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 invertingase.

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 (6, 13). Strains lacking
this ability have been detected amongst
those isolated from hares and belonging to se-
rological 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 conjugal plasmid,
pGC1 (6). This plasmid also enables host cells to ferment raffinose (6-O-
α-D-galactopyranosyl-1α-D-glucoside-2β-fructo-
franose).

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 10⁶ cells develops colonies (18).
In this communication, explanations are advanced
for this phenomenon and for the fermenta-
tion of raffinose by Lac⁺ strains of Y. enter-
ocolitica.

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.

Liquids cultures were grown in nutrient broth.

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iversity, Bundoora, Victoria 3083, Australia.

MacConkey base agar (Difco) supplemented with
either lactose or sucrose (1%, wt/vol) was used to
screen for colonies exhibiting the Lac⁺ or Scr⁺ char-
acter, 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 de-
scribed 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. en-
terocolitica strains were carried out on filters (6).
Matings were generally carried out overnight.

Fermentation tests. Tests were conducted in pept-
one water containing either bromocresol purple or
Andrade indicator (8) and appropriate sugars (1%,
w/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 invertingase. The Raf⁺ strains of Y. en-
terocolitica studied were able to grow on minimal
medium containing raffinose as the sole energy
TABLE 1. Bacterial strains and plasmids used

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Strains</th>
<th>Genus Markers (serotype)</th>
<th>Origin or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>W22708</td>
<td>Y. enterocolitica</td>
<td>r m*, str (0:9)</td>
<td>7</td>
</tr>
<tr>
<td>W277</td>
<td>Y. enterocolitica</td>
<td>wild type (0:9)</td>
<td>7</td>
</tr>
<tr>
<td>Ye 842</td>
<td>Y. enterocolitica</td>
<td>wild type (0:8, 19)</td>
<td>6</td>
</tr>
<tr>
<td>C900</td>
<td>E. coli K-12</td>
<td>thr leu thi lacY-Z+</td>
<td>5</td>
</tr>
<tr>
<td>3500</td>
<td>E. coli K-12</td>
<td>lacI thi</td>
<td>J. Langridge</td>
</tr>
<tr>
<td>K-12 Auxo</td>
<td>E. coli K-12</td>
<td>lac pro his trp phe</td>
<td>18</td>
</tr>
</tbody>
</table>

JCFLO F'lac

F'lacY+Z
pGC1
M.IP.232
Sac (H155)

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sugar included in fermentation medium*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sucrose</td>
</tr>
<tr>
<td>W22708</td>
<td>+</td>
</tr>
<tr>
<td>W22708(pGC1)</td>
<td>+</td>
</tr>
<tr>
<td>W22708(JCFLO)</td>
<td>+</td>
</tr>
<tr>
<td>W277</td>
<td>+</td>
</tr>
<tr>
<td>W277(F'lac)</td>
<td>+</td>
</tr>
<tr>
<td>Ye 842</td>
<td>+</td>
</tr>
<tr>
<td>Ye 842(pGC1)</td>
<td>+</td>
</tr>
</tbody>
</table>

* +, Fermentation; -, failure to ferment.

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

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>W22708</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W22708(JCFLO)</td>
<td>+</td>
<td>+</td>
<td>+ (10d)</td>
</tr>
<tr>
<td>W22708(pGC1)</td>
<td>+</td>
<td>+</td>
<td>+ (10d)</td>
</tr>
<tr>
<td>W277</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W277(F'lac)</td>
<td>+</td>
<td>+</td>
<td>+ (10d)</td>
</tr>
</tbody>
</table>

TABLE 4. Effects of different plasmids on the ability of E. coli C900 to ferment various carbohydrates

<table>
<thead>
<tr>
<th>Plasmids</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Raffinose</th>
</tr>
</thead>
<tbody>
<tr>
<td>C900</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C900(pGC1)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C900(M.IP.232)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C900(M.IP.232)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* +, 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 C900 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 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 x 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
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 melibitol (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 (M1P.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.

ACKNOWLEDGMENTS

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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. Buissiere, C. Coynaught, and L. Le Minor, Ann. Microbiol. (Paris) 128A:167-182, 1977). These authors conclude that fermentation 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 suggest 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.

LITERATURE CITED


FERMENTATION OF RAFFINOSE