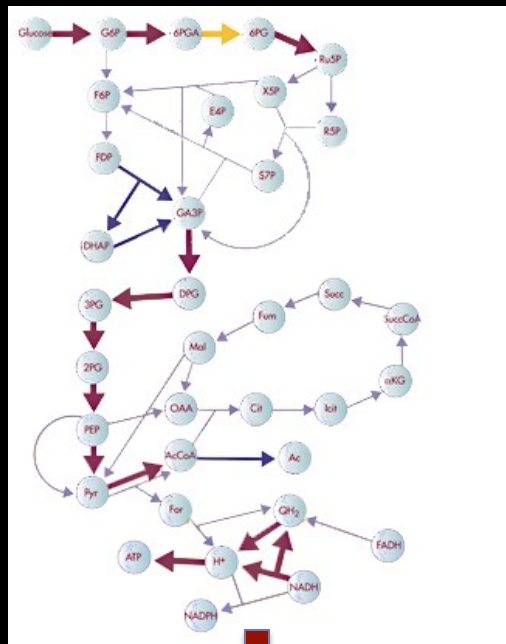


# Redireccionamiento de fluxos metabólicos

---

# Formação de produtos microbianos

## ■ Micro-organismos



## ■ Organismos obtidos na natureza

## ■ Produto interesse

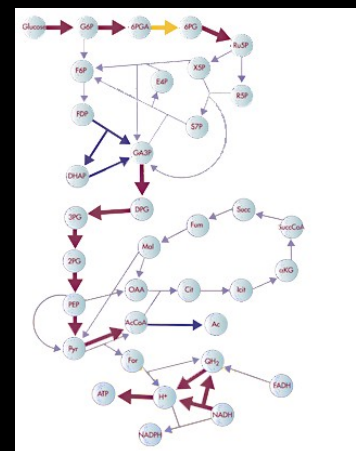
# Formação de produtos microbianos

## ■ Micro-organismos

### ■ Vias metabólicas

- Arranjo
- Coordenação

REDE



- Produto com aplicação produzido em escala comercial

# Necessidade de otimizar

---

- Reações de conversão
  - Propriedades cinéticas
  - Produtividades
  - Eficiência de conversão
  - Uso de matérias-primas
- Ação em diferentes pontos para tornar o produto comercialmente interessante

# Necessidade de otimizar

---

- Melhoramentos genéticos podem ser requeridos

- Avaliar investimento vs ganhos

# Como melhorar?

---

- Screening de linhagens/genes mais eficientes
- Técnicas de Engenharia Bioquímica
- Engenharia Genética, Biologia Sintética
- ...

# Como melhorar?

- Entender o metabolismo envolvido
  - Que rede metabólica é funcional no micro-organismo para chegar ao produto-alvo?

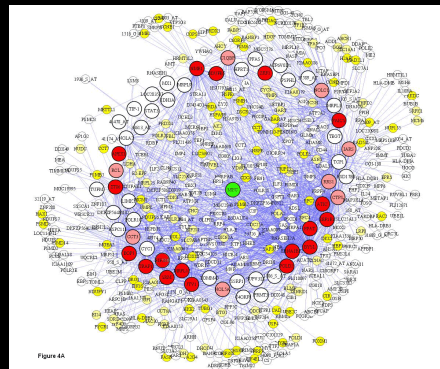
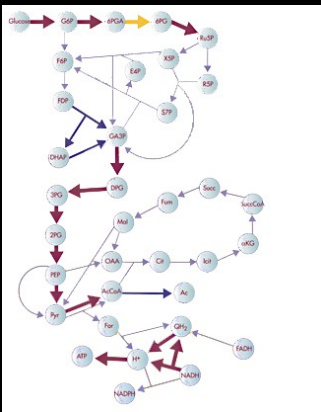
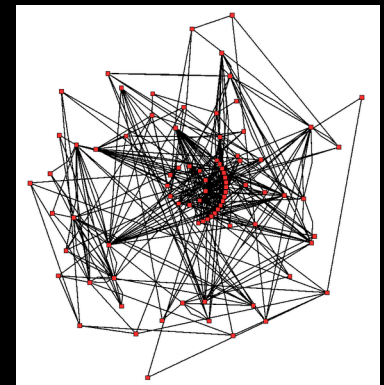
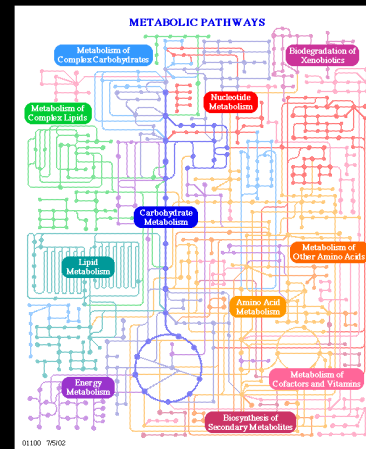


Figure 4A



# Aplicações de manipulação de vias

---

- Melhorar rendimento/produktividade
- Expandir a capacidade de uso de substratos
- Formação de novos produtos
- Melhorar propriedades gerais da célula
- Degradação de xenobióticos



# Aplicações de manipulação de vias

---

- Qualquer produto de origem microbiana pode ter a produção melhorada pela manipulação e redirecionamento das vias de sua rede metabólica

# Tópicos a considerar

---

- Complexidade da rede metabólica
- Regulação
- Coordenação das vias (envolvidas na formação do produto) com o metabolismo global

# Tópicos a considerar

---

- Diversos métodos têm sido aplicados com sucesso
- Aplicações mais efetivas para o melhoramento requerem
  - Modificações coordenadas de mais de uma etapa enzimática dentro da rede metabólica
- Destaca-se aqui o potencial da engenharia metabólica

# Aumento do rendimento e produtividade

---

- A maior parte dos processos industriais se inclui nesta categoria – requer melhoria de rendimento e produtividade
  - Rendimento  $\neq$  Produtividade

# Aumento do rendimento e produtividade

---

- Rendimento –
  - Afetado principalmente pelo custo da matéria-prima
  - Estratégia para o melhoramento:
    - redirecionamento dos fluxos metabólicos em direção à formação do produto desejado

# Aumento do rendimento e produtividade

---

- Produtividade –
  - Afetada por custo (capital) de equipamentos pra bioprocessar o produto
  - Depende da taxa de captação da matéria-prima pelo organismo:

# Aumento do rendimento e produtividade

---

- Produtividade –
  - Depende da taxa de captação da matéria-prima pelo organismo
  - Importante – não deve haver formação de subprodutos

# Aumento do rendimento e produtividade

---

- Produtividade –
  - Estratégia de melhoramento:
    - Amplificação dos fluxos metabólicos



# Aumento do rendimento e produtividade

---

## ■ Produtividade –

- Taxa de captação da matéria-prima pelo organismo:

- Valores muito baixos requerem:

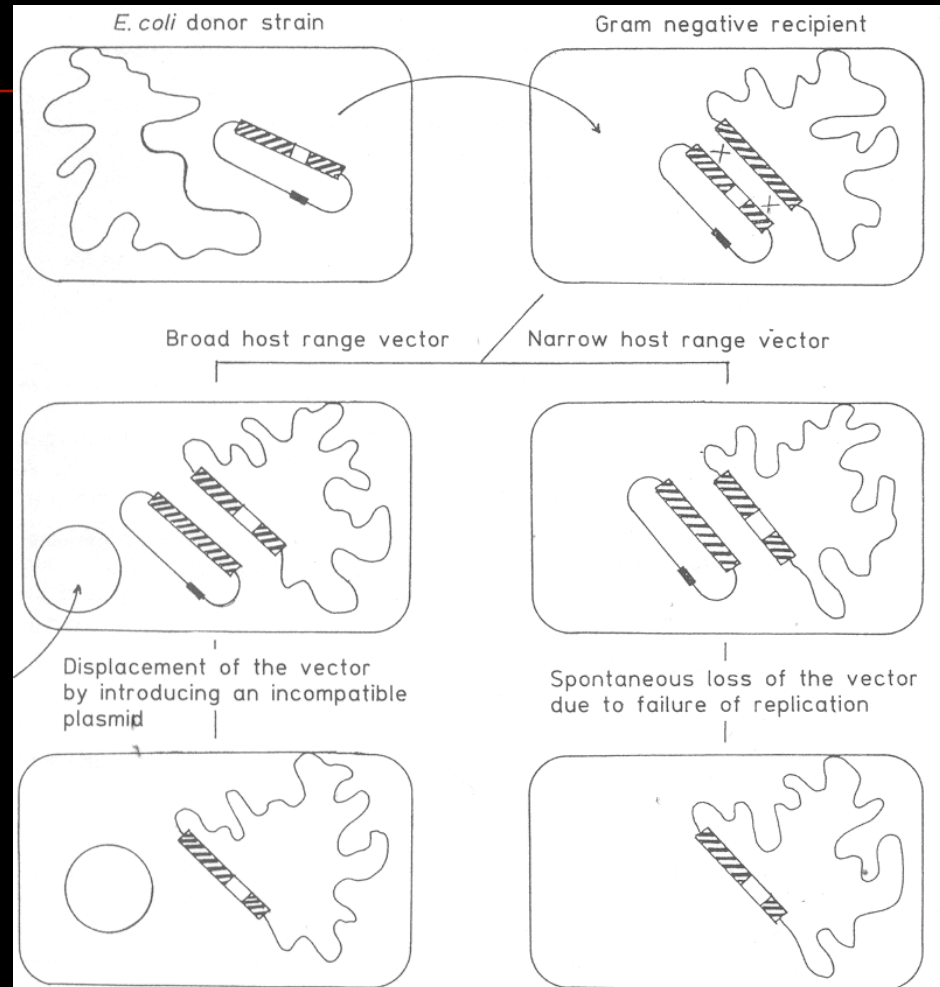
- a) melhoramento do sistema de transporte de substrato
- b) redirecionamento de fluxos

# Modificando vias

---


- Mutaç o cl ssica (agentes f sicos e qu micos)
- T cnicas de recombina o

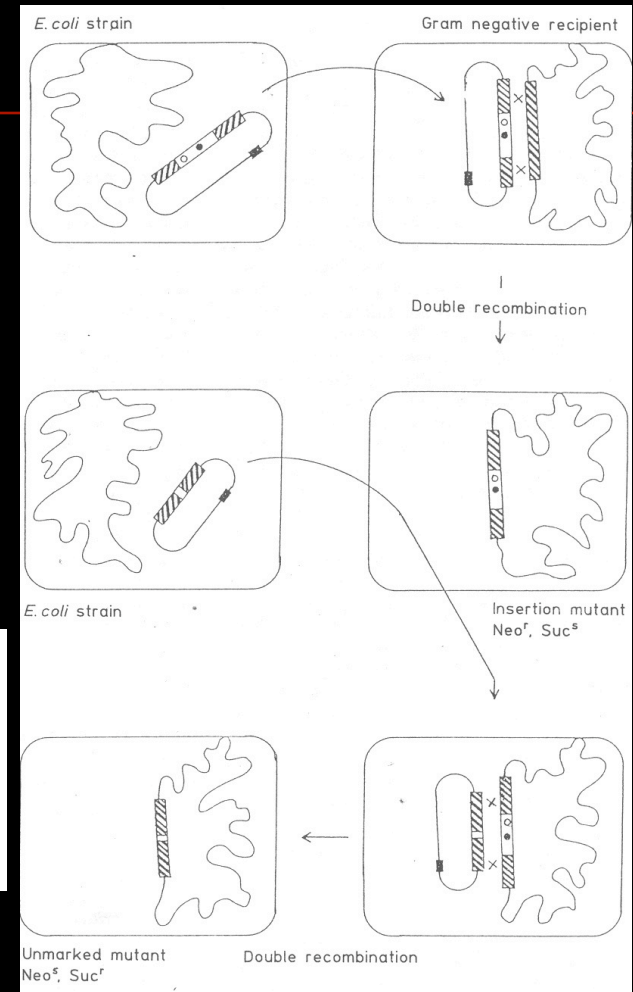
# Integração genômica de mutações com marcação



