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**EXPRESSION OF
CHITINASE GENE
FROM *Trichoderma virens* IN
*Escherichia coli***

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By
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INTRODUCTION





CHITINASE

➤ **Chitinase** are the **heterogeneous group of enzymes that catalyze the hydrolytic of chitin.**



CHITINASE



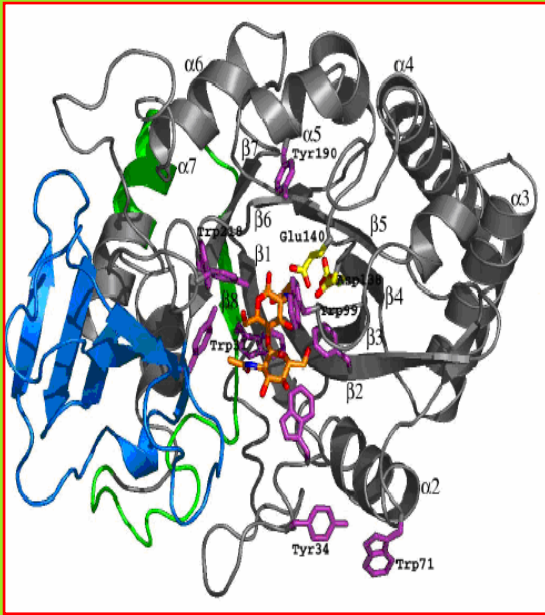
endochitinase

Random cleavage at
internal points in
the chitin chain and
generated low
molecular mass
multimers of GlcNAc

exochitinase

The progressive action
starting at the non-reducing
ends of chitin





Grey ribbon -- the backbone

Blue -- domain

Purple sticks -- Solvent-expose aromatic side chains lining the active site cleft

Orange -- NAG

SOURCES

Bacterial

Fungi

Serratia marcescens,
Enterobacter agglomerans
Aeromonas hydrophilia

Trichoderma harzianum,
Aspergillus niger and
Talaromyces emersonii

Ref: (Brurberg *et al.*, 2000).

Applications!!

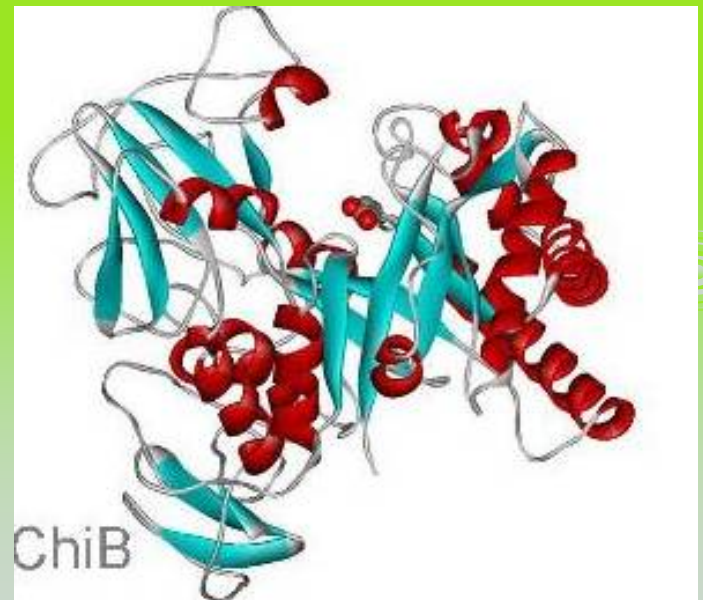
Target for biopesticides

Estimation of fungal biomass



Ref: Neetu et al., 2005)

The production of chitinase has received attention as one step in a bioconversion to treat waste.



Problem!!!

- Primary economic was estimated to account 12% of the TPC. **HIGH COST** due to high prices of commercially available chitinase.





however...!!!

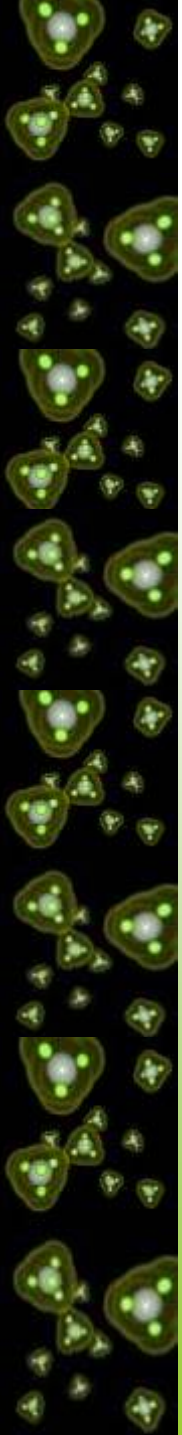
Genetic engineering technology provides a method to solve this problem.

Expressing the chitinase in *E.coli* help to developed new way to **produce the enzyme on a LARGE SCALE with LOWER COST**



