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Technical and Design Note

Development of a Computer Program to Predict the β-Lactamase Activity on the Basis of Ampicillin Fluorescence Values

H. V. Rao,[1] A. M. Masood,[1] and J. Mukherjee[1,2]

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Decrease in ampicillin fluorescence values (Ex. 385 n*M*, Em. 460 n*M*) were correlated with the increase in the pUC 19 plasmid-encoded β-lactamase activity of *Escherichia coli* DH5α. The fluorescence data were then utilized for the development of a computer program in MS DOS Q BASIC to predict the β-lactamase activity.

KEY WORDS: Ampicillin; fluorescence; prediction.

Culture fluorescence has developed into a widely applied, non-invasive, and real-time optical method for bioprocess monitoring during the last decade [1,2]. The expression of β-lactamase in recombinant *Escherichia coli* is often used as a model system to investigate the effects of cultivation conditions and operation modes in bioreactors. This communication describes the measurement of ampicillin fluorescence as an indicator of the activity of β-lactamase, a product of amp^R gene present in a pUC 19 plasmid of *E. coli* DH5α. A computer program was developed to predict the β-lactamase activity on the basis of ampicillin fluorescence values.

E. coli DH5α transfected with pUC 19 plasmid was cultivated in 2.5 L of M9 media in a 3.5-L bioreactor with 0.2% of either one of the carbon sources (glucose, maltose, or lactose) and 50 μg/ml ampicillin. The aeration rate, stirrer speed, and temperature were maintained constant at 0.5 vvm, 100 rpm, and 37°C, respectively. Assay for the extracellular β-lactamase was performed inside the spectrophotometer cuvette containing 50 m*M* phosphate buffer (pH 7) and ampicillin (0.5 mg/ml). The activity of β-lactamase was determined by measuring the rate of degradation of ampicillin [3]. Culture fluorescence (Ex. 385 nm, Em. 460) was measured in the Hitachi F 3010 fluorescence spectrophotometer until the stationary phase.

Up to the exponential phase, the values of ampicillin fluorescence were inversely related to the β-lactamase activity for all bioreactor runs. This correlation was lost when the organism was cultivated in a complex medium. Thus, this relationship is probably due to the growth of the organism in a chemically defined simple medium in which the interference to ampicillin fluorescence from other medium ingredients was minimum.

Program:

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PRESS F5 TO START

CLS
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[1] Department of Life Science and Biotechnology, Jadavpur University, Calcutta 700 032, India.

[2] To whom correspondence should be addressed. Tel.: 0091 33 473 1710. Fax: 0091 33 473 1484. E-mail: joymukherjee@vsnl.net

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LOCATE 8, 29
PRINT "CARBON SOURCE"
LOCATE 12, 27
PRINT "(1) GLUCOSE"
LOCATE 14, 27
PRINT "(2) MALTOSE"
LOCATE 16, 27
PRINT "(3) LACTOSE"
'COLOR 31, 0
LOCATE 20, 27
INPUT "ENTER YOUR CHOICE (1,2,3)"; D$
CLS

LOCATE 12, 1
IF D$ = "1" THEN 500
IF D$ = "2" THEN 600
IF D$ = "3" THEN 700
500
PRINT "GLUCOSE"
INPUT "ENTER CULTURE FLUORESCENCE
  VALUES (385 EXN & 460 EMN)"; F

501 IF F >= 84.03 AND F <= 86.24 THEN 502
  ELSE 503
502 F2 = 86.24
F1 = 84.03
L2 = 0
L1 = 0

GOTO 570

503 IF F >= 79.73 AND F < 84.03 THEN 504 ELSE 505
504 F2 = 84.03
F1 = 79.73
L2 = 0
L1 = .04
GOTO 570

505 IF F >= 74.42 AND F < 79.73 THEN 506 ELSE 507
506 F1 = 74.42
F2 = 79.73
L1 = .19
L2 = .04
GOTO 570

507 IF F >= 61.21 AND F < 74.42 THEN 508 ELSE 515
508 F1 = 61.21
F2 = 74.42
L1 = .19
L2 = .21
GOTO 570

515 IF F >= 53.09 AND F < 61.21 THEN 516 ELSE 517
516 F1 = 61.21
F2 = 53.09
L1 = .23
L2 = .21
GOTO 570

517 IF F >= 41.25 AND F < 53.09 THEN 518 ELSE 519
518 F1 = 41.25
F2 = 53.09
L1 = .23
L2 = .25
GOTO 570

519 IF F >= 31.41 AND F < 41.25 THEN 520 ELSE 521
520 F2 = 31.41
F1 = 41.25
L1 = .25
L2 = .27
GOTO 570

521 IF F >= 27.1 AND F < 31.41 THEN 522 ELSE 523
522 F2 = 27.1
F1 = 31.41
L2 = .6
L1 = .27
GOTO 570

523 IF F >= 23.88 AND F < 27.1 THEN 524 ELSE 525
524 F2 = 23.88
F1 = 27.1
L2 = .49
L1 = .6
GOTO 570

525 IF F >= 22.46 AND F < 23.88 THEN 526 ELSE 527
526 F2 = 22.46
F1 = 23.88
L2 = .41
L1 = .49
GOTO 570

527 IF F >= 21! AND F < 22.46 THEN 528 ELSE 575
528 F2 = 21!
F1 = 22.46
L2 = .41
L1 = .43
GOTO 570

570 LET A = L2 - L1
LET C = F2 - F1
LET B = F2 - F
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LET L = L2 − (A * B/C)

