

# Monitoring Virulence Factor Expression in *Yersinia pestis* in Real Time

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## ABSTRACT

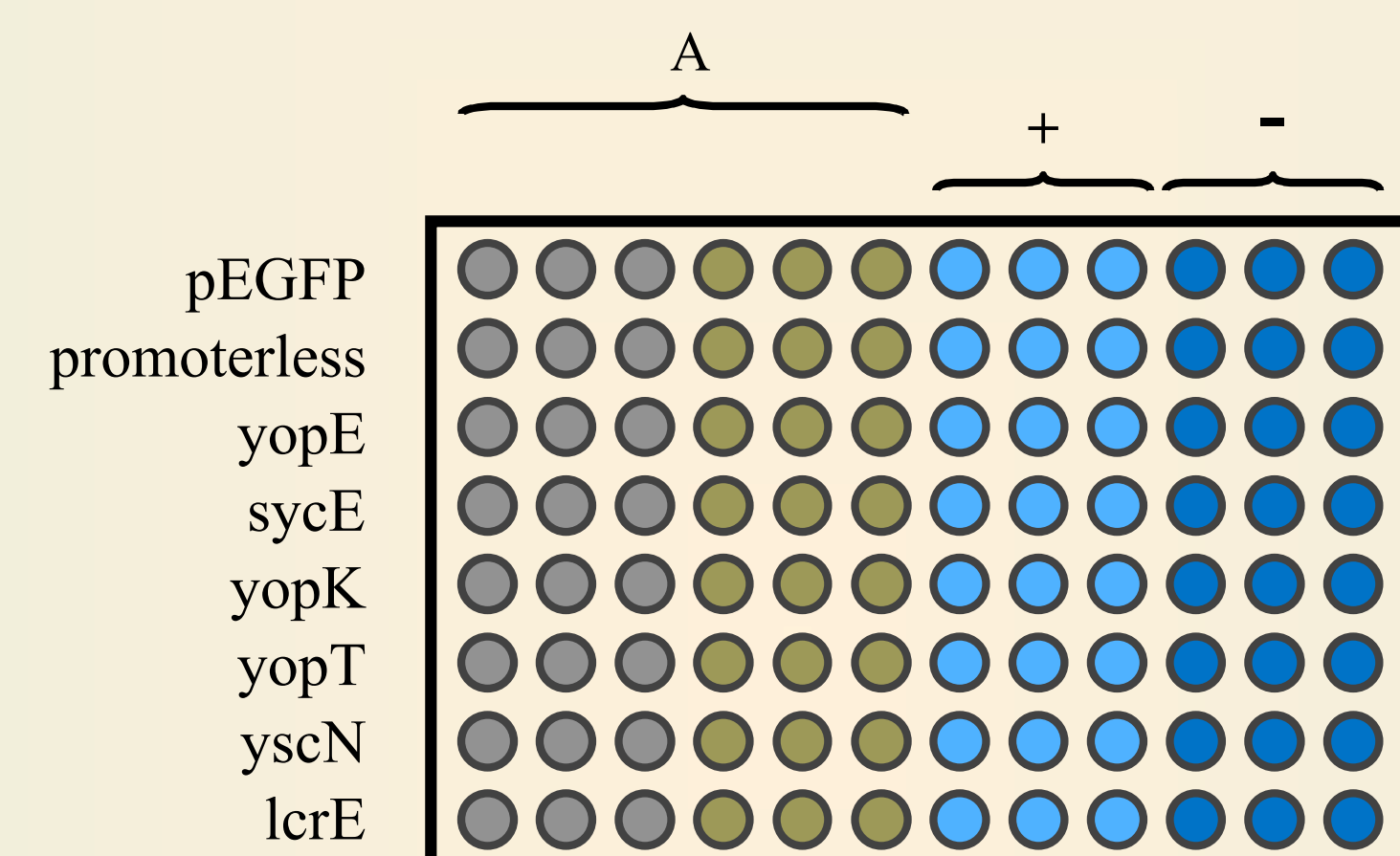
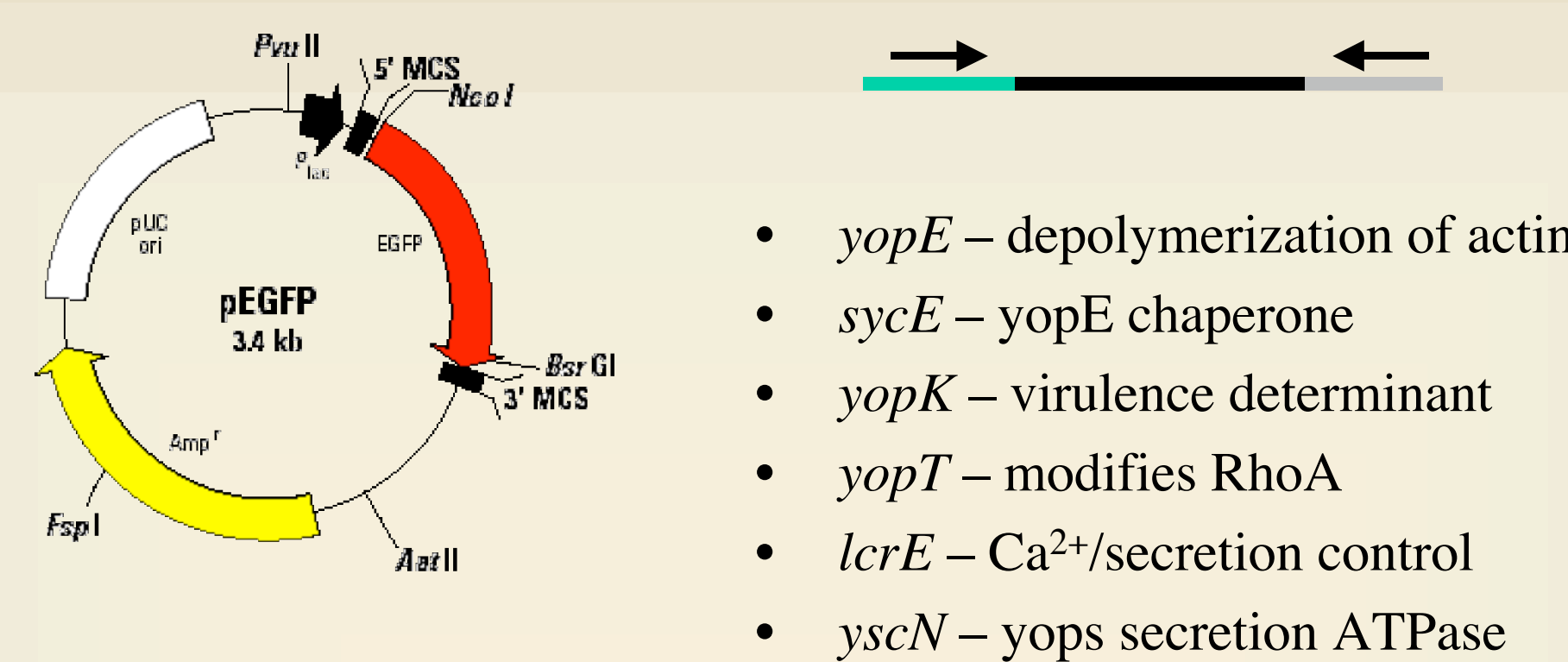
**Background:** *Yersinia pestis*, causative agent of plague, is one of the most virulent bacterial pathogens. The bacterium undergoes a global change in gene expression during the temperature transition associated with transfer from the flea vector to a warm-blooded host. We are interested in investigating the processes involved in the pathogenesis of *Y. pestis*.