Objective

**TO OVEREXPRESS the
chitinase gene in
Recombinant
Escherichia coli.**



Scope

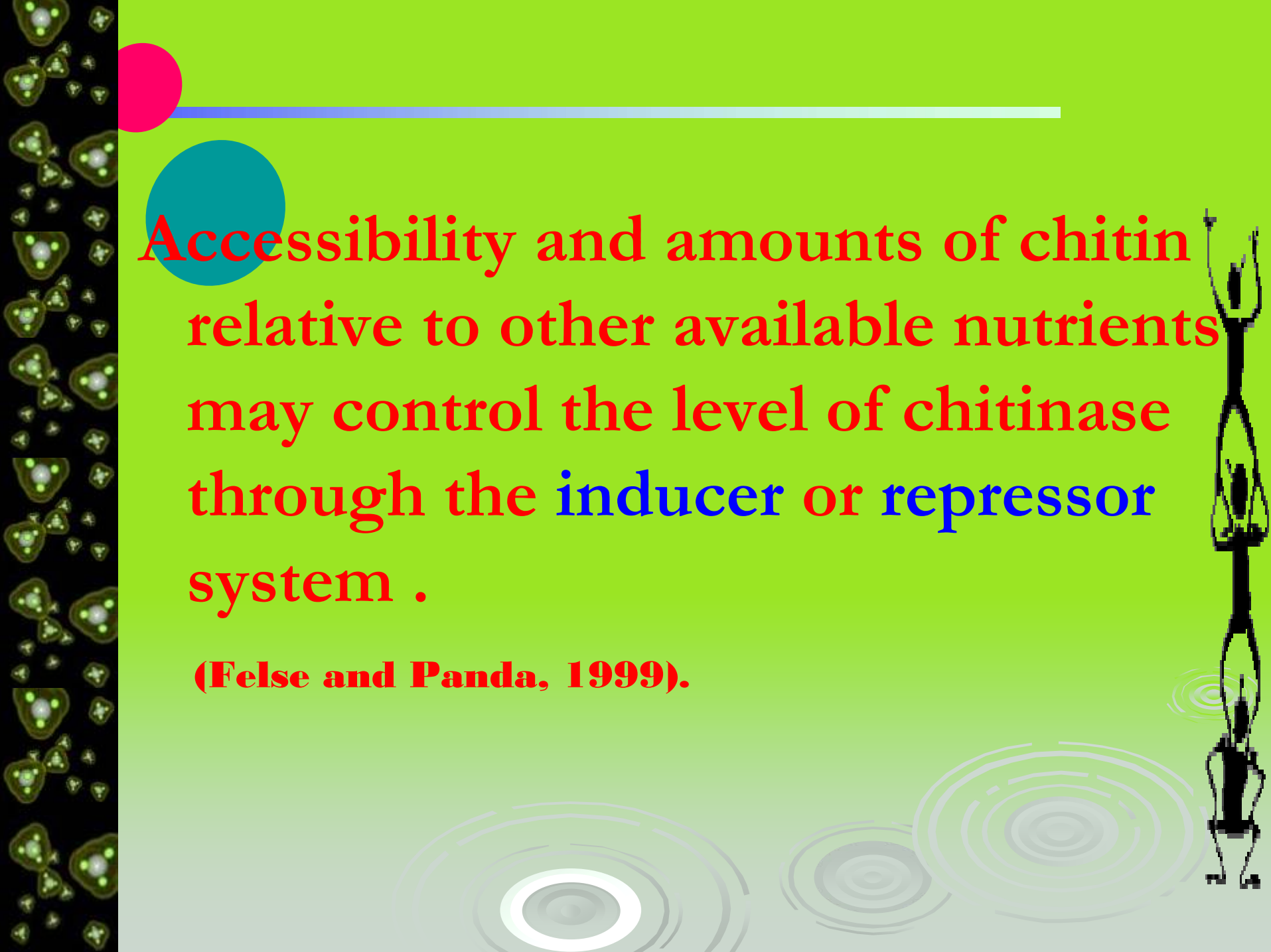
- TO STUDY THE EFFECT OF INDUCERS

- 1) IPTG
- 2) LACTOSE

- TO STUDY THE EFFECT OF REPRESSOR

- 1) GLUCOSE AND IPTG
- 2) GLUCOSE AND LACTOSE





Accessibility and amounts of chitin relative to other available nutrients may control the level of chitinase through the inducer or repressor system .

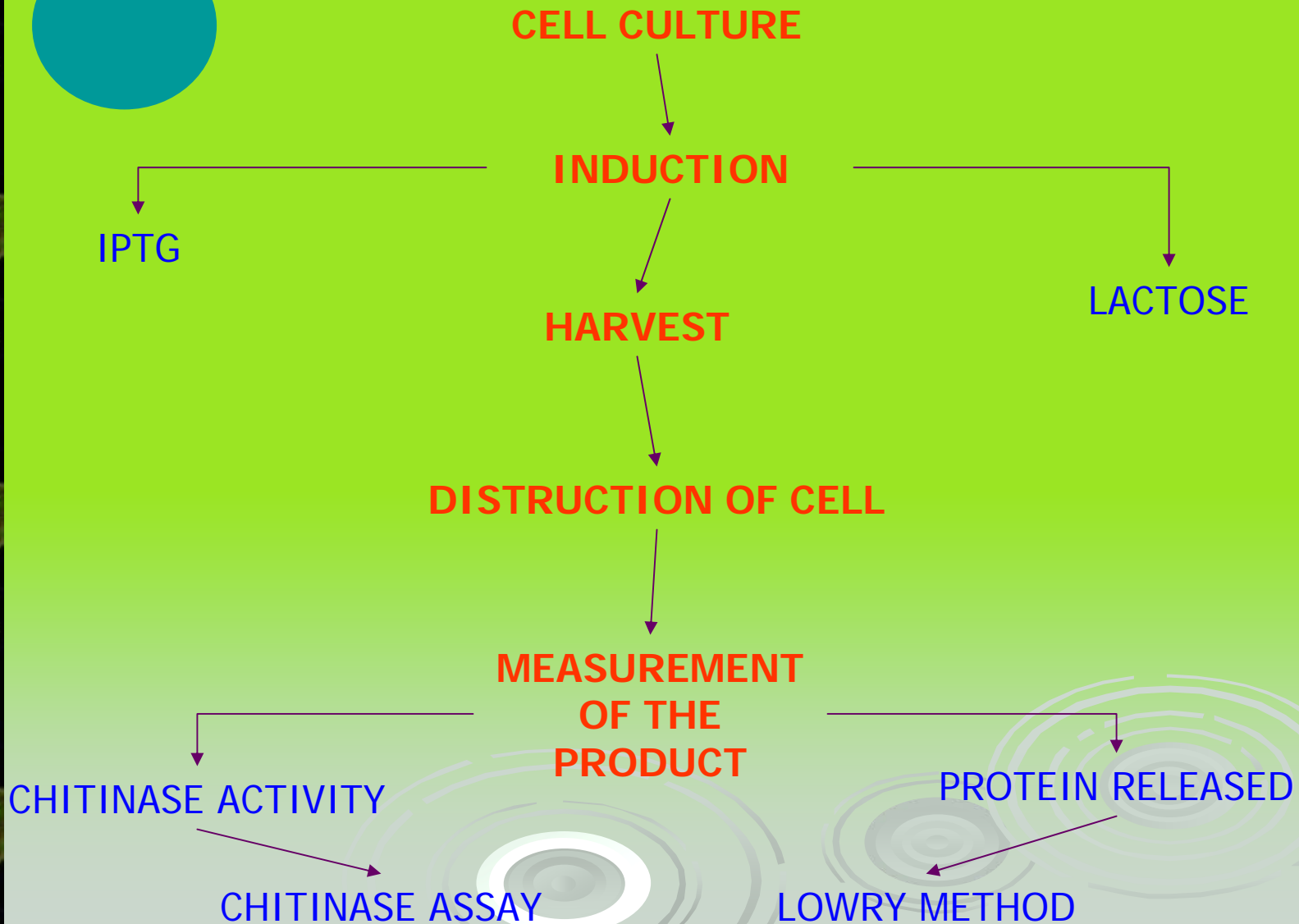
(Felse and Panda, 1999).