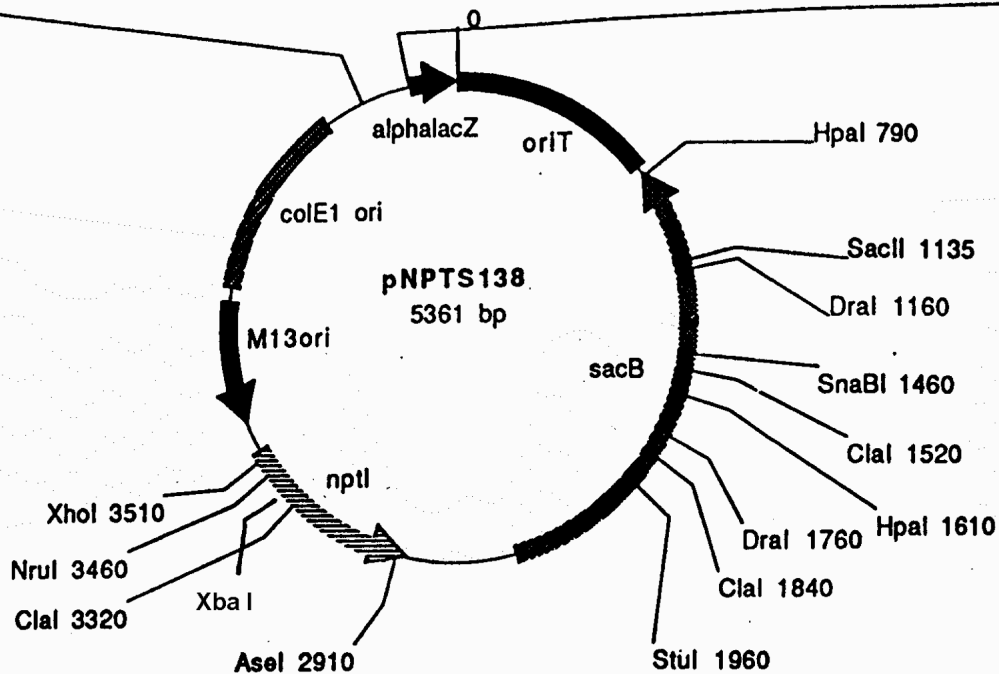
**Fig. 5.** Genomic integration of cloned DNA by double recombination events (gene replacement). A cloned (here mutagenized) fragment is transferred from *E. coli* into the Gram negative recipient by conjugation. A double crossing-over event between the genomic and the introduced DNA regions leads to the replacement of the wild type (genomic) with the mutated (cloned) DNA fragment. In order to select these marker exchange events, the recombinant vector plasmid has to be eliminated from the recipient. If the vector molecule is able to replicate in the host (broad host range vector) it is usually displaced by introducing a second plasmid of the same incompatibility group and selecting for the maintenance of the incoming plasmid. If vectors with a narrow replication host range are used (e.g., non-replicative pSUP vectors), they are automatically segregated and lost in the host organism.

# Integração genômica de mutações sem marcação

The fragment, cloned in a narrow host range vector, is mutagenized in *E. coli* by inserting the *nptI-sacB-sacR* cassette (symbolized by ). The recombinant plasmid is introduced into the Gram negative recipient and double recombination events are isolated by selection for neomycin resistance (Neo<sup>r</sup>; expressed from the *nptI* gene) and loss of the vector encoded resistance marker (marked by a black box). The insertion mutant, generated by this gene replacement is sucrose-sensitive (Suc<sup>s</sup>) due to the presence of the *sacB* gene. In a second step, most of the *nptI-sacB-sacR* cartridge is removed from the cloned fragment, resulting in an unmarked point mutation (frame shift). The recombinant molecule is introduced from *E. coli* into the insertion mutant, where a second double recombination event can be selected on plates containing sucrose. Only cells which have lost the *nptI-sacB-sacR* cartridge by marker exchange are able to grow on high sucrose concentrations (Neo<sup>s</sup>, Suc<sup>r</sup>).



SnaBI.17.SpeI.ApaI.MunI.NgoMl.KasI.HindIII.PstI.EcoRV.BamHI.EcoRI.NheI.EagI.MluI.BspEI.BsrGI.SphI.SalI.StuI.AflIII.T7



**Plasmid name:** pNPTS138

**Plasmid size:** 5361 bp

**Constructed by:** Dickon

**Construction date:** 11-94

**Comments/References:** pUC based plasmid with novel polylinker and M13 ori (not tested), nptI and sacB genes from pDELTA1 (EcoRV-XbaI) and oriT.

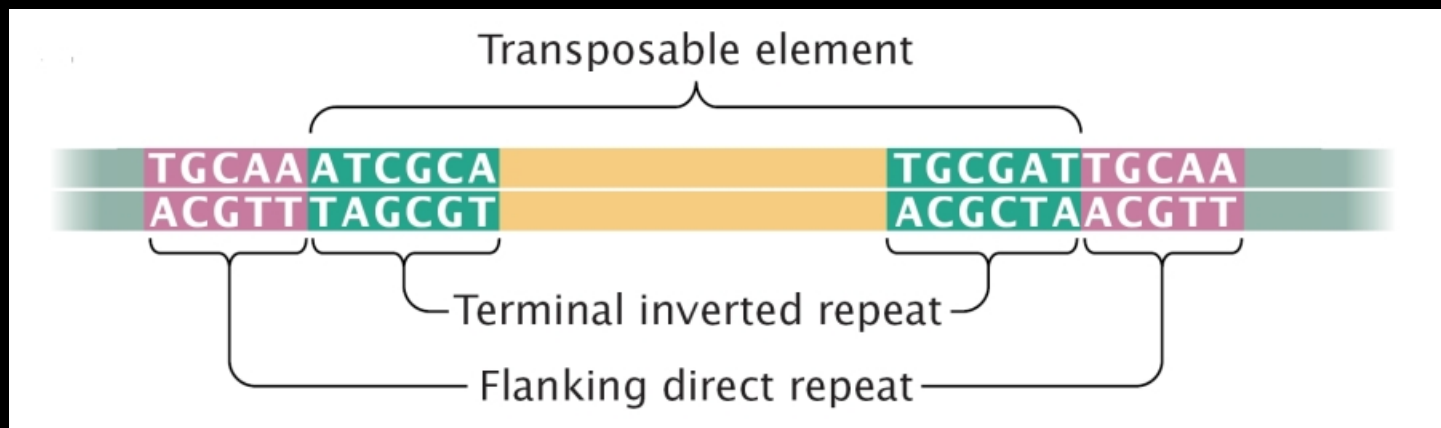
# Transposons “*jumping genes*”

Barbara McClintock

1930s

Cold Spring Harbor Laboratory – New York

Nobel Prize in 1983



Many transposable elements have common characteristics. Most transposable elements generate flanking direct repeats on each side of the point of insertion into target DNA. Many transposable elements also possess terminal inverted repeats

# Transposons “*jumping genes*”

---



Variation in kernel phenotypes is used to study transposon behavior.

Kernels on a maize ear show unstable phenotypes due to the interplay between a transposable element (TE) and a pigment

# Etanol

---

- Impactos
- Não compete com produção de alimentos
- Possibilidade do uso do bagaço de cana



# Etanol

---

- Várias matérias-primas renováveis podem ser usadas na sua produção
  - *Custos de produção são determinados pelo custo da matéria-prima*
- Açúcar de cana
- Grãos amiláceos (milho)
- Materiais lignocelulósicos
  - Resíduos agroindustriais (bagaço, madeira, biopolpação, resíduos municipais sólidos)

# Etanol

---

- Materiais lignocelulósicos

- Redução em até 25% nos custos de produção

- Lignocelulose

- Baixo custo por ser de difícil digestão, não compete com produção de alimentos
- Difícil digestão= dificuldade na conversão em açúcares mais facilmente utilizáveis

# Etanol

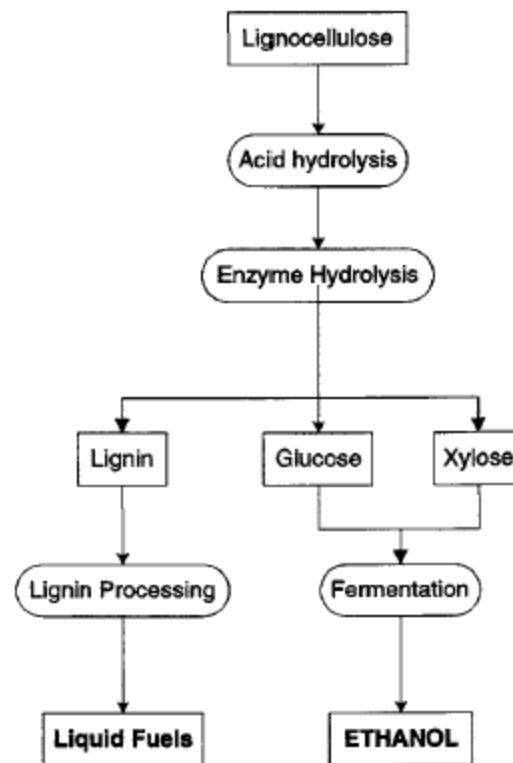
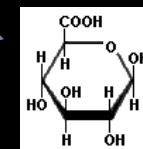
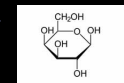
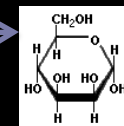
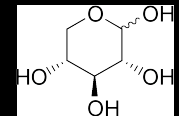
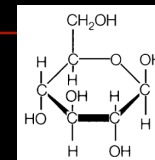


FIGURE 6.1 Conversion of lignocellulose to ethanol. Crystalline cellulose, the largest (50%) and most difficult fraction, is hydrolyzed by a combination of acid and enzymatic processes. During these steps 95-98% of the xylose and glucose is recovered. These monosaccharides subsequently are converted to ethanol by appropriate microorganisms.

# Etanol

## Carbohydrate Structural Polymers in Lignocellulose

Polymer	Monomer(s)	Typical % Total
Cellulose	Glucose	40
Hemicellulose	Xylose	30
	Mannose	
	Glucose	
	Galactose	
Lignin	Phenylpropane	25
Pectin	Uronic acids	5



Xilose é o segundo açúcar mais abundante na hemicelulose

# Etanol

Outros desafios:

- Mistura de açúcares
- Uso de pentoses
- Compostos tóxicos
- Levedura?
- Bactéria?

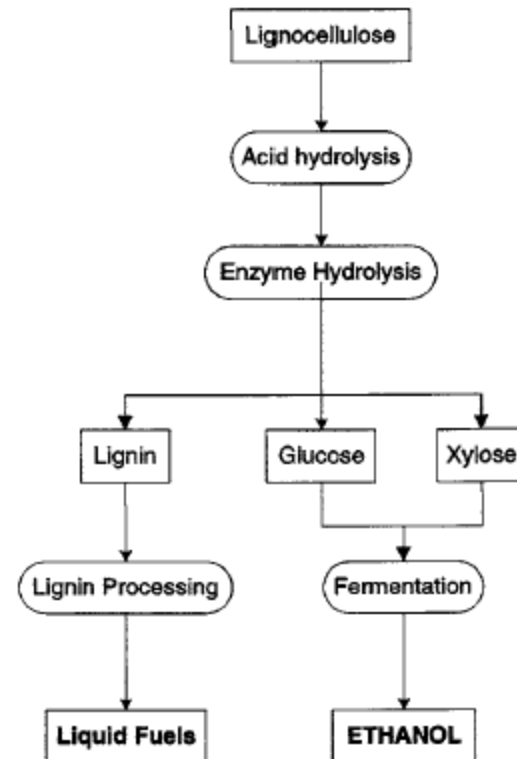


FIGURE 6.1 Conversion of lignocellulose to ethanol. Crystalline cellulose, the largest (50%) and most difficult fraction, is hydrolyzed by a combination of acid and enzymatic processes. During these steps 95-98% of the xylose and glucose is recovered. These monosaccharides subsequently are converted to ethanol by appropriate microorganisms.

