilization observed with the mutant were poorer than those of the parent strain. These confirm the positive relationship between motile, chemotactic cultures and solvent production. The low butanol production by the non-motile mutant suggests that the mutant has a lower butanol tolerance than does the parent.

Inhibition studies have shown that both growth and solvent production of C. acetobutylicum are subject to end-product toxicity. Growth inhibition studies confirmed that the non-motile mutant was less tolerant to solvents than was the parent.

A deficiency in membrane-bound ATPase activity was observed with the non-motile mutant but not in the parent strain. This deficiency in ATPase activity, lack of motility, and lower butanol tolerance may explain the low butanol production by the mutant.

On a percentage basis, greater inhibition of solvent production was observed in the parent than in the mutant suggesting that butanol toxicity during the solvent production phase is more profound in the presence of another target site (i.e. ATPase) in addition to the cell membrane. It was further suggested that during growth, butanol inhibition due to membrane disruption was more important than inhibition of ATPase.

Thus, chemotaxis prevents C. acetobutylicum from being confined in a toxic situation. Motile cells are more solventogenic because they can chemotactically respond to changes in their environment, and are less susceptible to product inhibition.

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ABBREVIATIONS

ABE	Acetone-Butanol-Ethanol
ATP	Adenosine 5'-tri-phosphate
ATPase	ATP phosphohydrolase
CCCP	Carbonylcyanidene-m-chloro-phenylhydrazone
CFU	Colony forming unit
CM	Chemotaxis Medium
CMMG	Cooked Meat Medium, supplemented with glucose
CoA	Coenzyme A
CW, CCW	Clockwise, Counterclockwise rotation
DCCD	N, N'-Dicyclohexylcarbodiimide
EDTA	Ethylenediaminetetraacetate
EMS	Ethyl-methanesulfonate
FCCP	Carbonylcyanide-p-tri-fluoromethoxyphenylhydrazone
MCP	Methyl-accepting chemotaxis protein
MNNG	N-methyl-N'-nitro-N-nitrosoguanidine
NAD^+, NADH	Nicotinamide adenine dinucleotide, and its reduced form
NYG	Nutrient Broth Yeast Extract Agar
OD	Optical density
PEP	Phosphoenolpyruvate
pHi	Internal or intracellular pH
Pi	Inorganic phosphate
PIPES	Piperazine N N'-bis-2-ethane-sulphonic acid
RCA	Reinfored Clostridial Agar
SSM	Semi-Synthetic Medium
TA	Tryptone Agar
WPA	Sulphuric acid-casein Whey Permeate Agar