METHODOLOGIES



METHODOLOGIES



Effect of inducers

IPTG
(0.1mM, 1.0mM, 1.5mM)

Lactose
(0.1, 0.5, 1.0, 1.5 and 2%)

Best concentration

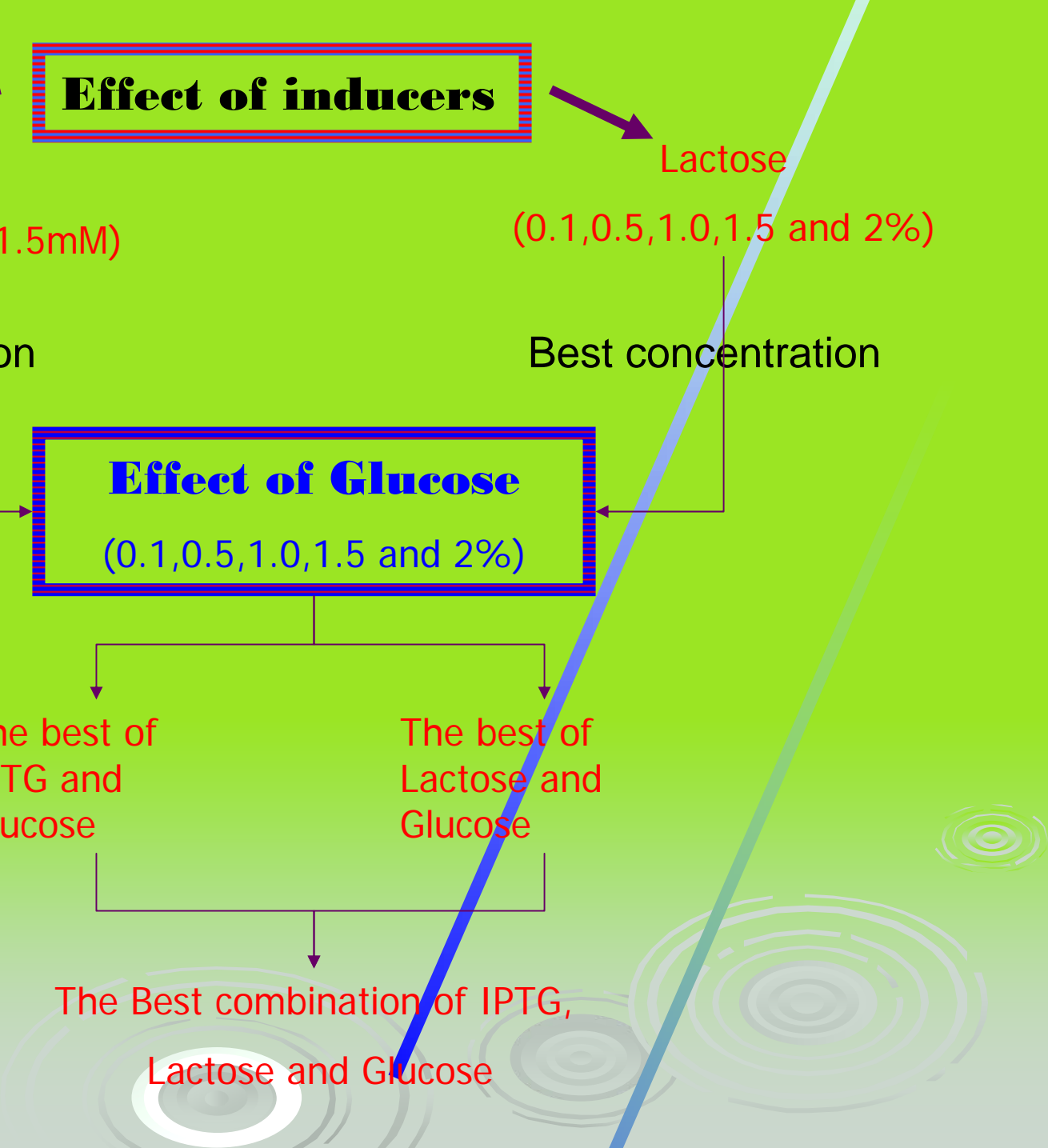
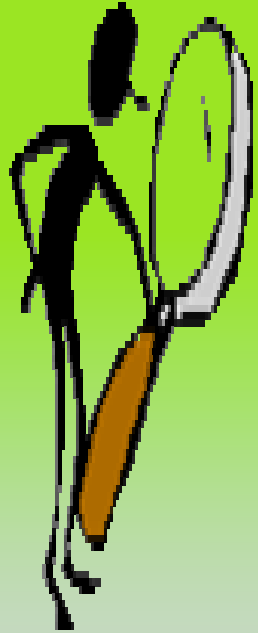
Best concentration

Effect of Glucose (0.1, 0.5, 1.0, 1.5 and 2%)

