



Natraj
D.No.: 45-1108

1994
Asmi Bio Tech









P. SIVA REDDY

T.S. SADASIVAN

M.S. SWAMINATHAN





M.S. SWAMINATHAN
Centre for Research



T.S. SADASIVAN

M.S. SWAMINATHAN

K.R. DRONAMRAJU



















M.K. SWAMINATHAN

N.R. SUBRAMANIAM

RANJANA S. RAJAN











SAMBASIVAN AUDITORIUM



























SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1982, MADRAS, INDIA





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1981 MADRAS, INDIA





ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA







INTERGENERIC HYBRIDISATION BETWEEN TRICHODERMA REESEI AND EREMOBOTHECIUM ASHBYII FOR THE CONVERSION OF CELLULOSE TO RIBOFLAVIN

B.P. LAKSHMI & T.S. CHANDRA *Dept. of Chemistry, IIT, Kharagpur - 726 705*

INTRODUCTION

With the advent of recombinant technology, it has been possible to induce genetic recombination in combination of organisms. This technique is now largely used for bringing together the desired characters in industrially important fungi.

In this study attempt has been made to fuse two industrially important fungi, the *Trichoderma reesei* (a hypercellulolytic organism, source of cellulase) & a potent riboflavin producer, *Eremobotrium ashbyi*. A protoplast fusion has been proved successful with hybrids producing both cellulase and riboflavin, suggesting fusion in fungi has not been altogether new, since the work by Shimizu et al., on other acid producing bacteria from *Trichoderma reesei* & *A. niger*.

MATERIALS AND METHODS

Organisms

Trichoderma reesei (DSM 1614) from DT, Delhi and *Eremobotrium ashbyi* (DSM 1614) from IIT, Kharagpur were used for the study. *E. ashbyi* was maintained in *T. reesei* at a concentration of 10^8 spores/ml.

Protoplast Production and Fusion

Conditions for protoplast production and regeneration were standardized (Table 1). The best substrate for protoplast release was added in good regeneration.

Equal numbers of protoplasts from the two strains were mixed together, centrifuged, and resuspended in 1.5 ml of sterile distilled water. Protoplasts were placed on minimal media plus bromocresol based on yeast cell membrane characteristics. The hybrids were transferred to plates with PDA, and observed for regeneration.

Enzyme Assays

Optimal enzymes were assayed by the IUPAC standard methods.

Analytical Methods

Riboflavin was estimated spectrophotometrically. DNA content was done as per the method of Bhatia¹.

RESULTS AND DISCUSSION

The rapid release of protoplasts from the *Eremobotrium* is evident as compared to that of *Trichoderma* (Table 1).

Phase 2 & 4 show the protoplasts of the two organisms under study and the presence of fusion when treated with PEG and illustrated microscopically when treated with methylene blue for *Trichoderma* (Table 2) and the auto fluorescence given in Table 3.

The *Trichoderma* under study with cellulase activities shows that the mutants are depressed in their activity levels as compared to the parent. To assess comparatively better enzyme levels of PDB, CMCase and β -glucosidase.

E. ashbyi shows negative values for cellulase activities. It produces around 2.5 mg/l of riboflavin (Table 2).

Table 2 depicts the fusion properties. Most of the hybrids from Phase 2 study, all of them show cellulase activity. DNA content of all the hybrids given 2 to be identical. Regeneration on PDA also confirms the fact. It can be observed that hybrids with high riboflavin yield have high cell wall susceptibility.

This is the only work in the protoplast fusion area where a hybrid is characterized also by its cell wall susceptibility to cell wall degrading enzymes.

TABLE 1
PROTOPLAST RELEASE AND REGENERATION CHARACTERISTICS OF THE ORGANISMS UNDER STUDY

Strain	Substrate	Enzyme	Concentration	Time	Yield
<i>T. reesei</i>	PDB	CMCase	10 ⁸	24	1.5
			10 ⁷	24	1.5
<i>E. ashbyi</i>	PDB	CMCase	10 ⁸	24	2.5
			10 ⁷	24	2.5
Hybrids	PDB	CMCase	10 ⁸	24	1.5
			10 ⁷	24	1.5