**Methods:** We have created a reporter system that places the expression of green fluorescent protein (GFP) under the control of promoter regions of virulence factors of *Y. pestis*. Six reporter plasmids with *gfp* under the control of *yopE*, *sycE*, *yopN* (*lcrE*), *yscN*, *yopK*, and *yopT*, as well as positive and negative control plasmids have been transformed into *Y. pestis* KIM D27. Expression of the virulence factor genes is then measured by the intensity of the fluorescence of the GFP reporter.

**Results:** The reporter system was tested using the well-characterized temperature transition. Shifting the growth temperature from 26 to 37°C results in up-regulation of the virulence factors in a calcium-dependant manner. The levels of virulence-factor gene expression were found to correlate well with values reported in the literature. We have also used this system to probe other factors that affect the pathogenesis of *Y. pestis*. Results from these studies will be presented.

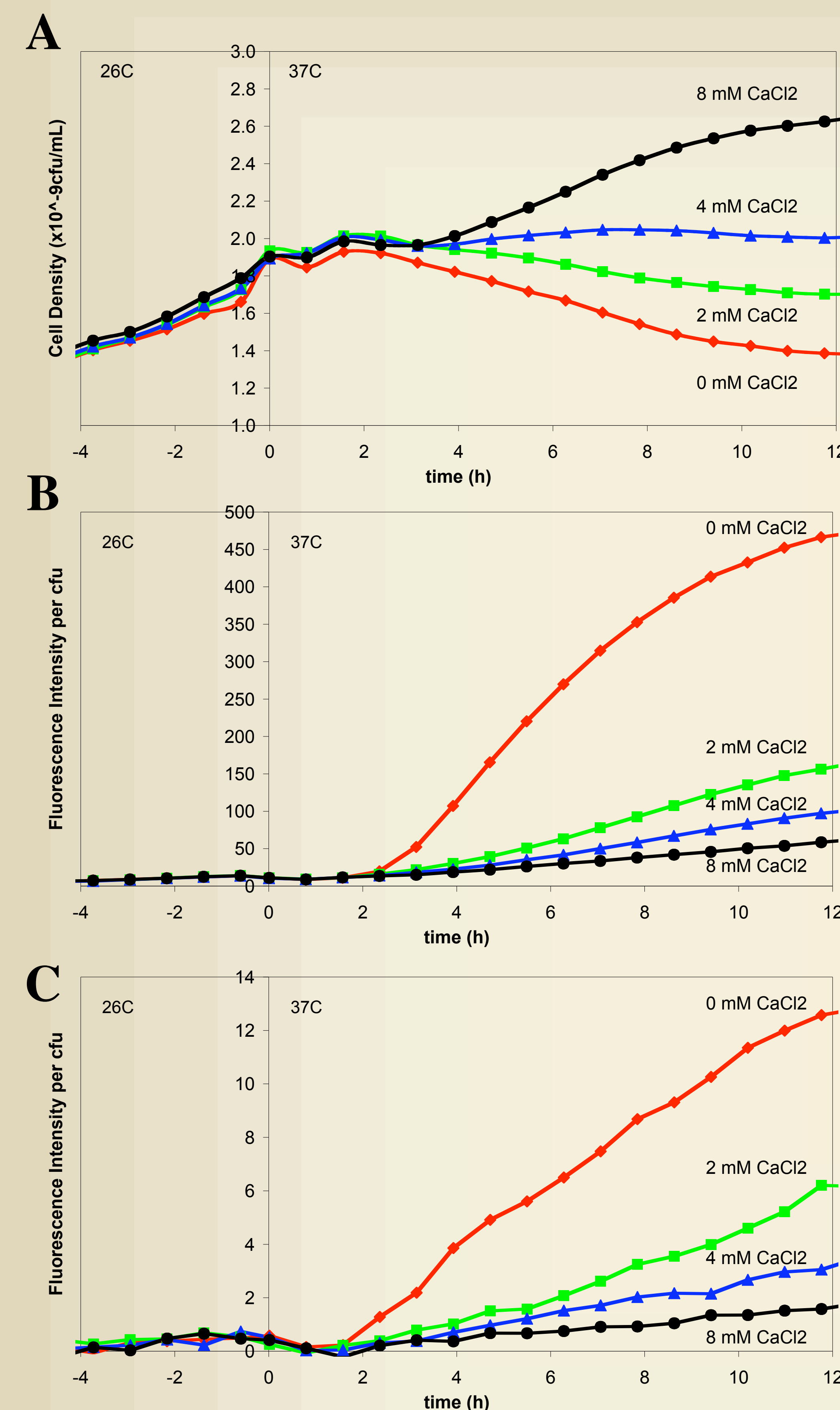
**Conclusions:** We have developed a system to study the expression of virulence factor genes in *Y. pestis* in real time using green fluorescent protein as an *in vivo* reporter.