The best of
IPTG and
Glucose

The best of
Lactose and
Glucose

The Best combination of IPTG,
Lactose and Glucose





Medium preparation



Cell culture, 17 h,
37C, 200rpm



Medium to shake
flask ratio , 1:5



After OD reach 0.46,
induced



Incubate 6h, 18C, 200rpm



Harvest and remove the
supernatant

CELL LYSES



Lysozymes and TE buffer were added



Incubate for 20min, at 27C, 170prm



Remove the pellet and ready for the assay

ASSAY



1 ml sample + 1ml cc

Incubate in water bath,
50C, 1h

Add 1ml 1%
NaOH and
incubate in boil
water for 5min



Moved to appendorf
tube, centrifuged
5000rpm, 5 min



1 ml product + 1ml DNS
were boiled for 10min



Chitinase OD were
measured







RESULTS AND DISCUSSION



INDUCERS

REPRESSOR



•IPTG

•LACTOSE

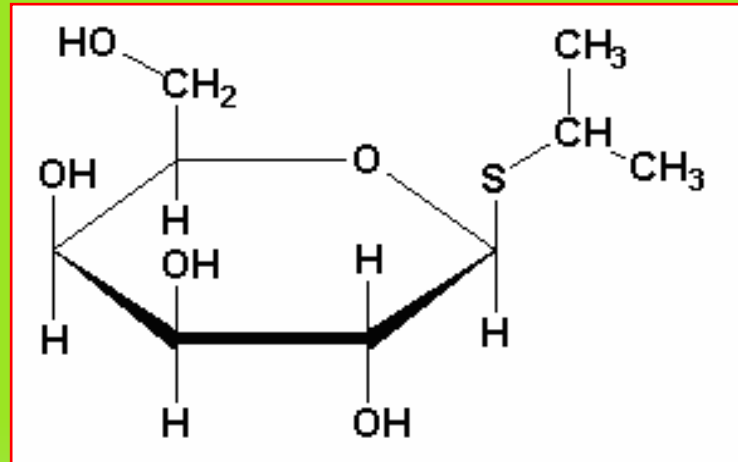
•INDUCER
COMPARISON

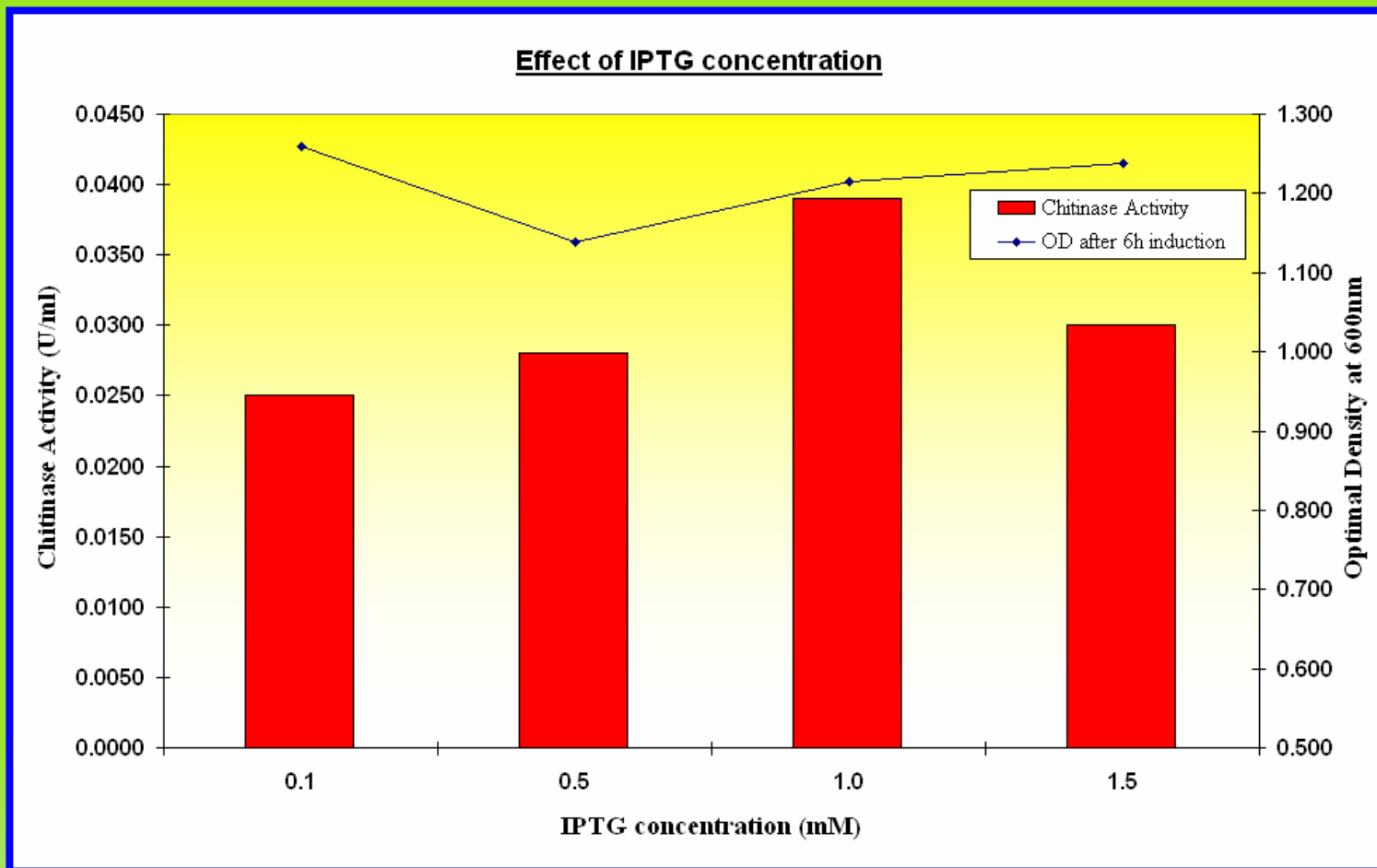
•GLUCOSE
AND INDUCERS

•GLUCOSE AND
CELL GROWTH

•THE BEST
COMBINATION

Isopropyl β -D-1-thiogalactopyranoside (IPTG)





Production of chitinase was influenced by IPTG where high and lower IPTG concentrations will INHIBIT the gene to expressed. Meanwhile, on the other hand it do not affected by the cell growth.

Optimum value

1mM of IPTG

inducer lower than
the optimum value

It was **TOO WEAK**
to yield much
foreign protein.

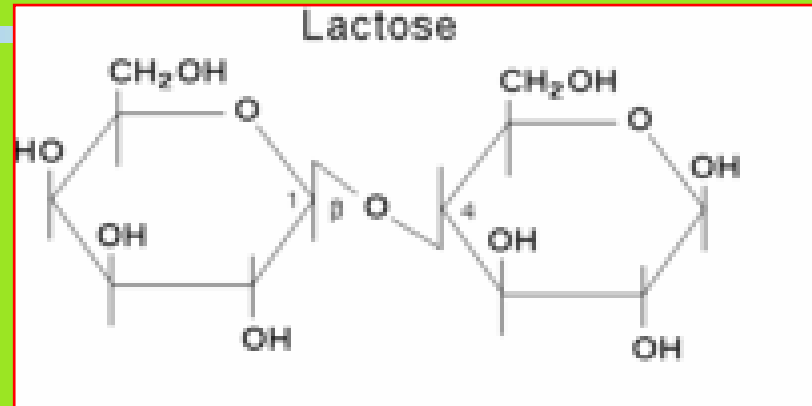
inducer level higher than
the optimum value

it was **TOO STRONG**
where the metabolism of cell is
influence drastically and
productivity reduce.

