Starch can be degraded by amylase produced by the animal itself and by anaerobic and aerobic bacteria existing in animal digestive tracts and in the environment. Naturally occurring *Escherichia coli* has not been found to produce amylase, but if an amylase gene could be expressed in harmless *E. coli* the transformed bacterium might produce abundant amylase. Though it has been reported that amylase gene from *Bacillus subtilis* has been expressed in *E. coli* (Lin and Hsu 1997), the use of different species of *B. subtilis* and different primers can produce different characteristics of amylases.

*B. subtilis, E. coli, and plasmid (pHMSXD1)* were provided by CSIRO and cultured in Luria–Bertani (LB) medium. Genomic DNA from *B. subtilis* was purified, and amylase gene from *B. subtilis* was amplified by polymerase chain reaction using three pairs of primers. The pHMSXD1 and amylase gene were partially digested with nucleases (Hind III and Xbal) and ligated with T4 DNA ligase. Ligation solution and *E. coli* cells were put into a pre-chilled cuvette (0.1 cm electrode gap); the electroporation apparatus was set to 1.7 kV. The transformed *E. coli* was aliquotted on to agar plates with starch (1%) and ampicillin (50 µg/ml); after overnight culture, the plates were stained by I2 and KI and the diameters of the haloes were measured as a semi-quantitative measure of amylase activity. For quantitative amylase measurement (Li *et al.* 1987), amylase in the culture supernatant was precipitated and incubated with a starch solution at 37°C for 7.5 min. Iodine was added and absorbance (A) was measured at 660 nm, whence:

\[
\text{Amylase activity (U/L)} = \left( \frac{A_{\text{blank}} - A_{\text{reaction}}}{A_{\text{blank}}} \right) \times 8000
\]

The results showed that the three kinds of amylase genes had similar molecular weights (2.3 kb). After the amylase gene was connected with a plasmid (pHMSXD1) and inserted into *E. coli*, the transformed *E. coli* expressed and excreted amylase. The activities of that excreted amylase and that from the initial *B. subtilis* were 1.0376 and 0.0226 U/ml in LB medium, respectively. After transformation in *E. coli*, the amylase activity was increased 45 times. The reasons may be: (i) the plasmid can replicate itself abundantly in the host (Brown 1990) and produce more amylase than the initial bacteria; (ii) the different regulatory and metabolic systems in *E. coli* could increase the secretion of amylase; (iii) the high voltage used during transformation could increase the expression of the amylase gene.

The transformed *E. coli* with high amylase activity may be suitable for use as a prebiotic/probiotic in animals.