# Melhoramento da produção de etanol

- Estudos iniciais: amplificação de PDC
  - Competição de outras vias
  - Produtos indesejáveis

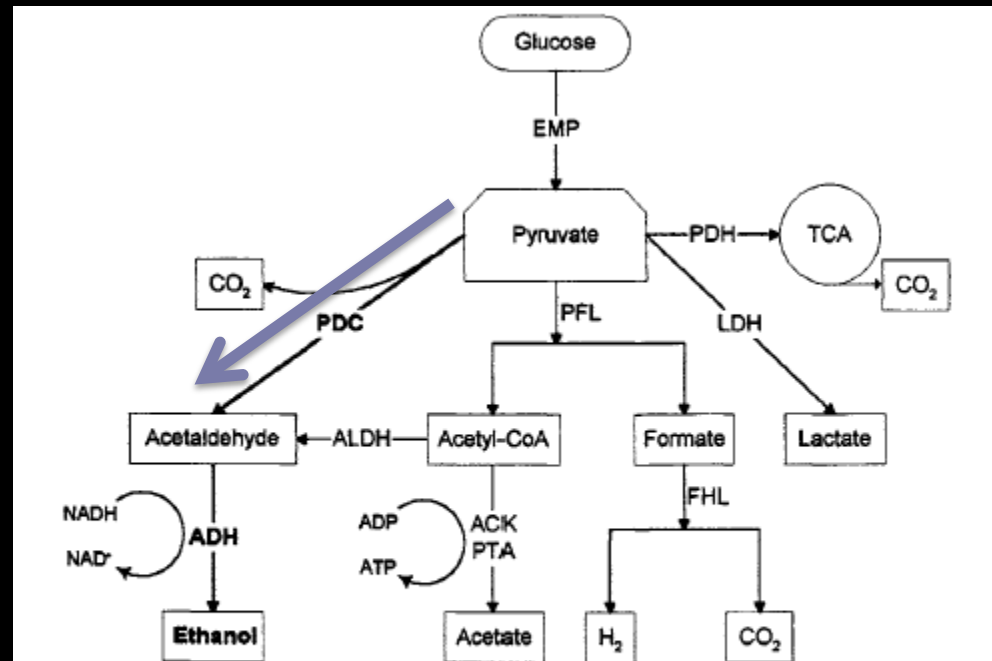


FIGURE 6.2 Competing pathways at the pyruvate branch point. Abbreviations: EMP, Embden-Meyerhof-Parnas enzymes and intermediates; PDC, pyruvate decarboxylase; ADH, alcohol dehydrogenase; PFL, pyruvate formate-lyase; ACK/PTA, phosphotransacetylase and acetate kinase; ALDH, acetaldehyde dehydrogenase; FHL, formate hydrogen lyase; LDH, lactate dehydrogenase; PDH, pyruvate dehydrogenase.

# Melhoramento da produção de etanol

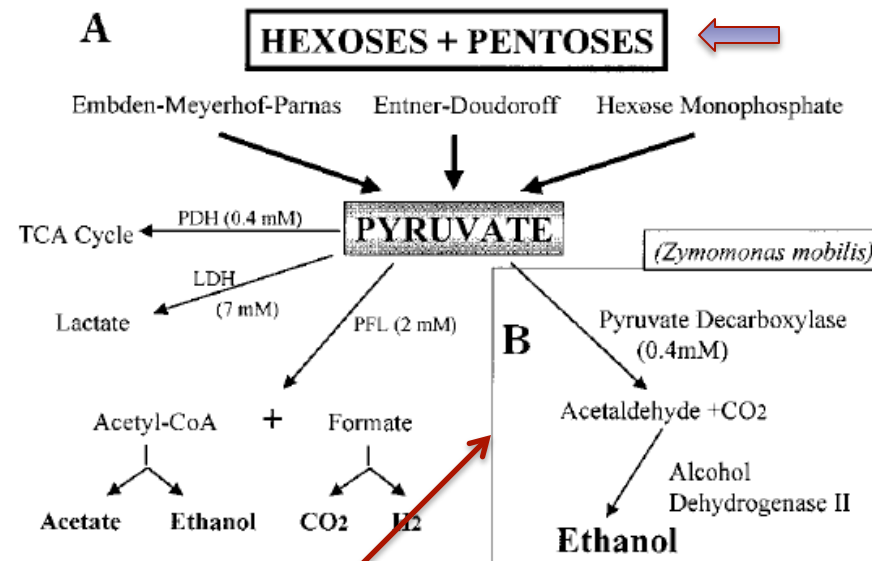


Figure 5. Fermentation pathways in recombinant *E. coli* that contain *Z. mobilis* genes for the conversion of pyruvate to ethanol, the **PET** operon (Ingram et al., 1987 and 1991). The PET operon containing pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase (*adhB*) has been used to engineer a similar ethanol pathway in many Gram negative bacteria. Succinate is also produced as a minor fermentation product, branching from phosphoenolpyruvate: (A) native *E. coli* pathways and (B) recombinant *Z. mobilis* homo-ethanol pathway.  $K_m$  values are shown in parenthesis.

Altos níveis de  
PDC e baixo  
 $K_m$

# Melhoramento da produção de etanol

TABLE 6.2 Comparison of Apparent  $K_m$  Values for Pyruvate for Selected *E. coli* and *Z. mobilis* Pyruvate-Acting Enzymes<sup>a</sup>

Organism	Enzyme	$K_m$	
		Pyruvate	NADH
<i>E. coli</i>	PDH	0.4 mM	0.18 mM
	LDH	7.2 mM	> 0.5 mM
	PFL	2.0 mM	
	ALDH		50 $\mu$ M
	NADH-OX		50 $\mu$ M
<i>Z. mobilis</i>	PDC	0.4 mM	
	ADH II		12 $\mu$ M

<sup>a</sup> Abbreviations: PDH, pyruvate dehydrogenase; LDH, lactate dehydrogenase; PFL, pyruvate formate lyase; ALDH, aldehyde dehydrogenase; NADH-OX, NADH oxidase; PDC, pyruvate decarboxylase; ADH II, alcohol dehydrogenase II.



# Melhoramento da produção de etanol

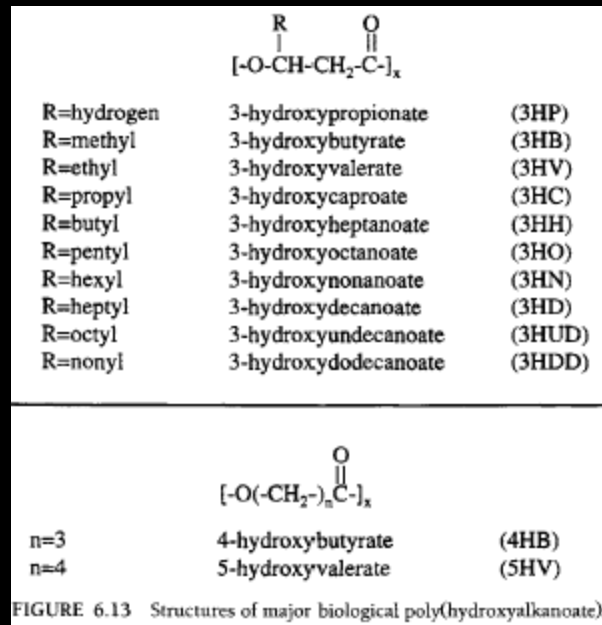
TABLE 6.3 Comparison of Fermentation Products during Aerobic and Anaerobic Growth of Wild-Type and Recombinant *E. coli*<sup>a</sup>

Growth	Plasmid	Fermentation Product (mM)			
		Ethanol	Lactate	Acetate	Succinate
Aerobic	None	0	0.6	55	0.2
	PLO1308-10 (PET)	337	1.1	17	4.9
Anaerobic	None	0.4	22	7	0.9
	PLO1308-10 (PET)	482	10	1.2	5.0

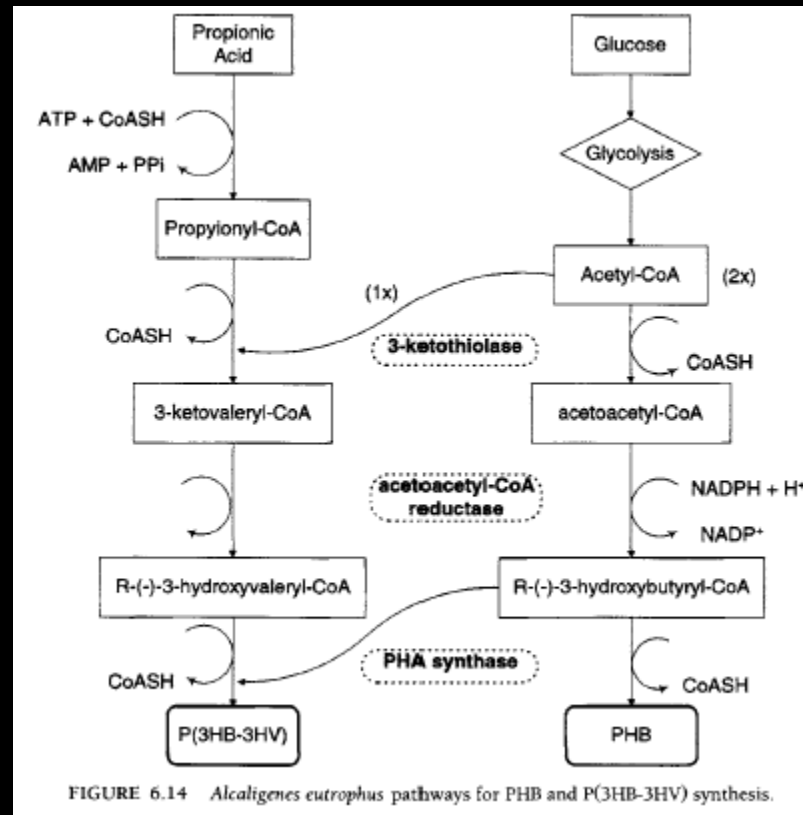
<sup>a</sup> From Ingram and Conway, 1988.

Construção similar em *Klebsiella oxytoca*  
US Patent 5,000,000 (Alterthum & Ingram)

# Melhoramento da produção de biopolímeros



# Melhoramento da produção de biopolímeros



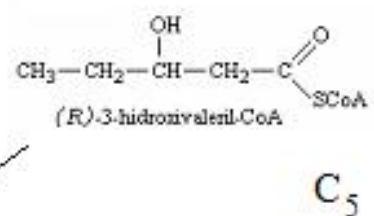
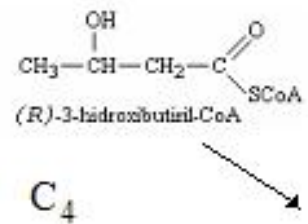
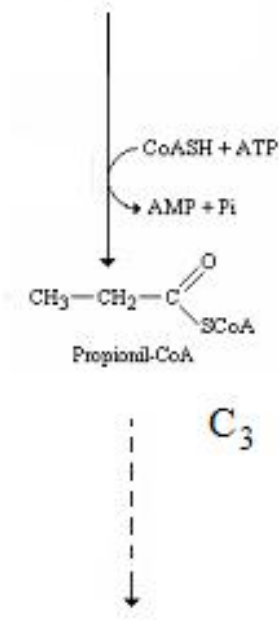
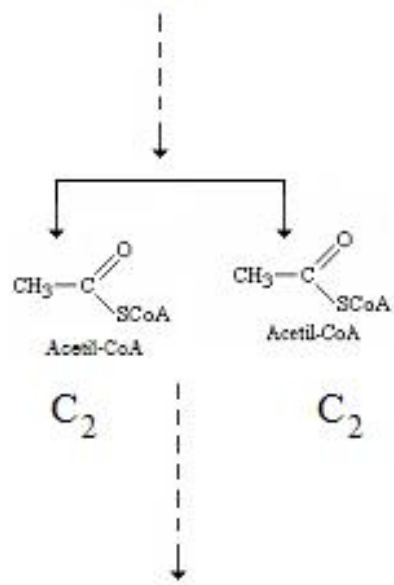
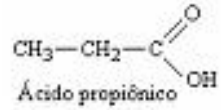
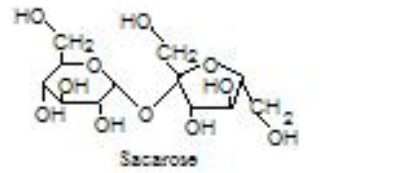
## Melhoramento da capacidade de uso de co-substratos

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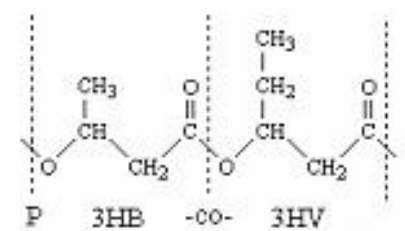
- 3HB-co-3HV tem propriedades melhoradas
- 3HV é obtido a partir de propionato
- A bactéria é pouco eficiente nesta conversão

