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Fermentation of Raffinose by Lactose-Fermenting Strains of *Yersinia enterocolitica* and by Sucrose-Fermenting Strains of *Escherichia coli*

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Introduction of plasmids carrying the *lacY* gene (lactose permease gene) into *Yersinia enterocolitica* results in cells being able to ferment both lactose and raffinose. Transfer of such plasmids into *Escherichia coli* C600 (*lacY*) confers ability to ferment lactose but not raffinose. Derivatives of C600 that ferment both lactose and sucrose (Lac$^+$ Scr$^+$ strains) are able to ferment raffinose, but do not grow well on raffinose minimal medium. Fermentation of raffinose by Lac$^+$ strains of *Y. enterocolitica*, and by Lac$^+$ Scr$^+$ strains of *E. coli*, is explained in terms of transport of raffinose via the *lac* permease and subsequent breakdown catalyzed by invertase.

Yersinia enterocolitica is recognized as generally being Lac$^-$ (i.e., unable to ferment lactose) despite the fact that cells normally hydrolyze *O*-nitrophenyl-β-D-galactoside (9, 13). Strains lacking this ability have been detected amongst those isolated from hares and belonging to serological group 2 (14). A strain isolated recently from a man's throat, strain 842, is able to ferment lactose and has been shown to owe its Lac$^+$ phenotype to the possession of a conjugative 33-megadalton plasmid, pGC1 (6). This plasmid also enables host cells to ferment raffinose (6-*O*-α-D-galactopyranosyl-1α-D-glucoside-2β-fructofuranoside).

Escherichia coli strains that acquire Scr (or Sac) plasmids, and thereby gain the ability to ferment sucrose, may also acquire the ability to ferment raffinose (Raf$^+$ character). However, when such Scr$^+$ cells are plated on a synthetic medium containing raffinose as the sole energy source, only 1 in 10^6 cells develops colonies (18). In this communication, explanations are advanced for this phenomenon and for the fermentation of raffinose by Lac$^+$ strains of *Y. enterocolitica*.

MATERIALS AND METHODS

Bacterial strains and plasmids. The strains and plasmids used are listed in Table 1.

Growth of bacteria. *E. coli* cells were grown at 37°C and *Y. enterocolitica* at 30°C.

Liquid cultures were grown in nutrient broth. MacConkey base agar (Difco) supplemented with either lactose or sucrose (1%, wt/vol) was used to screen for colonies exhibiting the Lac$^+$ or Scr$^+$ character, respectively. Clones of these types were selected by plating on minimal agar. This contained the mineral salts mixture described previously (10), either lactose or sucrose (0.2%, wt/vol), requisite amino acids (20 μg/ml each), and thiamine (5 μg/ml).

Transfer of genetic material. Transfer of the Scr plasmid Sac (H155) was performed at 22°C as described previously (18). Other bacterial conjugations involving only *E. coli* strains were performed by mixing equal volumes of late log phase cultures of the donor and recipient strains. Conjugations involving *Y. enterocolitica* strains were carried out on filters (6). Matings were generally carried out overnight.

Fermentation tests. Tests were conducted in peptone water containing either bromocresol purple or Andrade indicator (8) and appropriate sugars (1%, wt/vol). Tests were discontinued after 3 days.

RESULTS

The fermentation patterns of three strains of *Y. enterocolitica* carrying different *lac* plasmids are shown in Table 2. It is clear that pGC1, or F-prime factors carrying the *lac* genes of *E. coli*, confer on *Y. enterocolitica* the ability to ferment raffinose as well as lactose. This suggests that the Raf$^+$ character of these strains is related to the presence of the *lac* genes themselves and not to other plasmid genes. We propose that the Raf$^+$ phenotype results from entry of raffinose via the *lac* permease and subsequent attack by one or more chromosomally controlled enzymes such as invertase. The Raf$^+$ strains of *Y. enterocolitica* studied were able to grow on minimal medium containing raffinose as the sole energy

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TABLE 1. *Bacterial strains and plasmids used*