## METHODS



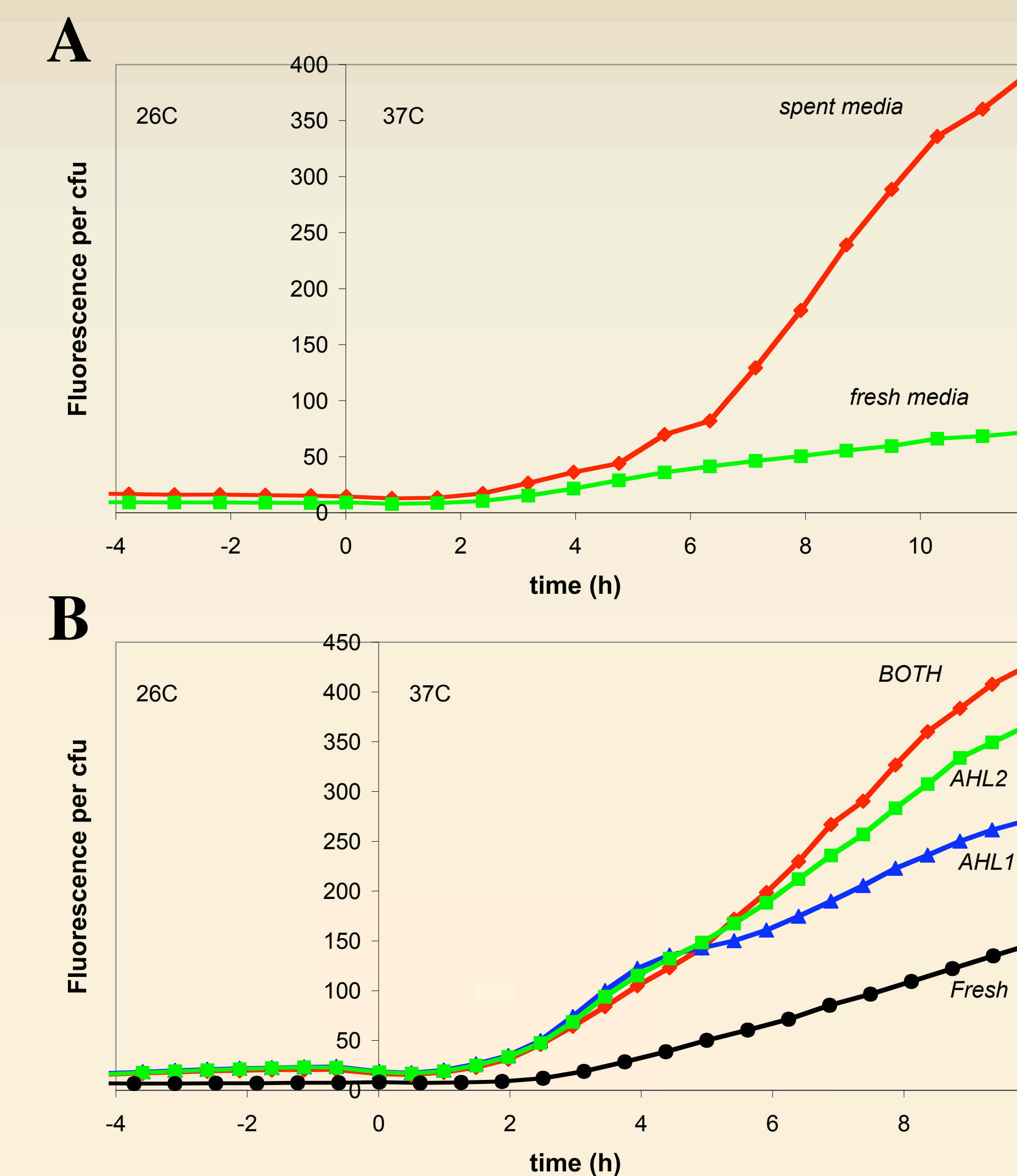
**Fluorescent Clones.** Promoter regions of virulence factors of *Y. pestis* were amplified by PCR and blunt-end ligated into pEGFP (Clontech) in place of the *lac* promoter (*PvuII/SmaI* cut sites). The reporter plasmids were transformed into *Y. pestis* KIM D27 and screened for temperature-inducible fluorescence. The plasmids from the clones were verified by PCR, gel electrophoresis, and sequencing.