REF: (Zheng *et al.*,2004)

BACK

Lactose

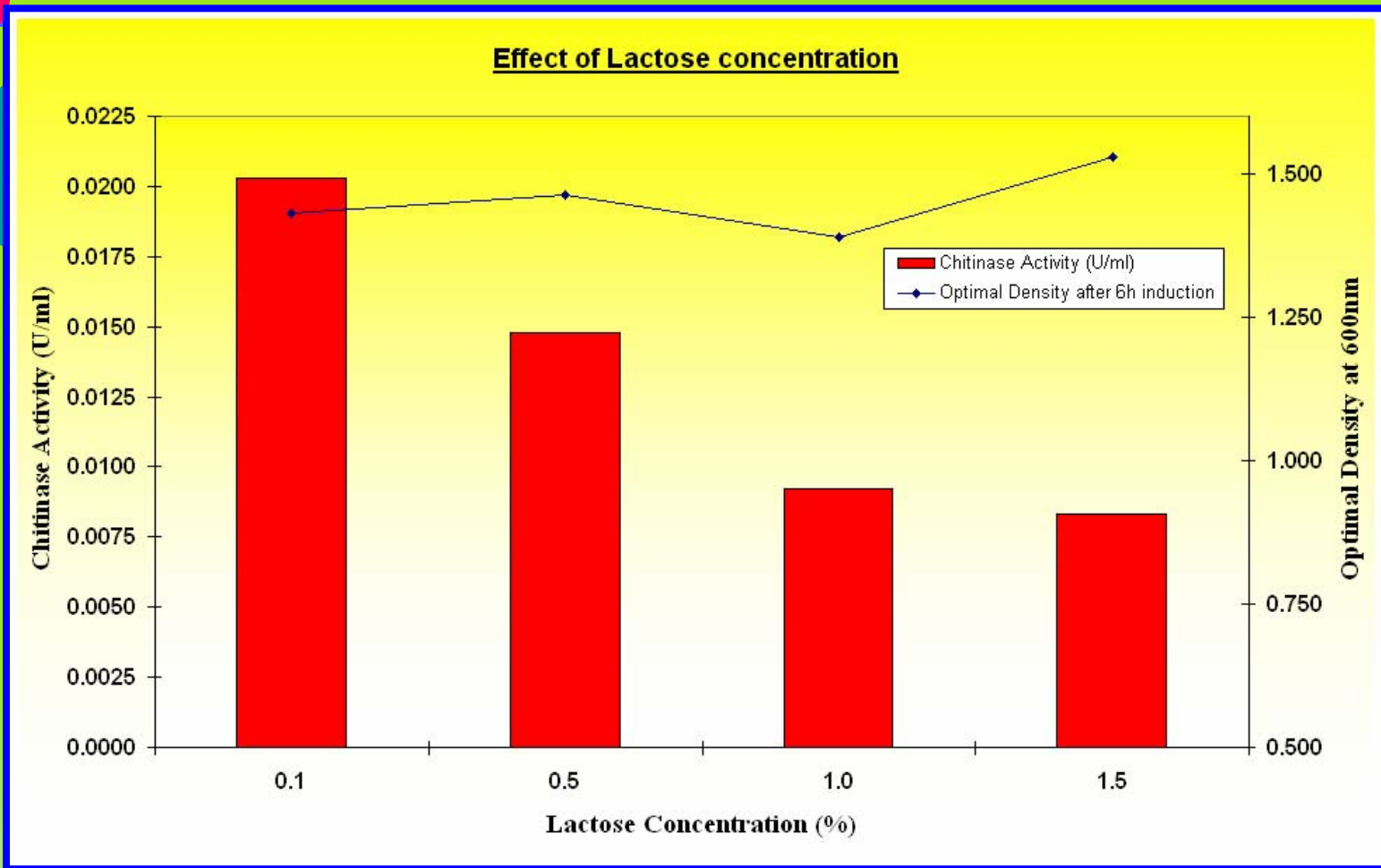


- Lactose has been proven to be as effective as IPTG for inducing recombinant proteins in *E. coli* and the use of lactose to enhance the solubility of the expressed protein has been reported

(Monteiro *et al.*, 2000)

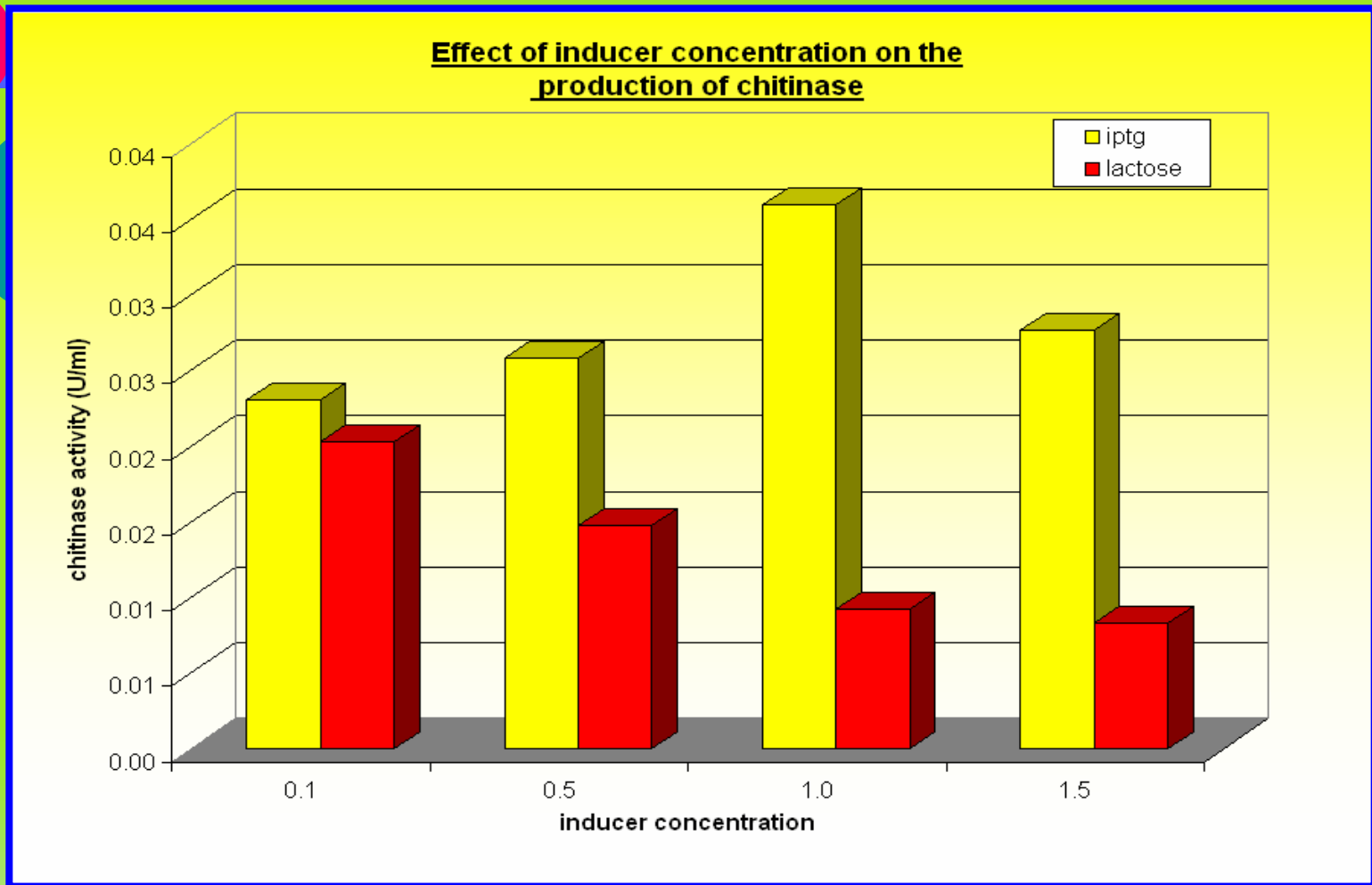
- low-cost and non-toxicity make lactose a potential for engineering products