REM PARAMETER OUTPUT
CLS
LOCATE 1, 27
PRINT "CULTURE FLUORESCENCE:"; F
'COLOR 1
PRINT "BETA-LACTAMASE ACTIVITY IN TERMS
  OF CHANGE IN AMP.OD:"; L
INPUT "DO YOU WANT TO CONTINUE (Y/N)"; C$
IF C$ = "Y" OR C$ = "y" THEN 500 ELSE 580
575 PRINT "FLUORESCENCE INPUT OUT OF
  RANGE ! PLEASE REDO"
580 END

600
PRINT "MALTOSE"
INPUT "ENTER CULTURE FLUORESCENCE
  VALUES (385 EXN & 460 EMN)"; F

601 IF F >= 80.02 AND F <= 86.46 THEN 602
ELSE 603
602 F2 = 86.46
F1 = 80.02
L2 = .026
L1 = 0

GOTO 670

603 IF F >= 67.12 AND F < 80.02 THEN 604 ELSE 605
604 F2 = 80.02
F1 = 67.12
L1 = .072
L2 = .026
GOTO 670

605 IF F >= 55.35 AND F < 67.17 THEN 606 ELSE 607
606 F1 = 55.35
F2 = 67.17
L1 = .126
L2 = .072
GOTO 670

607 IF F >= 44.74 AND F < 55.34 THEN 608 ELSE 609
608 F1 = 44.74
F2 = 55.34
L2 = .126
L1 = .246
GOTO 670

609 IF F >= 41.46 AND F < 44.74 THEN 610 ELSE 615
610 F1 = 44.74
F1 = 41.46
L1 = .335
L2 = .246
GOTO 670

615 IF F >= 39.95 AND F < 41.46 THEN 616 ELSE 617
616 F1 = 39.95
F2 = 41.46
L1 = .407
L2 = .335
GOTO 670

617 IF F >= 38.26 AND F < 39.95 THEN 618 ELSE 619
618 F1 = 38.26
F2 = 39.95
L1 = .416
L2 = .407
GOTO 670

619 IF F >= 32.26 AND F < 38.26 THEN 620 ELSE 675
620 F1 = 32.26
F2 = 38.26
L1 = .424
L2 = .416
GOTO 670

670 LET A = L2 − L1
LET C = F2 − F1
LET B = F2 − F
LET L = L2 − (A * B / C)

REM PARAMETER OUTPUT
CLS
LOCATE 1,27
PRINT "CULTURE FLUORESCENCE:"; F
'COLOR 1
PRINT "BETA-LACTAMASE ACTIVITY IN TERMS
  OF CHANGE IN AMP.OD:"; L
INPUT "DO YOU WANT TO CONTINUE (Y/N)"; C$
IF C$ = "Y" OR C$ = "y" THEN 600 ELSE 624
675 PRINT "FLUORESCENCE INPUT OUT OF
  RANGE ! PLEASE REDO"
624 END

700
PRINT "LACTOSE"
INPUT "ENTER CULTURE FLUORESCENCE
  VALUES (385 EXN & 460 EMN)"; F

701 IF F >= 81.02 AND F <= 82.32 THEN 702
  ELSE 703
702 F2 = 82.32
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F1 = 81.02
L2 = 0
L1 = 0

GOTO 770

703 IF F >= 68.24 AND F < 81.02 THEN 704 ELSE 705
704 F2 = 81.02
F1 = 68.24
L1 = .052
L2 = 0!
GOTO 770

705 IF F >= 57.92 AND F < 68.24 THEN 706 ELSE 707
706 F1 = 68.24
F2 = 57.92
L1 = .0676
L2 = .052
GOTO 770

707 IF F >= 43.78 AND F < 57.92 THEN 708 ELSE 709
708 F1 = 43.78
F2 = 55.34
L2 = .067
L1 = .098
GOTO 770

709 IF F >= 40! AND F < 43.78 THEN 710 ELSE 715
710 F1 = 43.78
F1 = 40!
L1 = .16
L2 = .098
GOTO 770

715 IF F >= 38.32 AND F < 40! THEN 716 ELSE 717
716 F1 = 38.32
F2 = 40!
L1 = .104
L2 = .16
GOTO 770

717 IF F >= 37.66 AND F < 38.32 THEN 718 ELSE 719
718 F1 = 37.66
F2 = 38.32
L1 = .119
L2 = .104
GOTO 770

719 IF F >= 31.62 AND F < 37.66 THEN 720 ELSE 775
720 F1 = 31.62
F2 = 37.66
L1 = .098
L2 = .119
GOTO 770

770 LET A = L2 - L1
LET C = F2 - F1
LET B = F2 - F
LET L = L2 - (A * B/C)

REM PARAMETER OUTPUT
CLS
LOCATE 1, 27
PRINT "CULTURE FLUORESCENCE:"; F
'COLOR 1
PRINT "BETA-LACTAMASE ACTIVITY IN TERMS
  OF CHANGE IN AMP.OD:"; L
INPUT "DO YOU WANT TO CONTINUE (Y/N)"; C$
IF C$ = "Y" OR C$ = "y" THEN 700 ELSE 724
775 PRINT "FLUORESCENCE INPUT OUT OF
  RANGE ! PLEASE REDO"
724 END
```

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