TABLE 2
PROPERTIES OF HYBRIDS UNDER STUDY

Phase	Strain	Substrate	Enzyme	Concentration	Time	Yield
1	<i>T. reesei</i>	PDB	CMCase	10 ⁸	24	1.5
				10 ⁷	24	1.5
2	Hybrids	PDB	CMCase	10 ⁸	24	1.5
				10 ⁷	24	1.5
3	<i>E. ashbyi</i>	PDB	CMCase	10 ⁸	24	2.5
				10 ⁷	24	2.5

1. Cell wall degrading enzymes
2. Cell wall degrading enzymes
3. Cell wall degrading enzymes
4. Cell wall degrading enzymes

HIGHLIGHTS

- Intergeneric fusion among *Trichoderma* and *Eremobotrium* feasible.
- CMCase is always expressed amongst the hybrids while PDB and β -glucosidase are not expressed quite well.
- Hybrids with high levels of riboflavin require easy cell wall breakdown which is a characteristic of the *E. ashbyi* parent.
- DNA content has doubled in all the hybrids under study.



REFERENCES

- Bhatia, S. K. (1971) Genetic recombination in fungi. *Journal of General Microbiology*, **65**, 1-10.
- Shimizu, T., Kato, T., & Kato, M. (1971) Genetic recombination in fungi. *Journal of General Microbiology*, **65**, 1-10.
- Shimizu, T., Kato, T., & Kato, M. (1971) Genetic recombination in fungi. *Journal of General Microbiology*, **65**, 1-10.
- Shimizu, T., Kato, T., & Kato, M. (1971) Genetic recombination in fungi. *Journal of General Microbiology*, **65**, 1-10.
- Shimizu, T., Kato, T., & Kato, M. (1971) Genetic recombination in fungi. *Journal of General Microbiology*, **65**, 1-10.









AGRICULTURAL BIOTECHNOLOGY

6-10 MARCH 1994 MADRAS, INDIA





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA



PROF
LI DNEVUSKENS

PROF
H S SVANIKANTHUS

THIRD
T V VENKATARAMAN

DR
G S SUDHAKAR

PROF
G L SUDHAKAR

DR
S S SUDHAKAR

SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA









SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA









AZIZ MIAZ

LI ZHEN

JOSEPH THOMAS







SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
MADRAS, INDIA





**TONAL
SERVICES**





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
19-20 MARCH 1994 MADRAS, INDIA





SECOND ASIA-PACIFIC
ON
AGRICULTURAL BIO
6-10 MARCH 1994











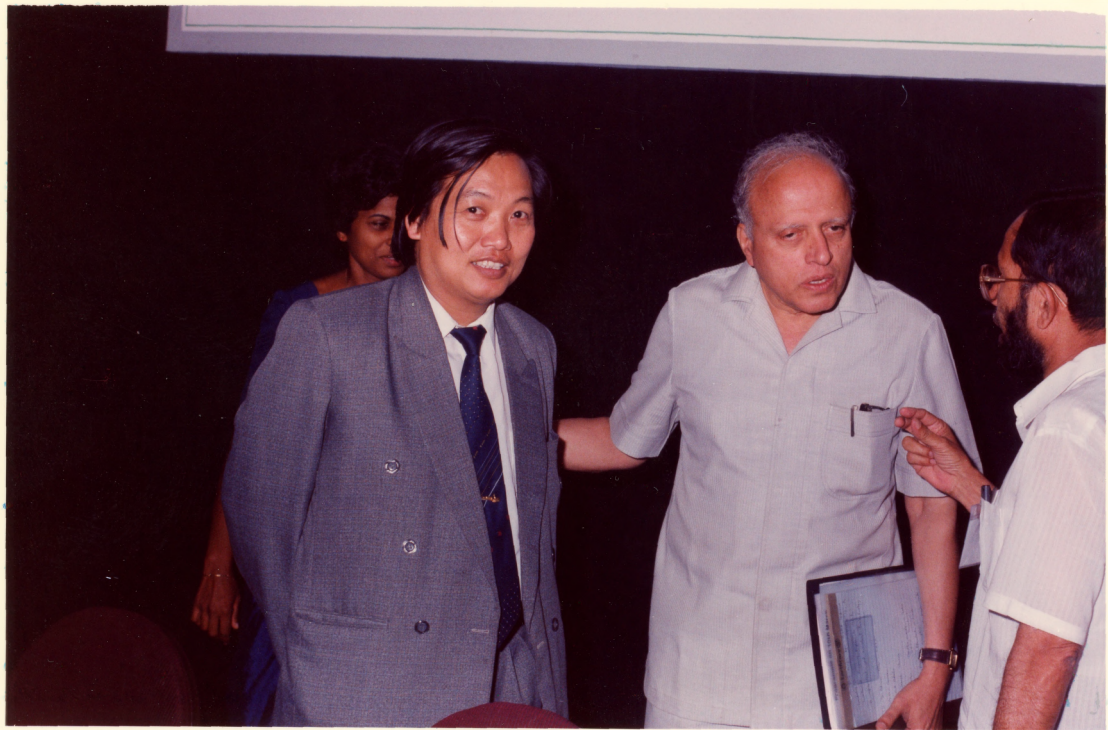
ON
URAL BIOTECHNOLOGY
RCH 1994 MADRAS, INDIA



SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA







SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA







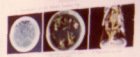
FORESTRY

MEDICINAL PLANTS AND FORESTRY

GENETIC APPLICATIONS

GENETIC FLUX
IN PINEAPPLE

CULTURE IN KIT. IMPROVEMENT



SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA





6-10 MARCH 1994 MADRAS



KUNUDU FERNANDO

S SWAMINATHAN

SUT
SRI





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA













**CONSERVATION OF CITRUS SINENSIS GERMPLAS
DORMANT ADVENTITIOUS SHOOT BUDS REGENERATED!**

B. D. SINGH AND RUCHI MAGGON
School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi

TISSUE CULTURE

BIODIVERSITY IN AGRICULTURE
BIOTECHNOLOGY IN AGRICULTURE



6-10 MARCH 1994 MADRAS, INDIA



SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA







ALPHAS, INDIA



GY
A











SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA









BIOTECHNOLOGY

OF FARM ANIMALS AND POULTRY

Biotechnological Interventions and Molecular Changes in Current Systems, and its Application in Industry Practice

ABSTRACT

INTRODUCTION

CONCLUSIONS

IMPLEMENTATION OF BIOTECHNOLOGY IN PULLED MILK SYSTEM

ABSTRACT

INTRODUCTION

RECENT AND PAST CONTRIBUTION OF BIOTECHNOLOGICAL INTERVENTION IN PULLED MILK

ABSTRACT

INTRODUCTION

SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA





SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA



MIAZ

LI ZHENSHENG

JOSEPH THOMAS

SHRI RAO

SHRI RAO



AGRICULTURAL BIOTECHNOLOGY

6-10 MARCH 1994 MADRAS, INDIA





MR GANESH

AGRICULTURE
6-10 MARCH 1994 MADRAS, INDIA







SECOND

AGRIC
6-10







**TONAL
SERVICES**



SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA



DR JOSEPH THOMAS



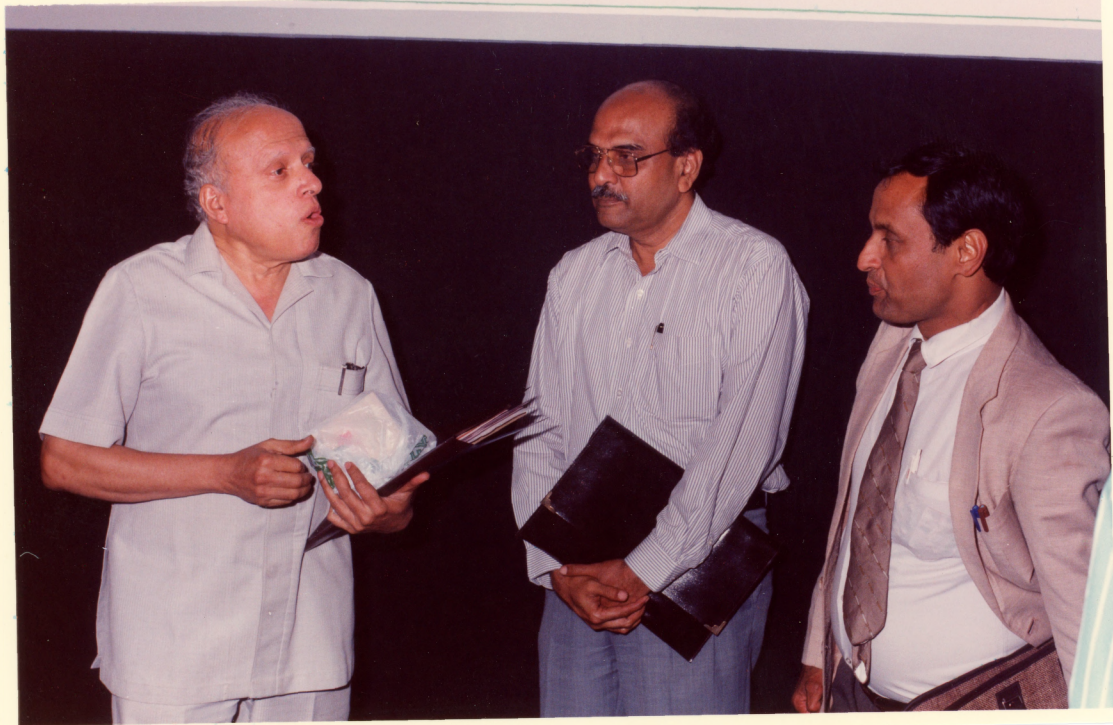
DR LI DECHAMBERA



DR S. SUNDARARAJAN















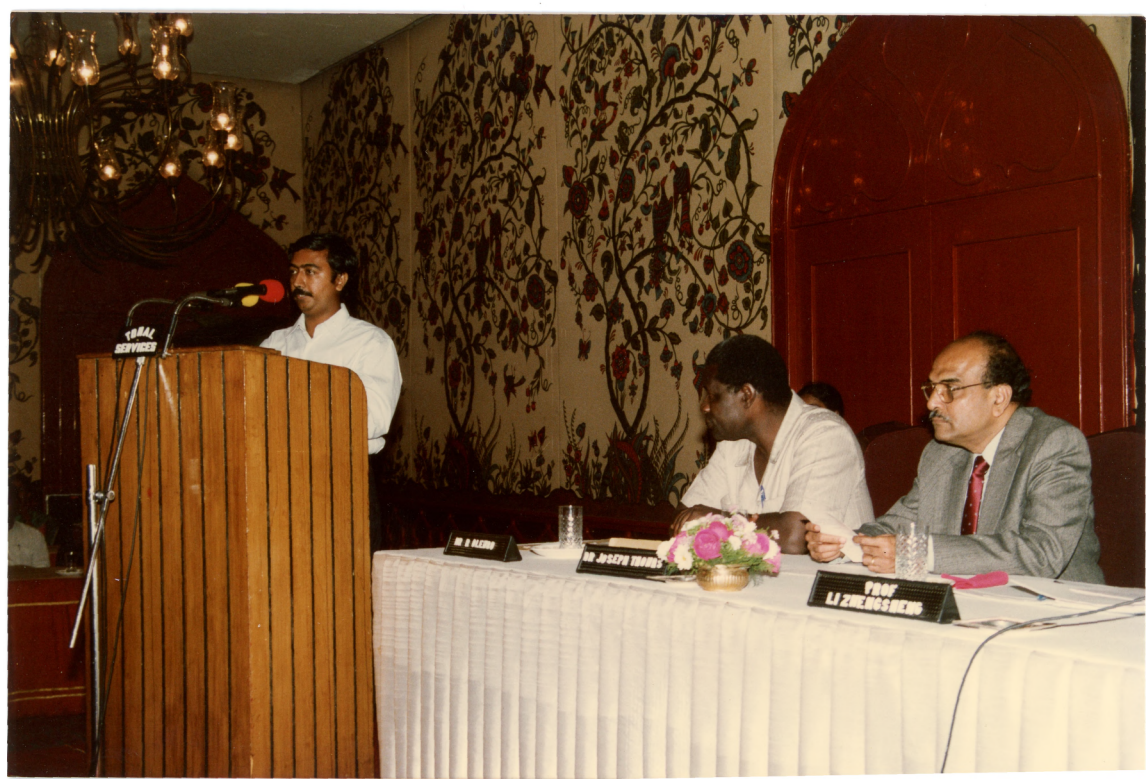
RICHARD JEFFERSON

INAPON

S PAR

SECOND ASIA-PACIFIC CONFERENCE
ON
AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA





TOTAL SERVICES

MR. A. ALI

MR. JOSEPH HODGES

PROF. LI ZHENGSHENG





AGRICULTURAL BIOTECHNOLOGY
6-10 MARCH 1994 MADRAS, INDIA



**ABDULLAH
KHAN**

PROF V L CHOPRA

DR C R BHATIA



