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 XL47.1 on minimal medium plates with starch as sole carbon source. Any recombinants which could release glucose, maltose or maltotriose 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 EcoRI 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 amyllose, amylopeptin 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 ClaI/ClaI 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 program (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 murine 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 deoxynucleate, 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 ClaI/ClaI 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...
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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