Plasmids	Strains	Genus	Markers (serotype)[a]	Origin or reference
	W22708	*Y. enterocolitica*	r^-m^+, *str* (0:9)	7
	W277	*Y. enterocolitica*	wild type (0:9)	7
	Ye 842	*Y. enterocolitica*	wild type (0:8, 19)	6
	C600	*E. coli* K-12	*thr leu thi lacY⁻Z⁺*	5
	3300	*E. coli* K-12	*lacI thi*	J. Langridge
	K-12 Auxo (Sac H155)	*E. coli* K-12	*lac pro his trp phe*	18
JCFLO			F′*lac*	1
F′*lac*			F′*lac*	P. Fredericq from Clowes strain 94
F′*lacY⁺Z⁻*				P. Fredericq from F′*lac*
pGC1			*lac⁺ tra⁺ fi⁻*	6
M.IP.232			*scr⁺ tra⁺ fi⁻*	12
Sac (H155)			*scr⁺ tra⁺*	18

[a] r^-m^+, Restriction deficient, modification proficient; *str*, streptomycin; *thr*, threonine; *leu*, leucine; *thi*, thiamine; *lac*, lactose; *pro*, proline; *his*, histidine; *trp*, tryptophan; *phe*, phenylalanine; *tra*, mediation of conjugation; *fi*, fertility inhibition.

TABLE 2. *Fermentation of sucrose, lactose, and raffinose by strains of Y. enterocolitica*

Strain	Sugar included in fermentation medium[a]		
	Sucrose	Lactose	Raffinose
W22708	+	−	−
W22708(pGC1)	+	+	+
W22708(JCFLO)	+	+	+
W277	+	−	−
W277(F′*lac*)	+	+	+
Ye 842(pGC1)	+	+	+
Ye 842(pGC1) segregants	+	−	−

[a] +, Fermentation; −, failure to ferment.

TABLE 3. *Growth (+) of Y. enterocolitica strains on solid media containing sucrose, lactose, or raffinose as sole C source*

Strain	Sucrose	Lactose	Raffinose
W22708	+	−	−
W22708(JCFLO)	+	+	+ (10d)
W22708(pGC1)	+	+	+ (10d)
W277	+	−	−
W277(F′*lac*)	+	+	+ (10d)

TABLE 4. *Effects of different plasmids on the ability of E. coli C600 to ferment various carbohydrates*[a]

Plasmids	Sucrose	Lactose	Raffinose
C600	−	−	−
C600(pGC1)	−	+	−
C600(M.IP.232)	+	−	−
C600(M.IP.232) (pGC1)	+	+	+
C600(M.IP.232) (F′*lacY⁺Z⁻*)	+	+	+

[a] +, Fermentation; −, failure to ferment.

source (MR medium), but growth was much slower (10 days instead of 3) than when lactose or sucrose was present (Table 3).

Further support for our hypothesis was obtained when the Lac⁻ *E. coli* strain C600 was supplied with both *lacY⁺* (carried on F′*lacY⁺Z⁻* or pGC1) and M.IP.232. Resulting strains were able to ferment both raffinose and lactose (Table 4), but grew only very slowly on MR medium. Strains supplied with *lacY* but not M.IP.232 were able to ferment lactose, but not raffinose.

As mentioned previously, acquisition of at least certain Scr plasmids can confer ability to ferment raffinose, without conferring ability to grow on MR medium. This association between the ability to ferment sucrose and the ability to ferment raffinose was investigated further by screening 330 Lac⁺ isolates of *E. coli* derived from human, porcine, and bovine sources for ability to ferment sucrose and/or raffinose. Initially, 12 isolates were found to ferment only sucrose and 12 to ferment only raffinose, whereas 185 fermented both sugars, and 121 fermented neither. When isopropylthiogalactoside, an inducer of β-galactosidase and β-galactoside permease, was included in the raffinose-peptone water at a concentration of 1.5×10^{-4} M, the 12 strains that initially fermented sucrose but not raffinose now fermented both. Production of acid by slow fermenters also occurred more rapidly in the presence of this inducer. These results support the concept that raffinose may be fermented when a sufficient amount of the substance is able to enter cells via the *lac* permease and when appropriate degradative enzymes are available. When the Scr plasmid Sac (H155) was transferred into strain 3300, which synthesizes the *lac* permease constitutively, eight out of eight Scr⁺ transconjugants (selected

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for ability to grow on sucrose minimal medium) were found to grow on MR medium.

DISCUSSION

The results presented above illustrate the importance of considering host-controlled functions during study of phenotypes influenced by plasmids. Moreover, it is now apparent that the Raf$^+$ phenotype may arise in two distinct ways.