> (Menzella *et al.*, 2002).



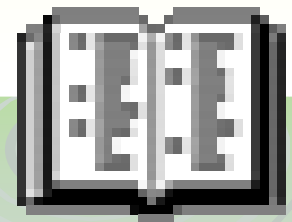
Production of chitinase **INVERSELY PROPORTIONAL** to the concentrations of **lactose** and independent to the cell growth.

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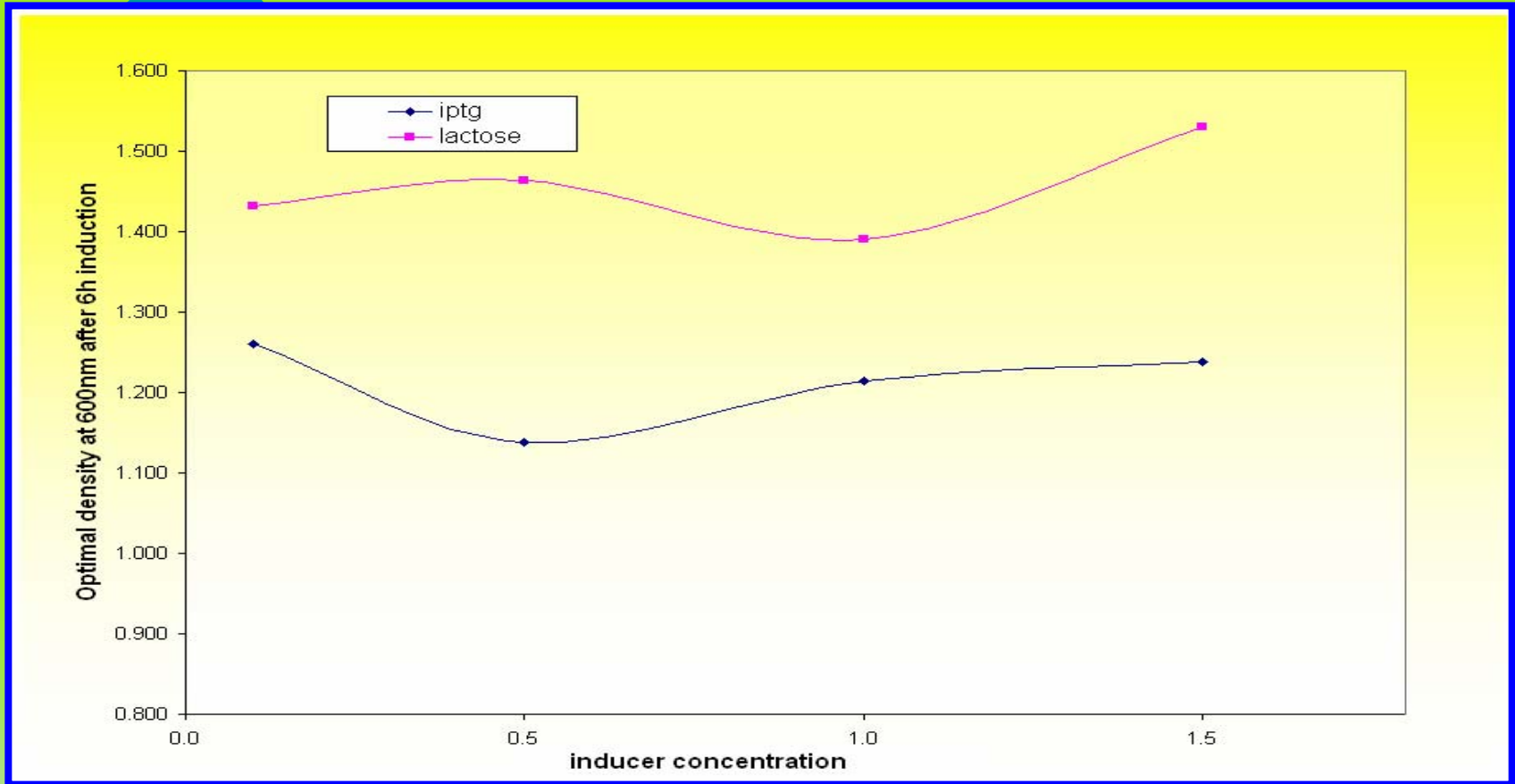


Results confirmed that **IPTG**, the frequently used inducer, could be completely substituted by **lactose**.

INDUCER	IPTG	LACTOSE	reference
ENTER THE CELL	Enter directly into the cell	With the help of <i>lac</i> permease	Donovan RS <i>et al.</i>, 1996
WORK	IPTG binds <i>lac</i> repressor protein directly without further change	Before binding to the <i>lac</i> repressor protein, lactose has to be converted into <i>allo</i>-lactose.	Beckwith <i>et al.</i>, 1987



EFFECT OF INDUCERS TO THE CELL GROWTH



Cells grow more rapidly in a presence of lactose

Why cells grow rapidly in a presence of lactose??