# Biossíntese de PHB-co-HV

Baixa eficiência de uso do propionato



PHA sintase



Teórico

$$Y_{\text{HV/Prp}} = 1,35 \text{ g/g}$$

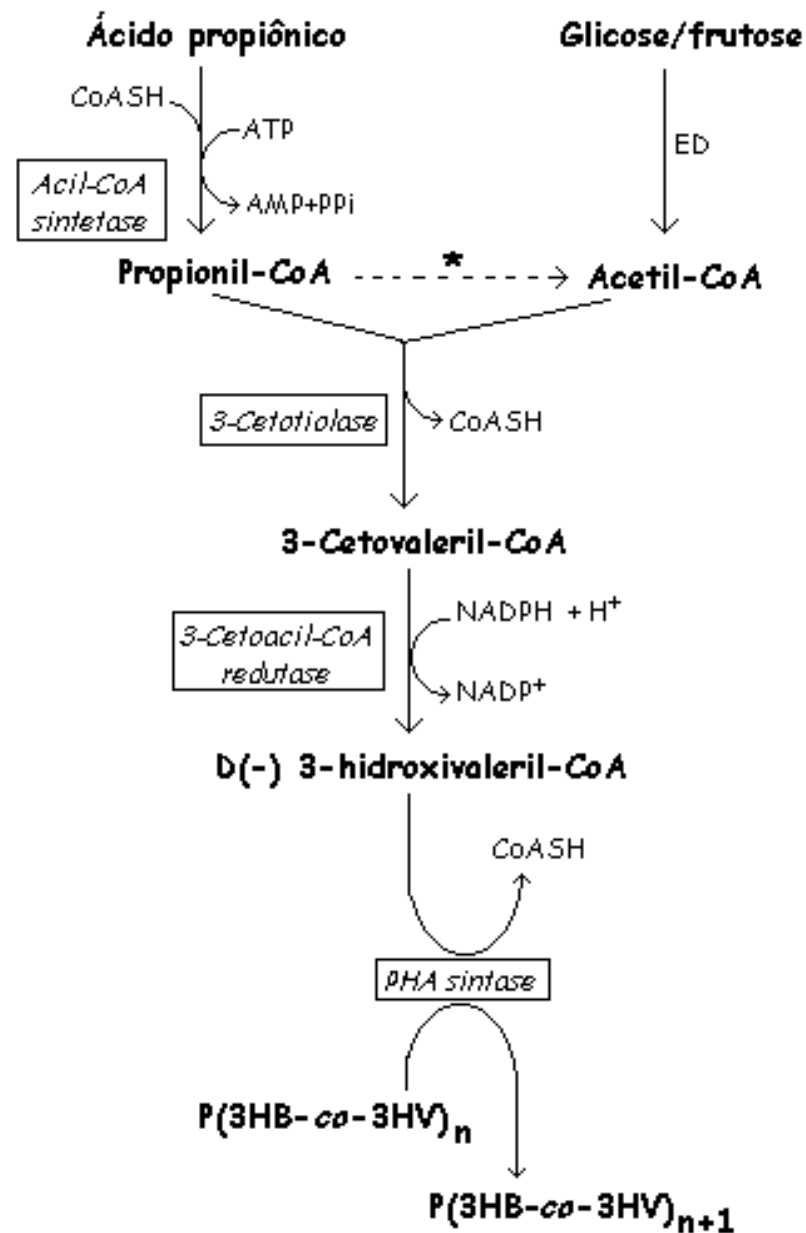
Real

$$Y_{\text{HV/Prp}} = 0,10 \text{ g/g}$$



**Table 2. Production of P(3HB-co-3HV) from glucose plus propionic acid.**

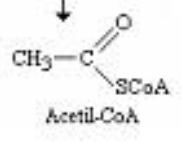
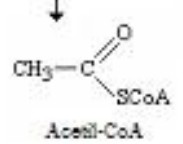
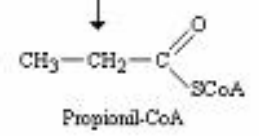
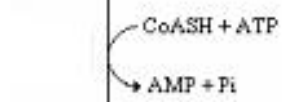
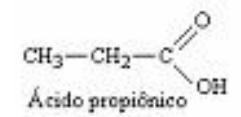
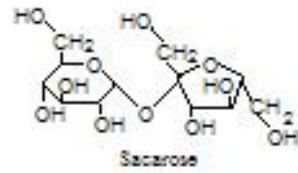
Strains	CDW <sup>c</sup> (g/L)	Residual Carbohydrates (%)	PHA			Y <sub>3HV/Prp</sub> <sup>b</sup>
			% CDW <sup>c</sup>	3HB mol%	3HV mol%	
<i>A. eutrophus</i> DSM 54	3,92	0,0	71,4	96,1	3,9	0,13
<i>A. latus</i> DSM 1123	0,95	101,8	14,6	55,0	45,0	0,07
<i>P.cepacia</i> DSM 50181	3,35	1,9	38,4	97,3	2,7	0,04
IPT-040	3,77	1,7	32,3	97,1	2,9	0,05
IPT-044	3,92	1,7	51,1	97,1	2,9	0,07
IPT-045	3,73	0,0	49,4	96,2	3,8	0,08
IPT-048	2,97	0,0	44,3	96,2	3,8	0,06
IPT-055	4,27	72,2	1,5	100,0	0,0	0,00
IPT-056	3,60	31,3	30,9	98,5	1,5	0,02
IPT-076	5,06	1,9	56,8	97,1	2,9	0,10
IPT-083	4,89	5,2	56,8	96,9	3,1	0,10
IPT-086 <sup>a</sup>	2,06	75,	39,0	89,9	10,1	0,09
IPT-098	5,90	0,0	17,7	94,7	5,3	0,07
IPT-101	2,98	41,8	32,3	95,4	4,6	0,05



Desvio do propionato a acetil-CoA

ED: Via de Entner-Doudoroff

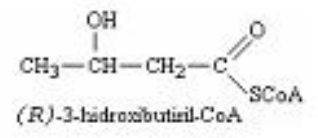
\* Vias de degradação de propionil-CoA



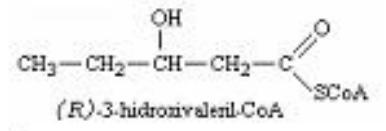
C<sub>2</sub>

C<sub>2</sub>

C<sub>3</sub>

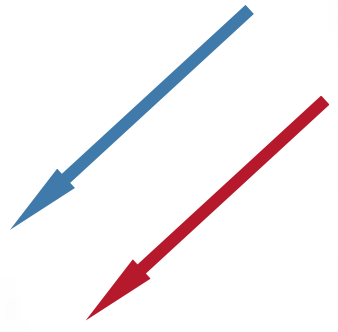
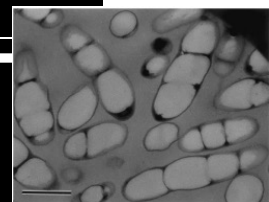
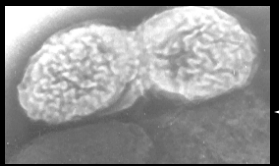
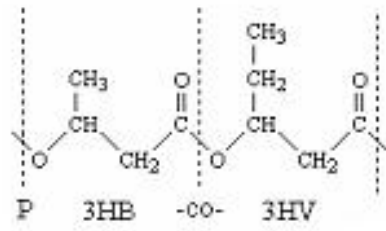