It has been shown recently that some strains of Raf$^+$ *E. coli* contain plasmids which control three functions associated with raffinose metabolism. These are a system which facilitates transport of raffinose (raf permease), an invertase, and an α-galactosidase (15). The last of these enzymes is distinct from that specified by the chromosomal *melA* gene (3, 16). Such strains grow well on MR media. In contrast, Scr$^+$ strains may not grow well on such media, and their ability to ferment raffinose may be regarded as being a side effect of their ability to ferment sucrose. Although the specificity of the sucrose-splitting enzyme present in Scr$^+$ cells (19) has not been determined, it seems likely that such an enzyme will be able to attack any raffinose that is able to enter cells. Entry of low levels of raffinose via the *lac* permease is not a new concept. Growth on MR media has been used to select strains that synthesize *lac* functions constitutively, and inclusion of isopropylthiogalactoside in such media results in improved growth of inducible strains (2).

At first sight, it seems that Scr$^-$ Lac$^+$ strains of *E. coli* should be able to ferment raffinose, since any of this substance which does enter via the *lac* permease should be attacked by the α-galactosidase specified by the *melA* gene. Synthesis of this enzyme is induced (16) when cells are grown at appropriate temperature in media containing melibiose (6-*O*-α-D-galactopyranosyl-D-glucopyranose) or melibitiol (6-α-D-galactopyranosyl-D-glucitol). However, raffinose is assumed not to act as an inducer of the *melA* gene product in *E. coli* K-12 (2), although it does serve as substrate for the enzyme (4) and does act as inducer in *E. coli* B (17). The presence of a sucrose-splitting enzyme in Scr$^+$ cells may lead to increased production of acid from raffinose in two ways. First, any sucrose released by α-galactosidase activity can be metabolized further in such cells. Second, action of invertase (β-fructofuranosidase) on raffinose would be expected to yield fructose, which is fermented by *E. coli* and by *Y. enterocolitica,* and melibiose, which is known to stimulate synthesis of *lac* functions (16) as well as α-galactosidase. Melibiose formed in this way may therefore lead to further uptake of raffinose by stimulating increased synthesis of *lac* permease.

Unlike *E. coli* K-12, most strains of *Y. enterocolitica* do exhibit invertase activity and are able to ferment sucrose. *Y. enterocolitica* cells do not normally ferment melibiose or raffinose (9, 13). The results shown in Tables 2 and 3 are consistent with the interpretation advanced above to account for fermentation of raffinose by Scr$^+$ strains of *E. coli.* Introduction of *lacY* into strains of *Y. enterocolitica,* which may be regarded as analogs of the *E. coli* strain C600 (M.IP.232), enables cells to transport both lactose and raffinose. Since enzymes are present that can attack both substances, such cells become both Lac$^+$ and Raf$^+$. Slow growth on MR media presumably reflects limited transport of raffinose by the *lac* permease. This interpretation is supported by the observation that Scr$^+$ derivatives of *E. coli* 3300 (*lacI*) grow better on MR medium than does the parent strain. It seems likely that the "mutational events" that enable Scr$^+$ strains of *E. coli* to grow on such media (18) are ones which affect transport of raffinose. In view of the recent findings of Guiso and Ullmann (11), it seems likely that *lac* plasmids from a variety of sources are able to confer the Raf$^+$ phenotype on *Y. enterocolitica.*

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ADDENDUM

When this paper was completed, the results of another work devoted to raffinose fermentation (in *Salmonella* and *E. coli*) appeared (J. Buissière, C. Coynault, and L. Le Minor, Ann. Microbiol. (Paris) **128A:**167–182, 1977). These authors conclude that fermenation of raffinose occurs only when α-galactosidase, invertase, and relevant permeases (sucrose and melibiose) are present. The reason for the need for α-galactosidase activity is not clear, since action of invertase on raffinose should release fructose, which may be further fermented. In the present communication, emphasis is placed on the significance of the *lac* permease in facilitating entry of raffinose, and the presented results do not sugest any need for the α-galactosidase activity when an invertase and a *lac* permease are present and expressed. It is evident from results presented in each paper that when phenotypes influenced by plasmids are being studied, consideration must be given to both chromosome-controlled and plasmid-controlled functions.

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