Microbiology Comment provides a platform for readers of *Microbiology* to communicate their personal observations and opinions in a more informal way than through the submission of papers.

Most of us feel, from time to time, that other authors have not acknowledged the work of our own or other groups or have omitted to interpret important aspects of their own data. Perhaps we have observations that, although not sufficient to merit a full paper, add a further dimension to one published by others.

In other instances we may have a useful bit of methodology that we would like to share.

The Editors hope that readers will take full advantage of this new section and use it to raise matters that hitherto have been confined to a limited audience.

John Freer
Editor-in-Chief

Artefactual detection of amylase activity

A recent paper in this journal by Gómez *et al.* (4) reported the effects of a cloned *Bacillus* lipoprotein on envelope integrity in *Escherichia coli*, a phenomenon they initially detected while seeking recombinants which expressed amylase activity. We would like to report a similar experience of detecting 'unwanted' amylase expression.

Bacteriophage λ is a favoured vector for the initial screening of recombinant gene banks, and is particularly useful for the detection of recombinants expressing foreign genes which may be difficult to maintain stably on plasmid vectors. We have previously used λ to clone the genes encoding various enzymes of the oral bacterium *Streptococcus mutans* which either cleave sucrose or form polymers from sucrose and we have successfully detected recombinants of interest by direct screening for activity in phage plaques. *S. mutans* has also been reported to degrade starch, though no reports of the biochemistry or genetics of the enzyme(s) responsible have appeared. We therefore screened a gene bank constructed from chromosomal DNA of *S. mutans* strain Ingbritt in λ L47.1 on minimal medium plates

with starch as sole carbon source. Any recombinants which could release glucose, maltose or maltosaccharides from starch would give a surrounding halo of *E. coli* growth, as described for sucrase activity (6). Several recombinants were found and these were all shown to give areas of clearing on agar containing 0.5% soluble starch, stained with Lugol's iodine. One recombinant was chosen for further study and a 7.3 kb *Eco*R1 fragment subcloned into plasmid pACYC184, to give plasmid pSB3. *E. coli* carrying this fragment gave the anticipated phenotype of causing a zone of starch clearing on starch-agar plates.

A number of different enzymes – amylase, cyclodextrin glucanotransferase, glycogen-branching enzyme, isoamylase and pullulanase – can all give clearing of starch. To investigate the activity encoded by pSB3, we therefore applied a wide range of assays to cell extracts of *E. coli* carrying this plasmid, including thin-layer chromatography, HPLC analysis of starch breakdown products, action on amylose, amylopectin and pullulan, and effect on spectral properties of starch-iodine complexes. In none of these tests was it possible to demonstrate any novel activity that was not present in the host *E. coli*. These results suggested either that a cloned streptococcal enzyme was active on agar plates but not in the other assays, or that no cloned gene was being expressed and all activity around colonies was due to release of *E. coli* amylase.

Subcloning of the DNA insert in pSB3 revealed that starch-clearing activity was associated with a 2.6 kb *Cl*A/*Cl*A fragment, which could only be cloned in pUC vectors in one orientation. This fragment was subjected to nucleotide sequencing and the sequence compared with the databases by the BLASTN programme (1). The result of this analysis showed clearly that the sequenced fragment consisted entirely of DNA derived from λ , corresponding to bases 43823–46437 and it thus appears that, during subcloning from our original λ recombinant, we had introduced λ DNA into pSB3. The cloned region of the λ chromosome carried the Q, S and R genes which are involved in the lytic pathway. Gene Q is regulatory, the S product is believed to act on the cell membrane (5) and the R product is a murein transglycosylase (2). Expression of these genes from pSB3 in *E. coli* might be anticipated to damage the cell envelope and potentially release enzymes from the

periplasm, and perhaps also the cytoplasm. To test this possibility, *E. coli* carrying pSB3 was compared with a control carrying pACYC184 for release of DNase, RNase and alkaline phosphatase by agar plate assays. Presence of pSB3 caused all three enzymes to be released. In addition, *E. coli* carrying pSB3 showed enhanced sensitivity to Acridine Orange and sodium deoxycholate, two agents normally excluded by the outer membrane. Despite these alterations in permeability, the presence of pSB3 did not affect the growth rate of *E. coli* in Luria Broth. Nor were the cells abnormally osmotically fragile. The damage caused by the λ lysins expressed from pSB3 is thus sublethal, yet has a significant effect on the envelope permeability properties. However, high-level expression of S and R genes under the control of the *lac* promoter results in massive culture lysis (5), and this would explain the inability to clone the *Cl*A/*Cl*A fragment in both orientations.

The integrity of the envelope of *E. coli* is highly susceptible to disruption and hence release of periplasmic enzymes may be caused by a range of factors, including damage to the peptidoglycan layer (3). In the present instance, release of amylase (and other enzymes) can be explained by the direct effect of the λ lysins. In the instance described by Gómez *et al.*, the 'artefactual' release of amylase has been shown to be due to expression

► GUIDELINES

Communications should be in the form of letters and should be brief and to the point. A single small Table or Figure may be included, as may a limited number of references (cited in the text by numbers, and listed in alphabetical order at the end of the letter). A short title (fewer than 50 characters) should be provided.

Approval for publication rests with the Editor-in-Chief, who reserves the right to edit letters and/or to make a brief reply. Other interested persons may also be invited to reply. The Editors of *Microbiology* do not necessarily agree with the views expressed in *Microbiology Comment*.

Contributions should be addressed to the Editor-in-Chief via the Editorial Office.

of a foreign lipoprotein in *E. coli* (4). In devising strategies for screening gene banks for expression of foreign genes, one must therefore be alert to the confusion that can arise due to peripheral effects on the host *E. coli*. Meanwhile, we still believe that our original λ recombinant carries the *S. mutans* amylase gene...

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