C<sub>4</sub>

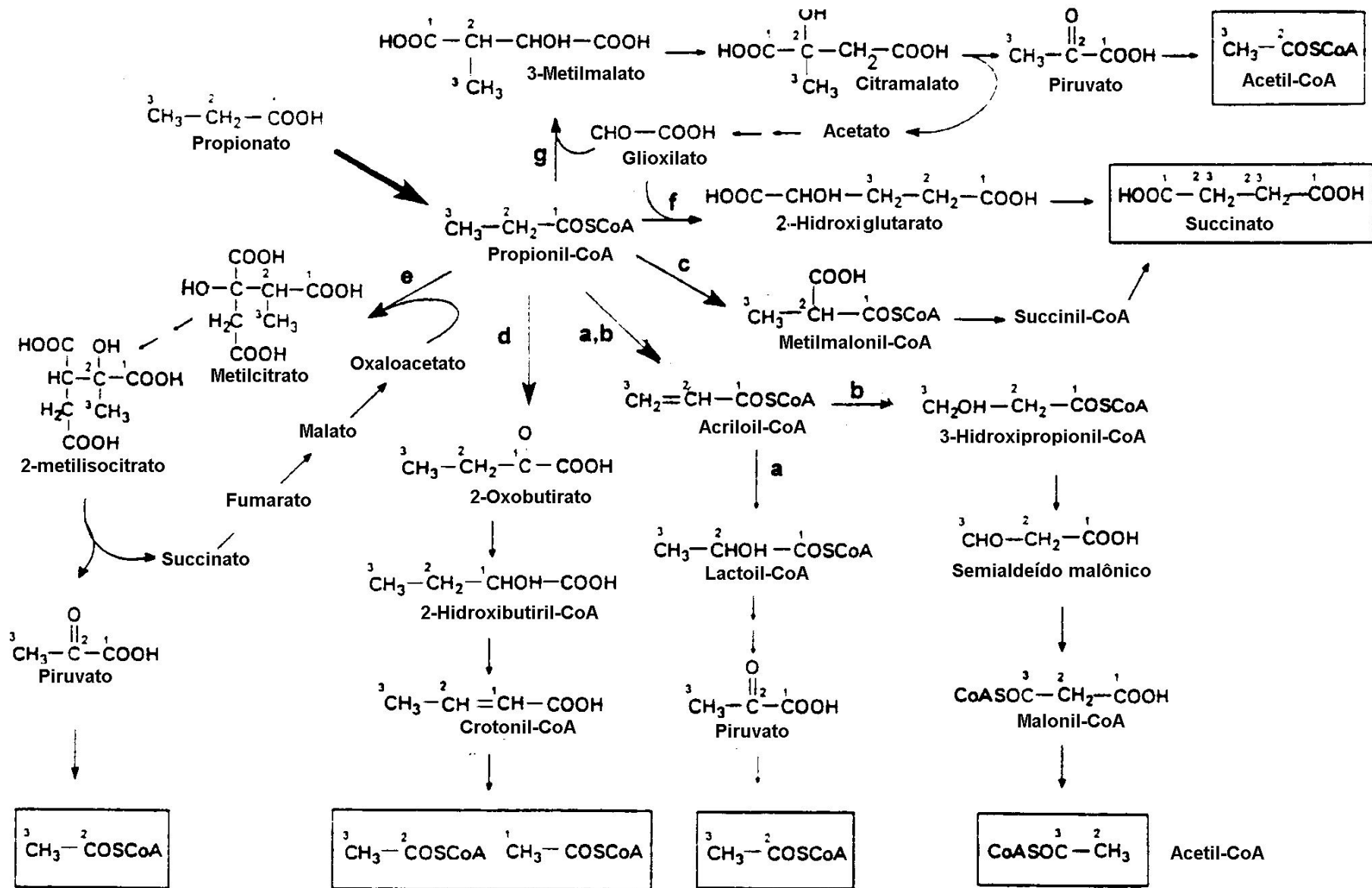


C<sub>5</sub>

PHA sintase

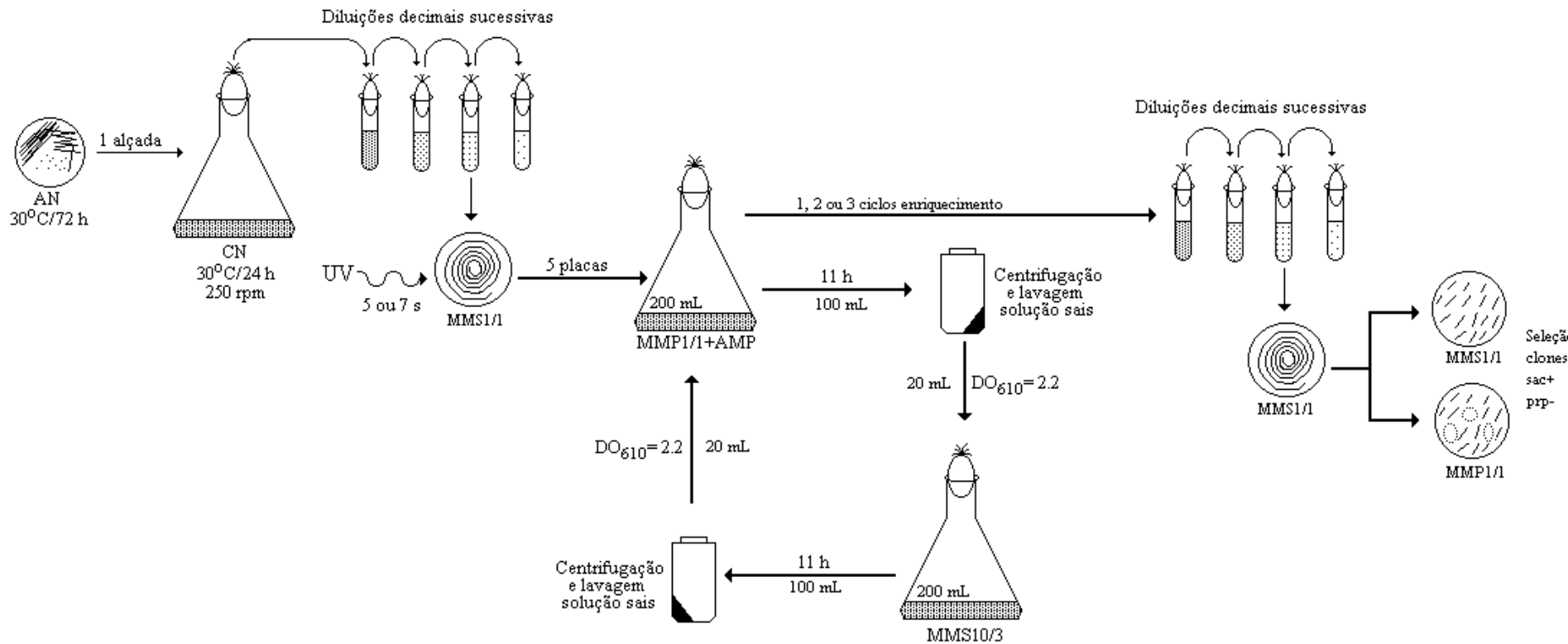






Textor et al., 1997 modificado

# Obtenção de mutantes $prp^- sac^+ PHA^+$



# Grupos fenotípicos de mutantes prp de *Burkholderia* sp 101

Group number	Phenotype <sup>a</sup>	Number of mutants
Wild type	suc <sup>+</sup> acet <sup>+</sup> prp <sup>+</sup> lac <sup>+</sup> pyr <sup>+</sup>	-
I	suc <sup>+</sup> acet <sup>-</sup> prp <sup>-</sup> lac <sup>-</sup> pyr <sup>-</sup>	219
II	suc <sup>+</sup> acet <sup>-</sup> prp <sup>-</sup> lac <sup>?</sup> pyr <sup>-</sup>	5
III	suc <sup>+</sup> acet <sup>-</sup> prp <sup>-</sup> lac <sup>+</sup> pyr <sup>-</sup>	3
IV	suc <sup>+</sup> acet <sup>+</sup> prp <sup>-</sup> lac <sup>+</sup> pyr <sup>+</sup>	13

suc = sucrose / acet = acetate / prp = propionate / lac = lactate / pyr = pyruvate

Efficiency of *B. sacchari* mutants in converting propionic acid to 3HV units.

Strain	Phenotype	Results		
		3HB mol%	3HV mol%	$Y_{3HV/prp}$ (g/g) <sup>c</sup>
IPT 101 <sup>d</sup>	wild type	93.8	6.2	0.10
IPT 183	I	84.1	15.9	0.34
IPT 185	II	82.6	17.4	0.35
IPT 190	III	80.1	19.9	0.37
IPT-195	IV	39.0	61.0	0.81
IPT 196	IV	33.2	66.8	0.78
IPT 189	IV	44.7	55.3	0.81

Teórico

$Y_{HV/Prp}=1,35$  g/g

Incrementos na produção de unidades HV empregando diferentes estratégias, como resultado de trabalho multidisciplinar para produzir o copolímero PHB-co-HV, com propriedades melhoradas

Linhagem bacteriana	Ferramentas empregadas	Y <sub>3HV/Prp</sub> * (g/g)
<i>Burkholderia sacchari</i>	Microbiologia - Isolamento de solo	0,10 <sup>♠</sup>
Mutante 189	Melhoramento genético	0,81 <sup>+</sup>
Mutante 189	Engenharia bioquímica	1,34 <sup>º</sup>

\*Fator de conversão de ácido propiônico em unidades HV

<sup>♠</sup>Gomez *et al.*, 1996 <sup>+</sup>Silva *et al.*, 2000 <sup>º</sup>Rocha ,2002 (Rocha et al., 2008)

# Produção industrial melhorada

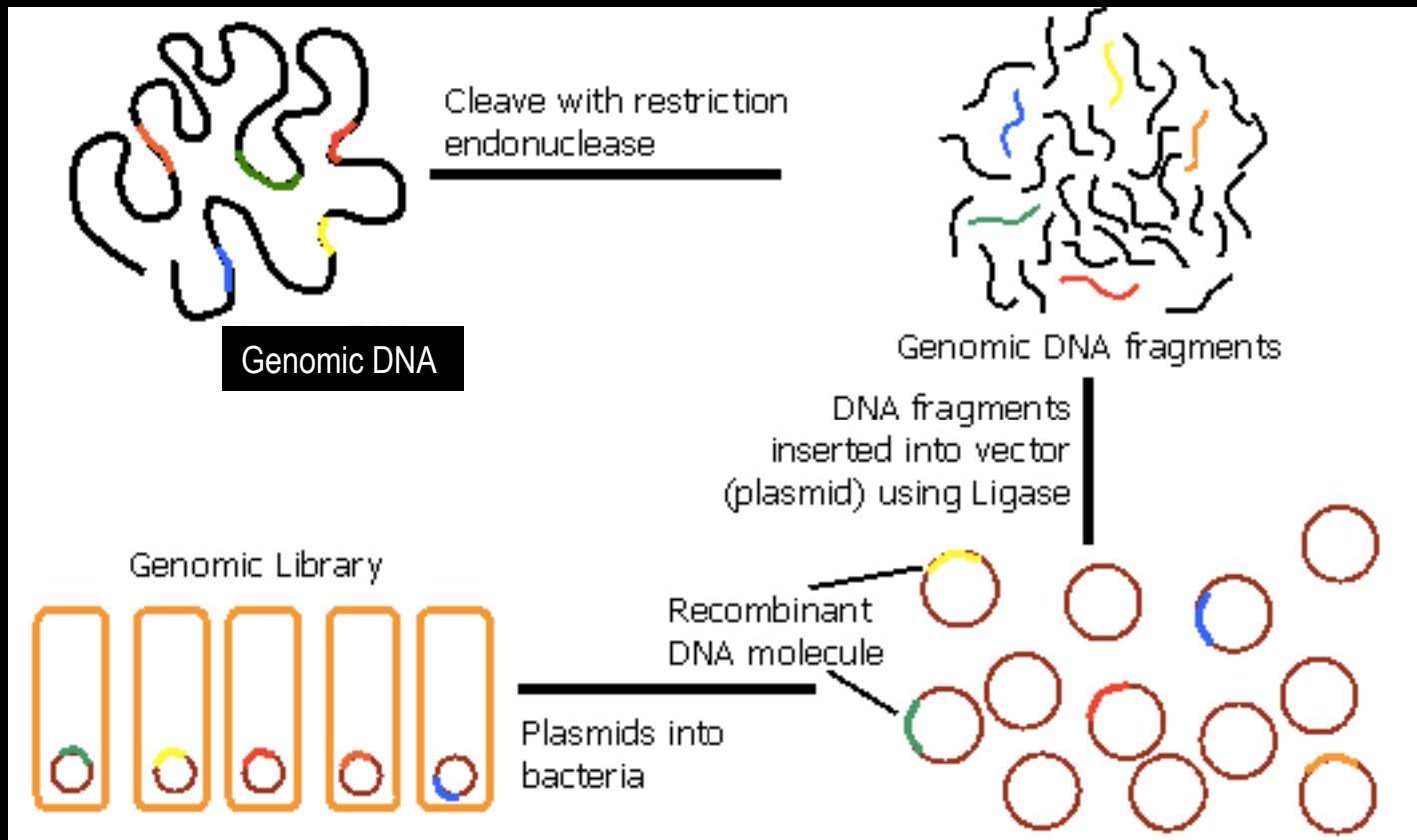
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## Experimentos em biorreator

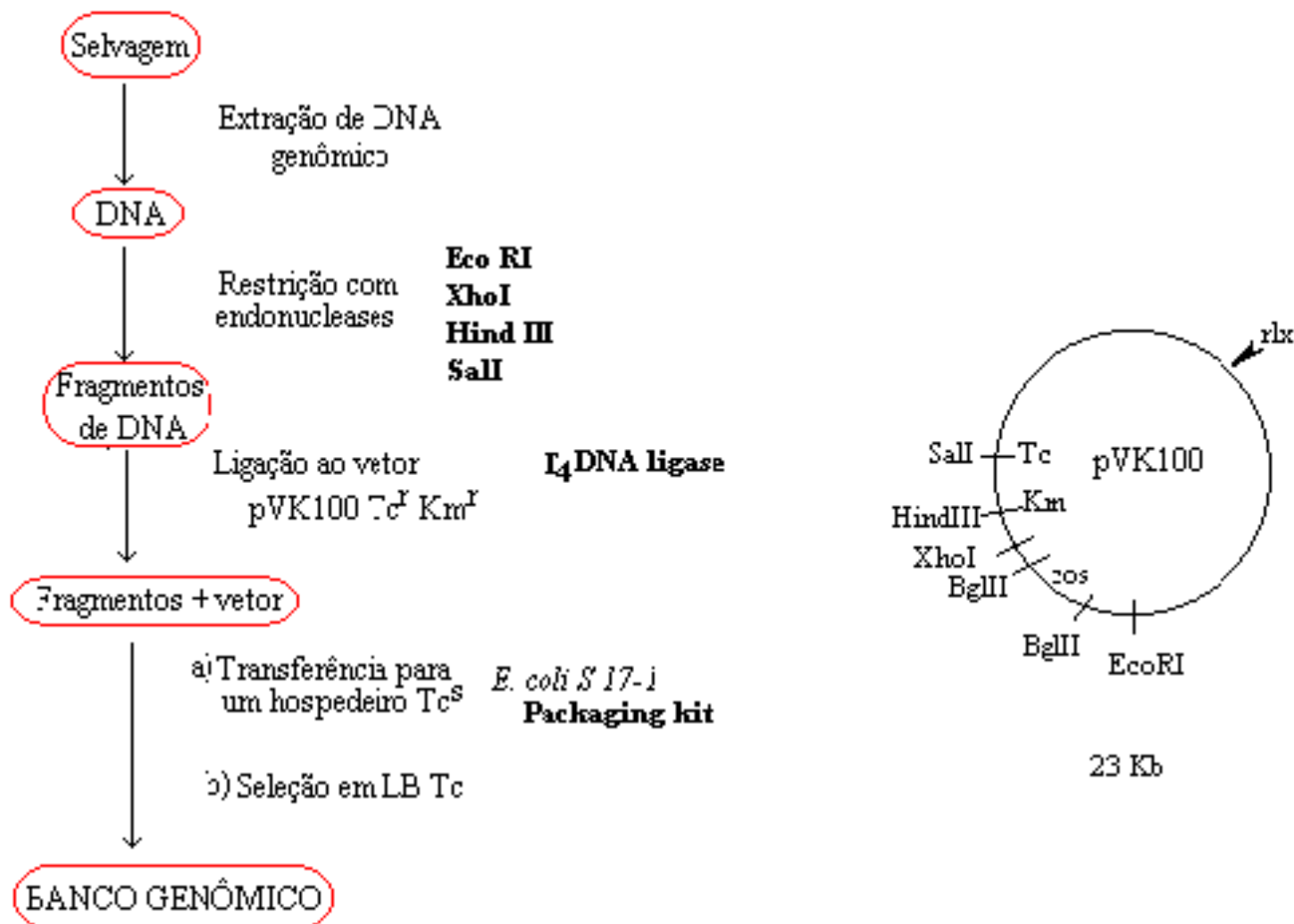
variam a taxa de alimentação  $\mu_{prp}/sac$   
atingem o máximo teórico 1,35 g/g

Mas qual era o gene afetado no mutante, afinal?