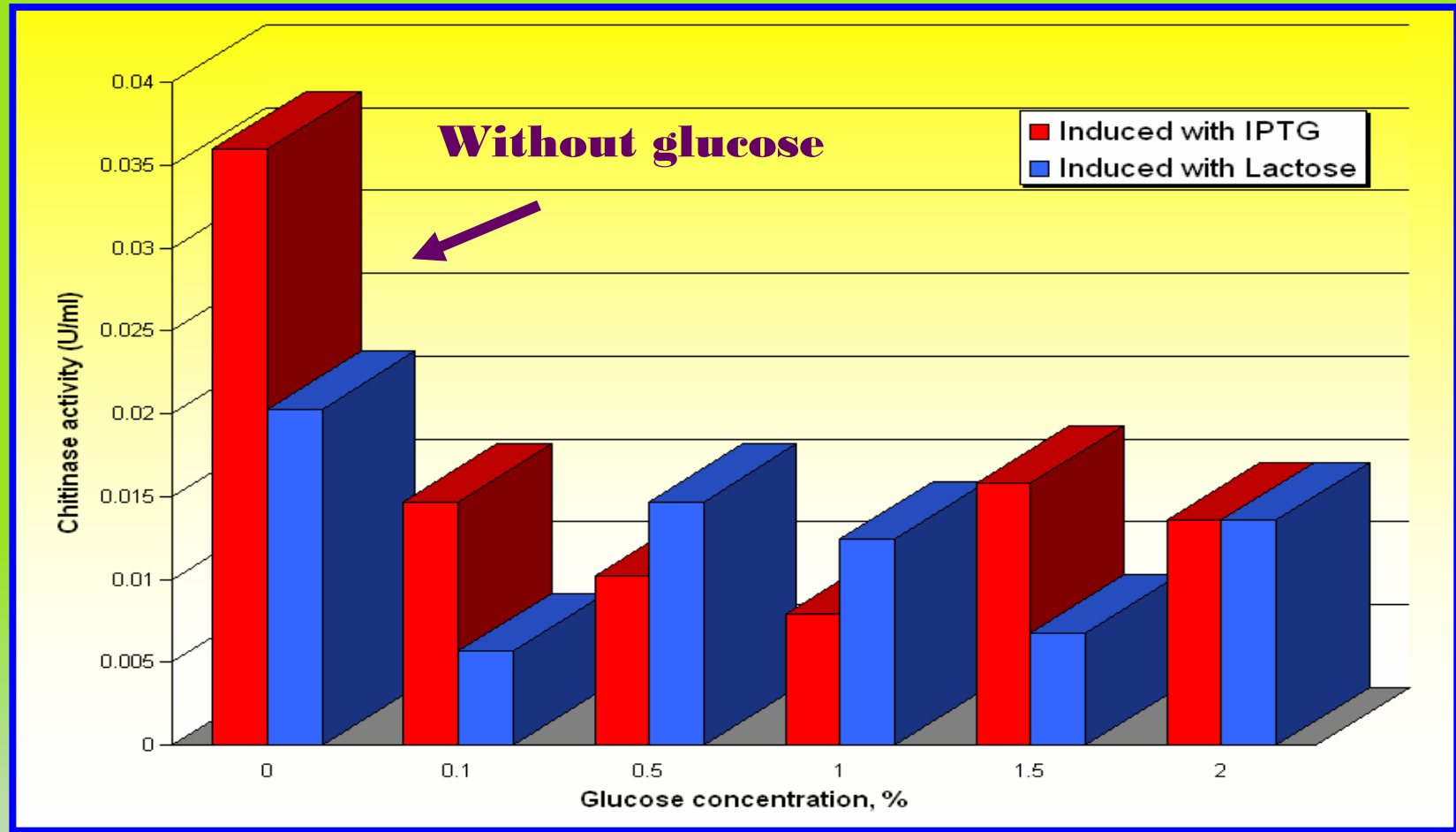


- Lactose can work as **INDUCER** and **CARBON SOURCE** simultaneously, therefore, more cell recovery was observed when lactose was added to the growth medium.

(Weng *et al.*, 2005)

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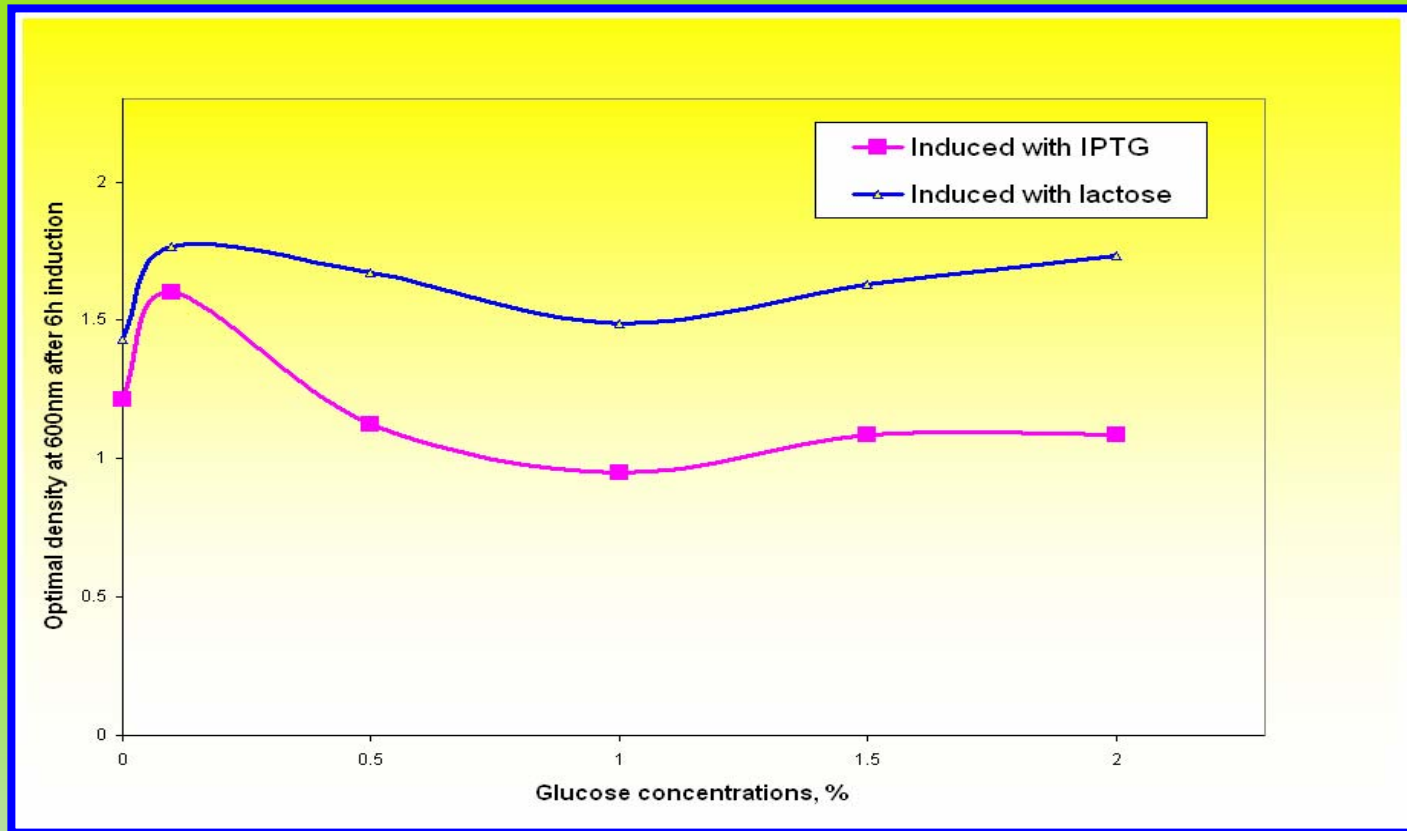
EFFECT OF GLUCOSE