**Fluorescence Measurements.** Cultures of *Y. pestis* KIM D27 reporter clones were grown to saturation, collected by centrifugation, washed three times with fresh media and resuspended in fresh media supplemented according to the desired experiment and then aliquoted into 96-well plates. All spectrophotometric measurements are averages of triplicate wells. Plates of cultures are incubated with shaking in a Victor2 plate reader (Perkin Elmer) with temperature control. Optical density measurements were performed using a 600 nm filter (10 nm full-width half-maximum). Fluorescence readings were performed using 485 nm excitation and 510 nm emission filters (10 nm full-width half-maximum). The fluorescence measurements are corrected for autofluorescence using the emission at 580 nm.

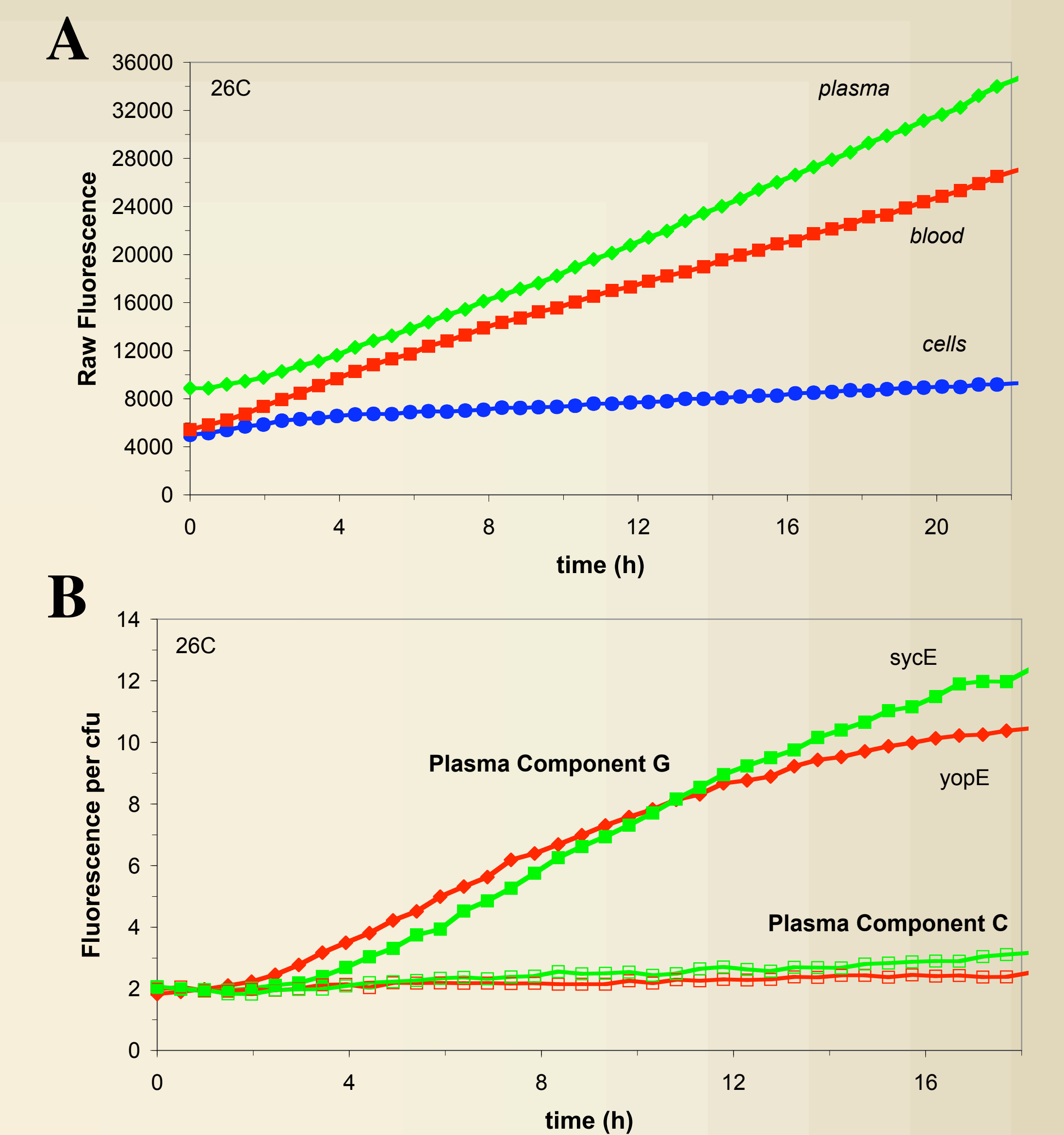


**Figure 1.** Effect of calcium on the growth (A) and induction of GFP under the control of promoters for *yopE* (B) and *yscN* (C) over the temperature shift from 26 to 37°C.

## RESULTS



**Figure 2.** Effect of spent media (A) and supplementation with acyl homoserine lactones (B) on the induction of GFP under the control of promoter for *yopK* over the temperature shift from 26 to 37°C.



**Figure 3.** Effect of blood, red and white blood cells and plasma (A) and supplementation with two different plasma components (B) on the induction of GFP under the control of promoters for *yopE* and *sycE* at 26°C.

**Table 1.** Data from the thermal induction of virulence factors in *Y. pestis*.

	Background (% of <i>lac</i> )	Initial Rate of Induction	Rate Ratio -Ca:+Ca
<i>yopE</i>	15	70	11
<i>yopK</i>	13	40	6
<i>sycE</i>	15	6	1
<i>yscN</i>	0.8	2	7
<i>lcrE</i>	3.3	2	3
<i>yopT</i>	4	1	2