# Cosntrução de uma biblioteca genômica



# Construção de biblioteca genômica de *Burkholderia* sp IP<sup>-</sup> 101



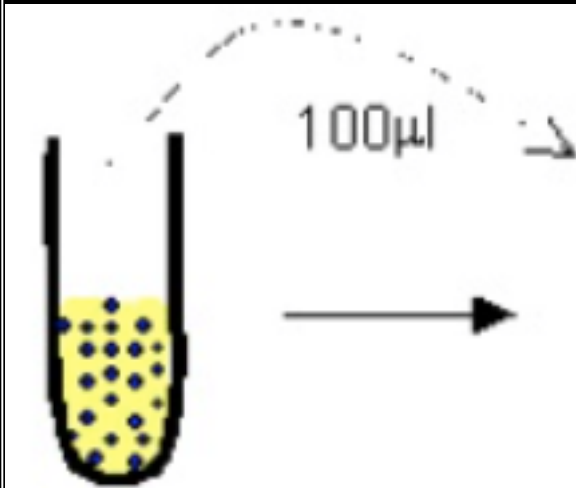


# Minicomplementação

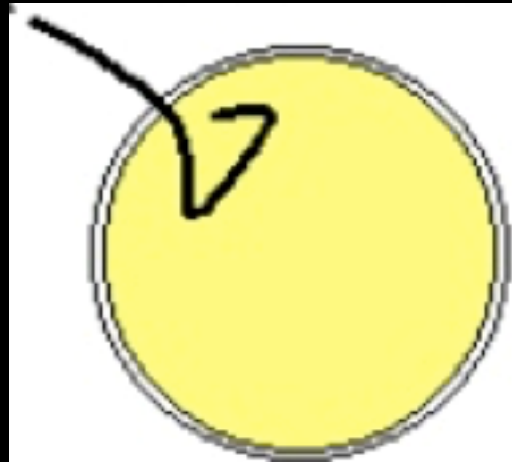
Biblioteca genômica da selvagem 101 em *E. coli*

MM Sacarose + antibiótico

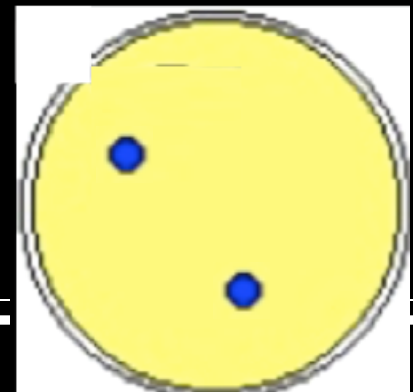
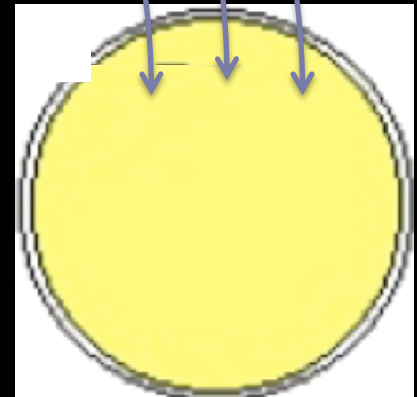
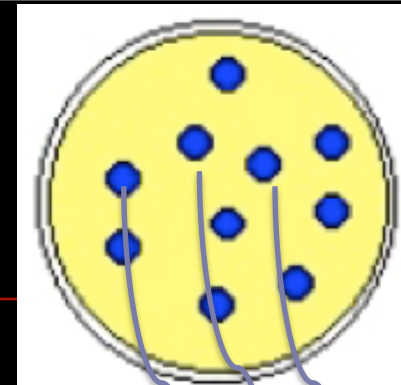
MM Propionato + antibiótico



Cultura do mutante 189



Semeadura por espalhamento formando um tapete



## Clones do banco genômico capazes de complementar o mutante IPT 189

Clone	Plasmídio	Tamanho dos Fragmentos Eco RI (Kb)						Total
		2.0	3.5	5.0	12.0	8.0	10.0	
2929	p2929	2.0	3.5					5.5
17227	p17227	2.0	3.0	5.0	12.0			22.0
1762	p1762	1.5	3.5	5.0	8.0	10.0		28.0
4116	p4116	2.0	2.5	3.5	4.5	7.0	10.0	29.5

Subclone	Plasmídio	Tamanho dos subfragmentos EcoRI (Kb)			
		E <sub>2</sub>	Outros		Total
A <sub>26</sub>	pA <sub>26</sub>	2.0	3.0	5.0	10.0
B <sub>6</sub>	pB <sub>6</sub>	2.0			2.0
B <sub>7</sub>	pB <sub>7</sub>	2.0	3.0		5.0
B <sub>10</sub>	pB <sub>10</sub>	2.0			2.0
B <sub>27</sub>	pB <sub>27</sub>	2.0			2.0
C <sub>14</sub>	pC <sub>14</sub>	2.0			2.0

E<sub>2</sub> → seqüenciamento → citrato sintase (?)

subclonagem

digestão

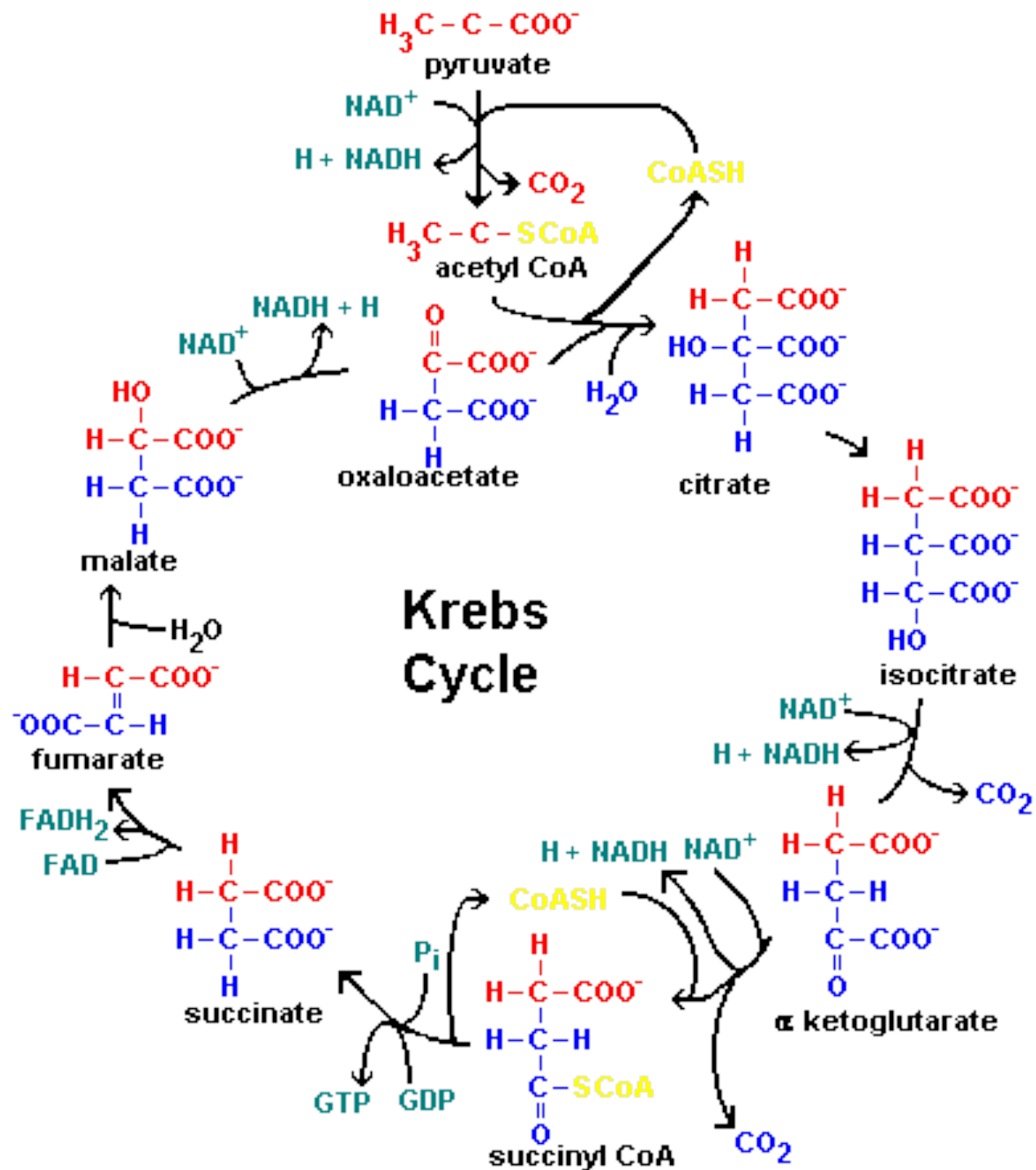
SalI, SmaI, XhoI, ApaI, PstI, HindIII, EcoRV

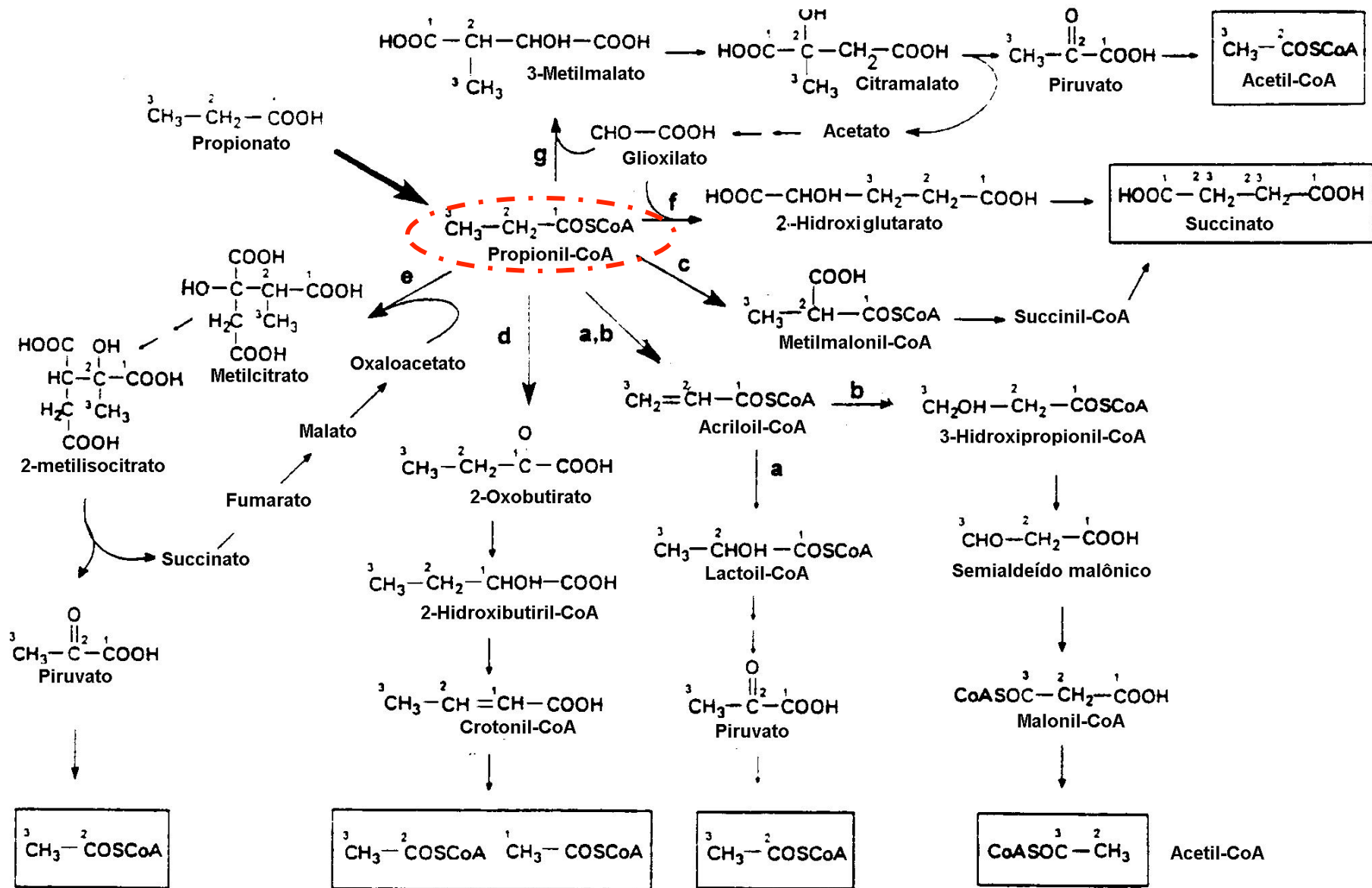
e EcoRI

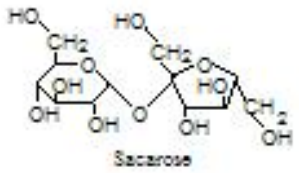
S<sub>1</sub> 1.1 Kb

seqüenciamento

citrato sintase (?)