The additional of glucose **DEPRESSED the expression**

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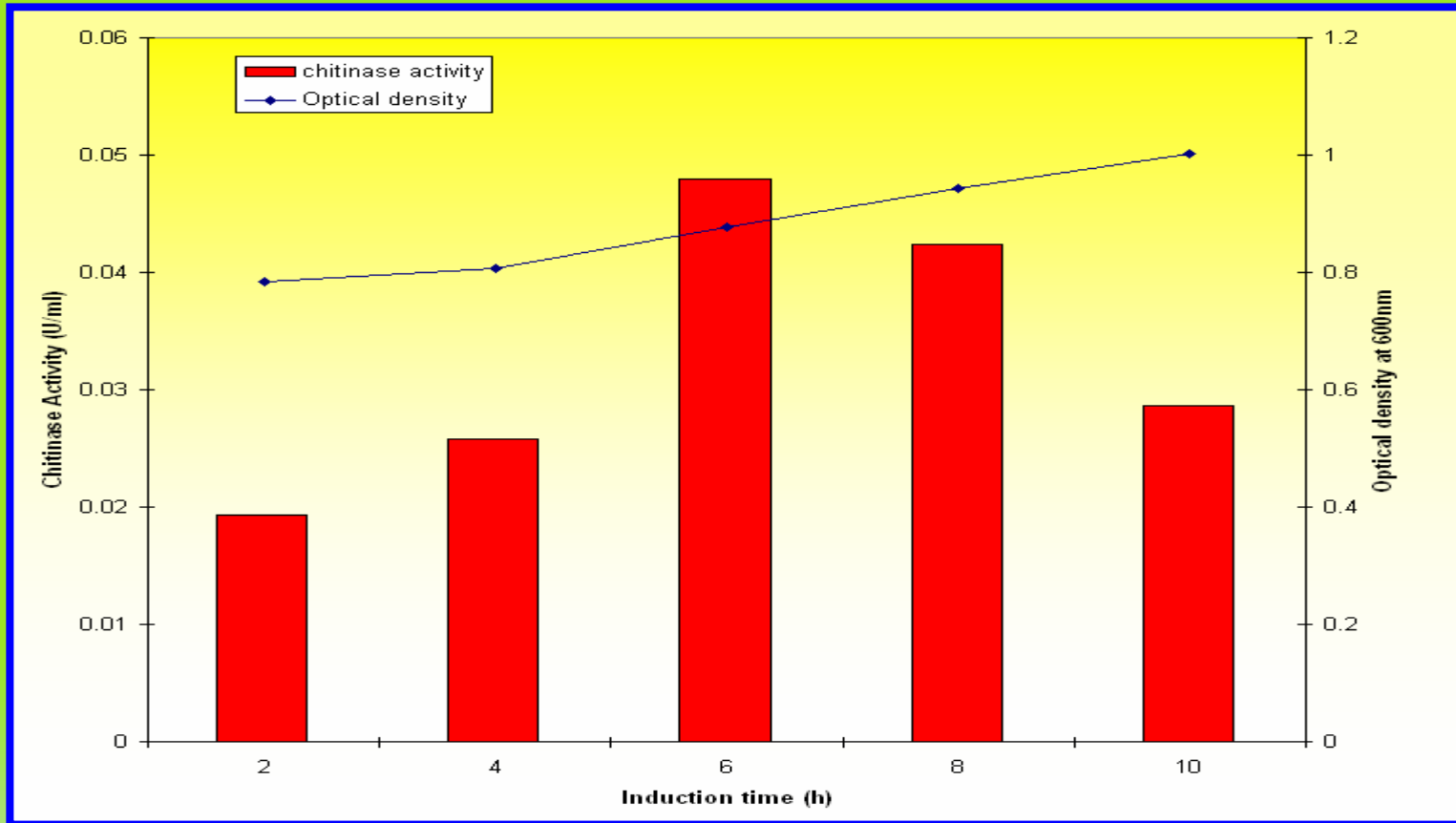
CELL GROWTH WITH GLUCOSE



**CELLS GROW FASTER IN A PRESENCE
OF GLUCOSE AND LACTOSE**

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THE BEST COMBINATION



The combination of IPTG and lactose can overcome the repression effect by glucose. The production of chitinase increased in a bell curve where the maximum is situated at the exponential phase of the cell growth.



PROJECT ● ○ ●

CONCLUSION



SCOPE 1

IPTG : 1mM

**LOW AND HIGH CONCENTRATION INHIBIT
THE EXPRESSION**

LACTOSE :0.1%

**ABLE TO INDUCE CHITINASE AND
PRODUCTIVITY DECREASED ALONG WITH
LACTOSE CONCENTRATION**

SCOPE 2

GLUCOSE

**DECREASED THE
PRODUCTIVITY**



ThaNK yOU!!



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Reetarani S. Patil, Vandana Ghormade and Mukund V. Deshpande



Target for biopesticides

- **Chitin present in the exoskeleton of insects.**



Estimation of fungal biomass

- **A strong correlation has been reported between chitinase activity and fungal population in soils**

Medical

➤ **chitinase can be employed in human health care, such as making ophthalmic preparations with chitinase and microbiocides.**

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Mosquito control

- **The lethal time for larvae with chitinase is 24 h compare to 48 h without chitinase.**