- The background levels at 26°C are expressed as a percentage of the values measured for the (leaky) *lac* promoter.
- The rates of induction are normalized to that of *yopT*, the least strongly induced gene in this study.
- The column labeled "rate ratio" represents the ratios of the rates of induction without calcium supplementation relative to the rates with calcium supplementation (4 mM CaCl<sub>2</sub>).
- All values determined from at least six biological replicates measured in triplicate.

## DISCUSSION

- We have prepared a reporter system in which the expression of GFP is controlled by promoters for virulence factors in *Y. pestis*.
- The six reporter clones show varying degrees of background fluorescence at 26°C suggesting that the expression of some of the virulence factors is more tightly regulated.
- The six reporter clones show varying rates of initial GFP production upon temperature transition suggesting that the different promoters are induced to different extents.
- The effect of calcium on the induction during the temperature shift is different for the six reporter clones.
- The thermal induction of the virulence factor genes in this study is controlled by a quorum-sensing system.
- There is a component in blood plasma that up-regulates virulence factor expression in *Y. pestis* at 26°C.

## CONCLUSIONS

GFP can be successfully exploited as a reporter of the induction of virulence factors in *Y. pestis*. Using this system we have shown that quorum sensing plays an important role in the regulation of virulence-factor expression and we have discovered that there is a component in blood plasma that induces virulence-factor expression without the temperature transition.

## ACKNOWLEDGEMENTS

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