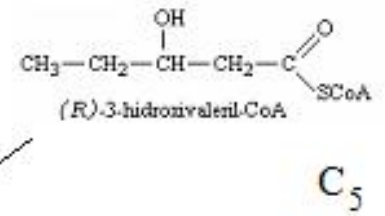
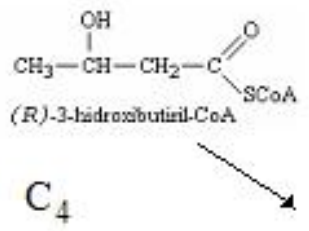
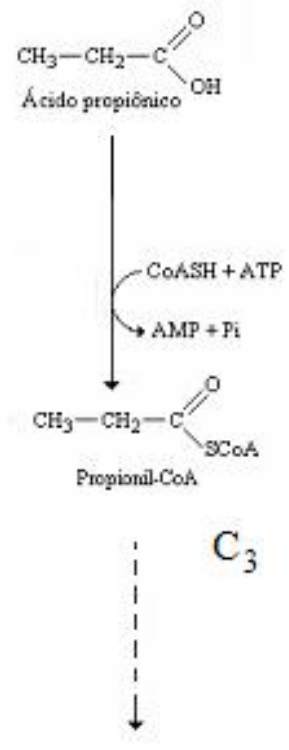
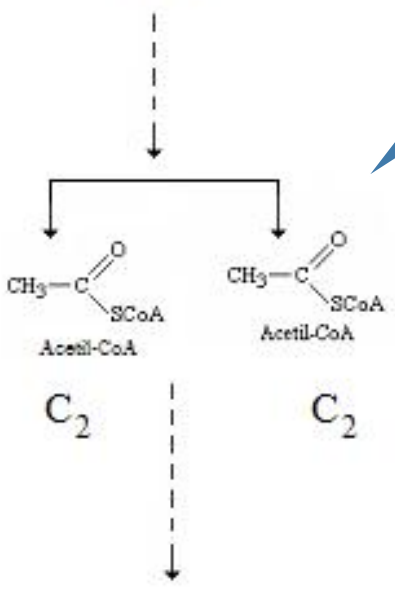




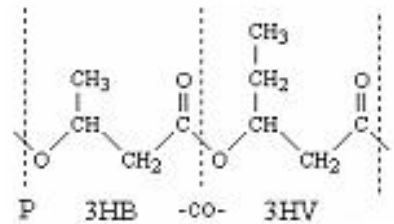


$\alpha$ -oxidação

2MCC



PHA sintase



Teórico

$Y_{\text{HV/Prp}} = 1,35 \text{ g/g}$

Real

$Y_{\text{HV/Prp}} = 0,10 \text{ g/g}$

# Catabolismo de propionato em

*Burkholderia sacchari*

**Propostas**  
**Pelo menos duas vias**  
**envolvidas:**  
**alfa-oxidação**  
**ciclo de 2-**  
**metilcitrato**

Identification of the 2-Methylcitrate Pathway Involved in the Catabolism of Propionate in the Polyhydroxyalkanoate-Producing Strain *Burkholderia sacchari* IPT101<sup>T</sup> and Analysis of a Mutant Accumulating a Copolyester with Higher 3-Hydroxyvalerate Content

C. O. Brämer,<sup>1</sup> L. F. Silva,<sup>2</sup> J. G. C. Gomez,<sup>2</sup> H. Priefert,<sup>1</sup> and A. Steinbüchel<sup>1\*</sup>

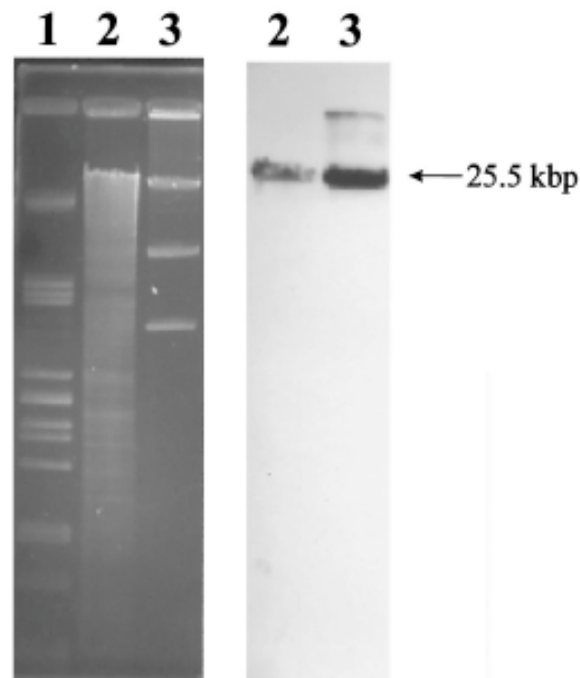
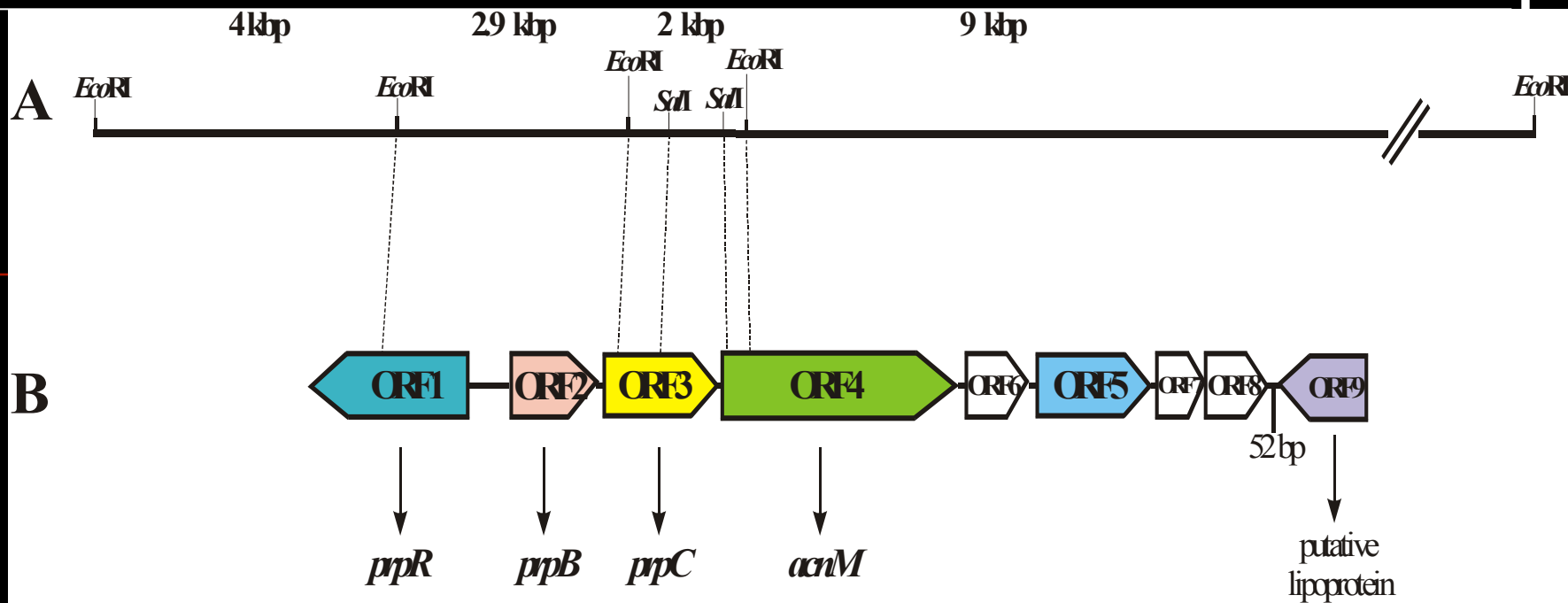
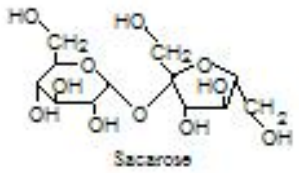


FIG. 1. Southern hybridization of *E. coli* S17-1 clones, harboring *Hind*III-digested genomic DNA of *B. sacchari* IPT101<sup>T</sup> in cosmid pHC79, identified by colony hybridization by using the 2-kbp *Eco*RI fragment as a probe. Lane 1, *Pst*I-digested  $\lambda$  DNA; lanes 2, *Hind*III-digested genomic DNA of *B. sacchari* IPT101<sup>T</sup>; lanes 3, *Hind*III-restricted hybrid cosmids of the *E. coli* S17-1 clone.



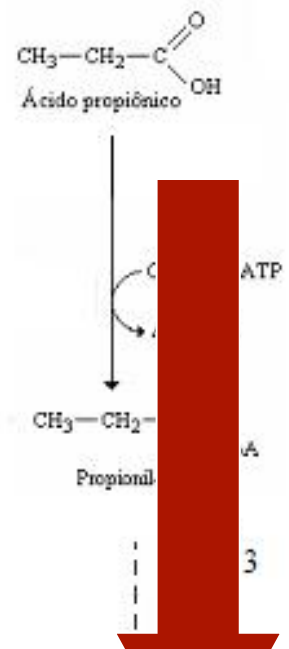
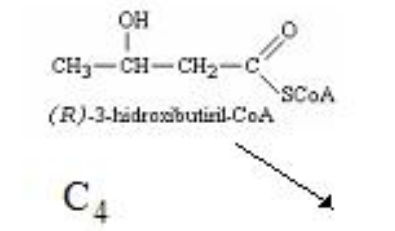
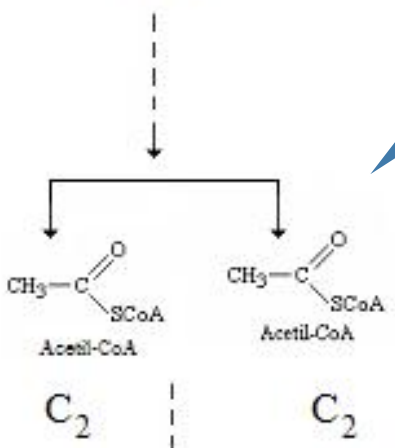
**Figure 2:** (A) Arrangement of four *EcoRI* subfragments on the 25.5-kbp *HindIII* fragment of *B. sacchari* IPT101<sup>T</sup>; (B) Organisation of the *prp* locus in *B. sacchari* IPT101<sup>T</sup>.



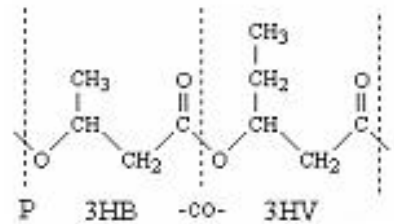


$\alpha$ -oxidação

2MCC



PHA sintase



Teórico

$Y_{HV/Prp} = 1,35 \text{ g/g}$

Real

$Y_{HV/Prp} = 0,10 \text{ g/g}$

Identificação e inativação de vias competidoras

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**Disruption of the 2-methylcitric acid cycle and evaluation of poly-3-hydroxybutyrate-co-3-hydroxyvalerate biosynthesis suggest alternate catabolic pathways of propionate in *Burkholderia sacchari***

**Erica Mendes Pereira, Sonia Regina Silva-Queiroz, José Gregório Cabrera Gomez, and Luiziana Ferreira Silva**

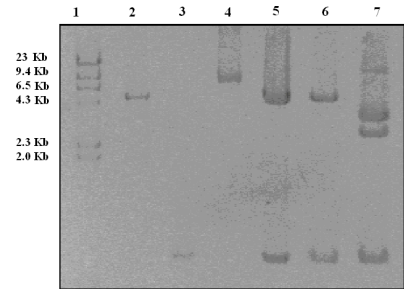
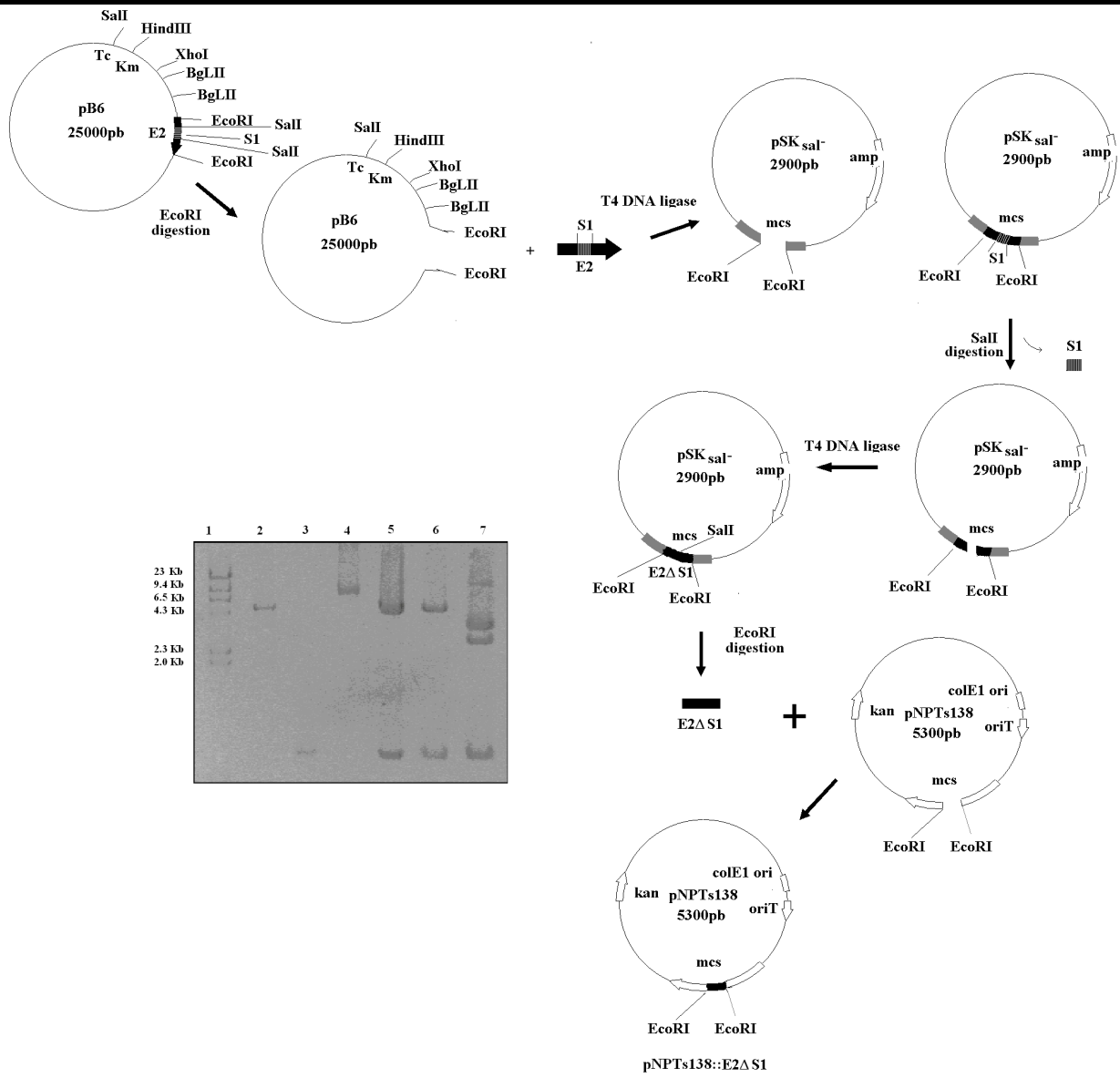
Can. J. Microbiol. 55: 688–697 (2009)

doi:10.1139/W09-018

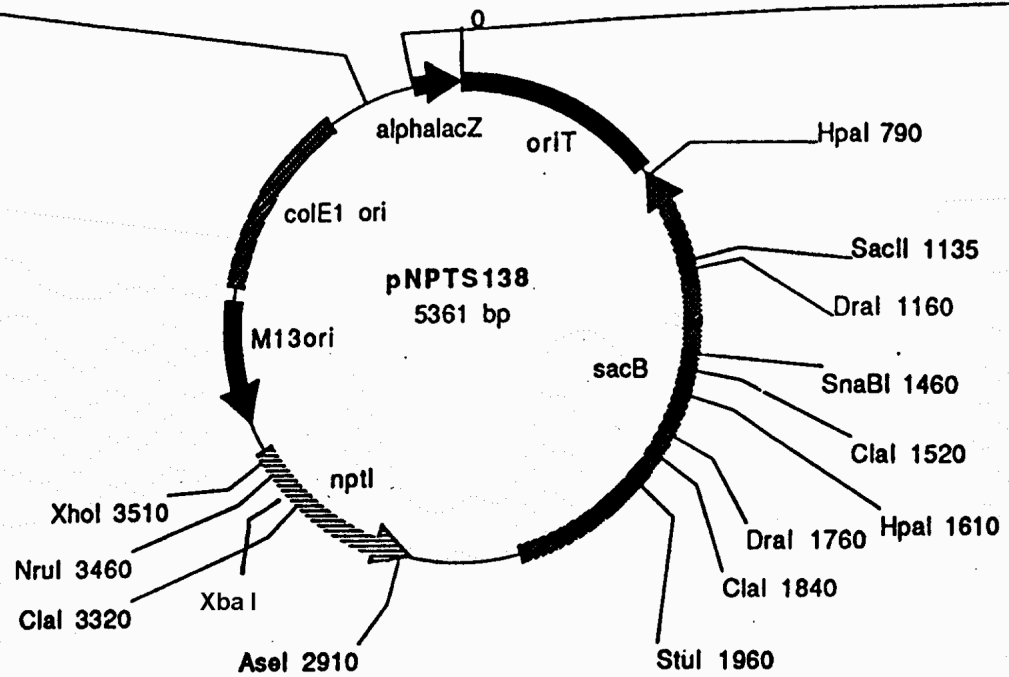
Published by NRC Research Press







SnaBI.17.SpeI.ApaI.MunI.NgoMl.KasI.HindIII.PstI.EcoRV.BamHI.EcoRI.NheI.EagI.MluI.BspEI.BsrGI.SphI.SalI.StuI.AflIII.T7



**Plasmid name:** pNPTS138

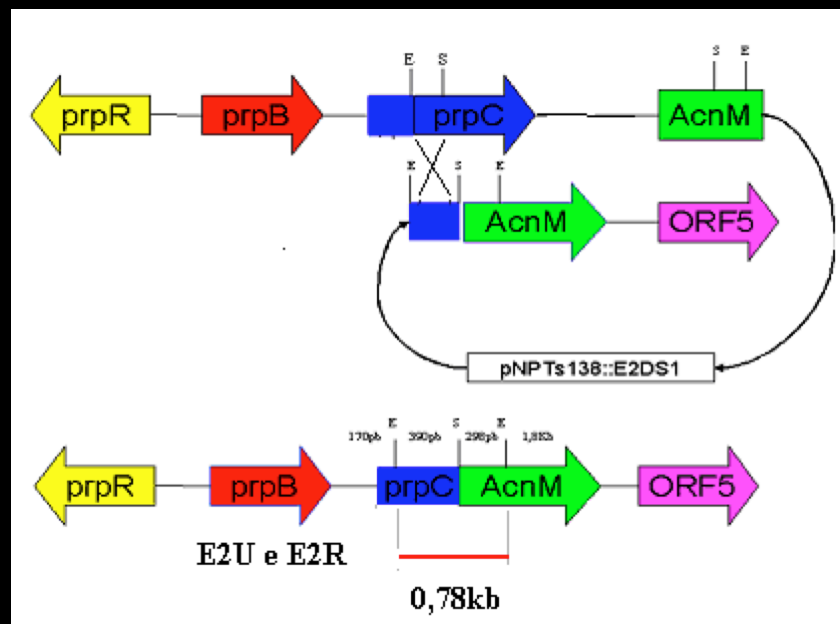
**Plasmid size:** 5361 bp

**Constructed by:** Dickon

**Construction date:** 11-94

**Comments/References:** pUC based plasmid with novel polylinker and M13 ori (not tested), *nptI* and *sacB* genes from pDELTA1 (EcoRV-XbaI) and *oriT*.

# Inativação do gene



Esquema indicativo da ocorrência do segundo evento de recombinação homóloga, bem como da deleção sítio específica de parte dos genes *prpC* e *acnM*.

**Table 2.** Comparison of poly-3-hydroxybutyrate-co-3-hydroxyvalerate accumulation by *Burkholderia sacchari* and *prp* mutants.

Bacterial strain	Glucose (g·L <sup>-1</sup> )		Propionate (g·L <sup>-1</sup> )		CDM (g·L <sup>-1</sup> )	Polyhydroxyalkanoate (PHA)			Y <sub>3HV/prp</sub> (g·g <sup>-1</sup> )
	Initial	Final	Initial	Final		% CDM	3HB (mol %)	3HV (mol%)	
Wild type	5.17	2.01	0.95	0.00	3.36	41.98	93.36	6.64	0.09±0.02
IPT 189	5.17	2.26	0.95	0.53	2.81	36.92	43.59	56.40	0.90±0.14
EM1	5.17	1.79	0.95	0.29	3.02	46.23	44.44	55.55	0.81±0.14
EM2	5.17	2.80	0.95	0.55	2.83	37.78	42.16	57.84	0.96±0.11

**Note:** PHA, total polymer content; CDM, cell dry mass; HB, 3-hydroxybutyrate fraction on PHA; Y<sub>3HV/prp</sub>, 3-hydroxyvalerate (3HV) yield from propionate (*prp*); 3HV, 3-hydroxyvalerate fraction on PHA; IPT 189, *prp* UV mutant; EM1, *prp* mutant with pNPTSE2ΔS1 integrated to the genome; and M2, *prp* mutant deleted on *prpC* and *acnM* regions after second recombination event and plasmid excision. Experiments representing the average of 3 repetitions in a mineral salts medium with propionate (1 g·L<sup>-1</sup>) and glucose (5 g·L<sup>-1</sup>) and nitrogen-free (MMPG 1/5/0).



# 3HV content is dependent on propionic acid concentration

Table 3. Efficiency of *Burkholderia sacchari* and *prp* mutants in accumulating 3-hydroxyvalerate units under different propionate concentrations.

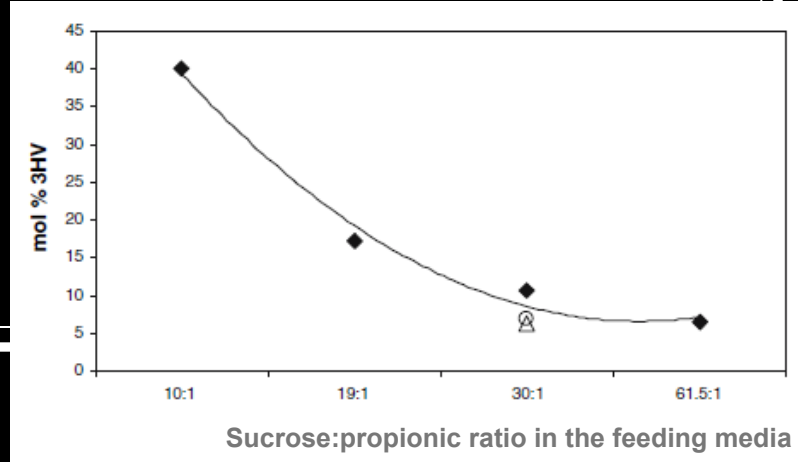
Bacterial strain	Glucose (g·L <sup>-1</sup> )		Propionate (g·L <sup>-1</sup> )		Polyhydroxyalkanoate (PHA)			
	Final*	Initial	Final	CDM (g·L <sup>-1</sup> )	% CDM	3HB (mol %)	3HV (mol %)	Y <sub>3HV/prp</sub> (g·g <sup>-1</sup> )
<i>Burkholderia sacchari</i> wild type	2.15	0	0	4.67	57.38	100.00	0.00	0
	2.15	0.21	0	4.72	49.60	98.46	1.54	0.20
	2.15	0.51	0	4.67	48.99	97.74	2.26	0.12
	2.15	0.96	0	4.51	53.33	96.14	3.86	0.11
<i>Burkholderia sacchari</i> UV mutant IPT 189	2.18	0	0	3.9	49.57	98.90	1.10	0
	2.18	0.21	0	3.25	41.25	82.12	17.88	1.29
	2.18	0.51	0.24	2.92	38.96	81.00	19.00	0.92
	2.18	0.96	0.56	2.92	43.47	84.95	15.05	0.54
<i>Burkholderia sacchari</i> mutant EM1	1.59	0	0	3.60	43.94	92.43	7.57	0
	1.59	0.21	0	3.60	61.70	91.10	8.90	1.07
	1.59	0.51	0	2.92	41.42	85.47	14.53	0.40
	1.59	0.96	0.31	3.10	38.84	86.30	13.70	0.29
<i>Burkholderia sacchari</i> mutant EM2 <i>acnM/prpC</i> deletion	2.45	0	0	2.85	49.21	98.65	1.35	0
	2.45	0.21	0	3.36	49.48	82.48	17.52	1.57
	2.45	0.51	0.23	2.88	37.15	82.78	17.22	0.74
	2.45	0.96	0.64	2.83	35.20	84.07	15.93	0.56

**Note:** PHA, total polymer content; CDM, cell dry mass; 3HB, 3-hydroxybutyrate fraction on PHA; 3HV, 3-hydroxyvalerate fraction on PHA; Y<sub>3HV/prp</sub>, 3HV yield from propionate (prp); IPT 189, *prp* UV mutant; EM1, *prp* mutant with pNPTSE2ΔS1 integrated into the genome; and EM2, *prp* mutant deleted on *prpC* ad *acnM* regions after second recombination event and plasmid excision.  
\*Initial glucose concentration in mineral medium was 5.15 g·L<sup>-1</sup>.

2MCC is more operative at low *prp* concentrations.

A second *prp* catabolic pathway does exist?

Control on 3HV content



# Fim

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