

Introduction

- ❖ **Microbial biotransformation** is a biological process whereby an organic compound is modified or converted to a new product reversibly or irreversibly by micro-organism.
- ❖ It is one of the tools of **Green Chemistry**

Objective

- ❖ Study the microbial transformation of some of the substituted coumarins.
- ❖ Standardisation and establishing the conditions suitable for biotransformation.
- ❖ Identification and characterisation of the transformed products, using NMR, Mass spectra and HPTLC.

Methods

- ❖ Nine biotransformation experiments were performed, where, random combination of four micro-organisms and five substrates (JSN-1 to JSN-9) showing optimum recovery of the final compounds were selected.
- ❖ The bio-transformations were selected from the preliminary study to scale-up the process, based on the results of TLC obtained.

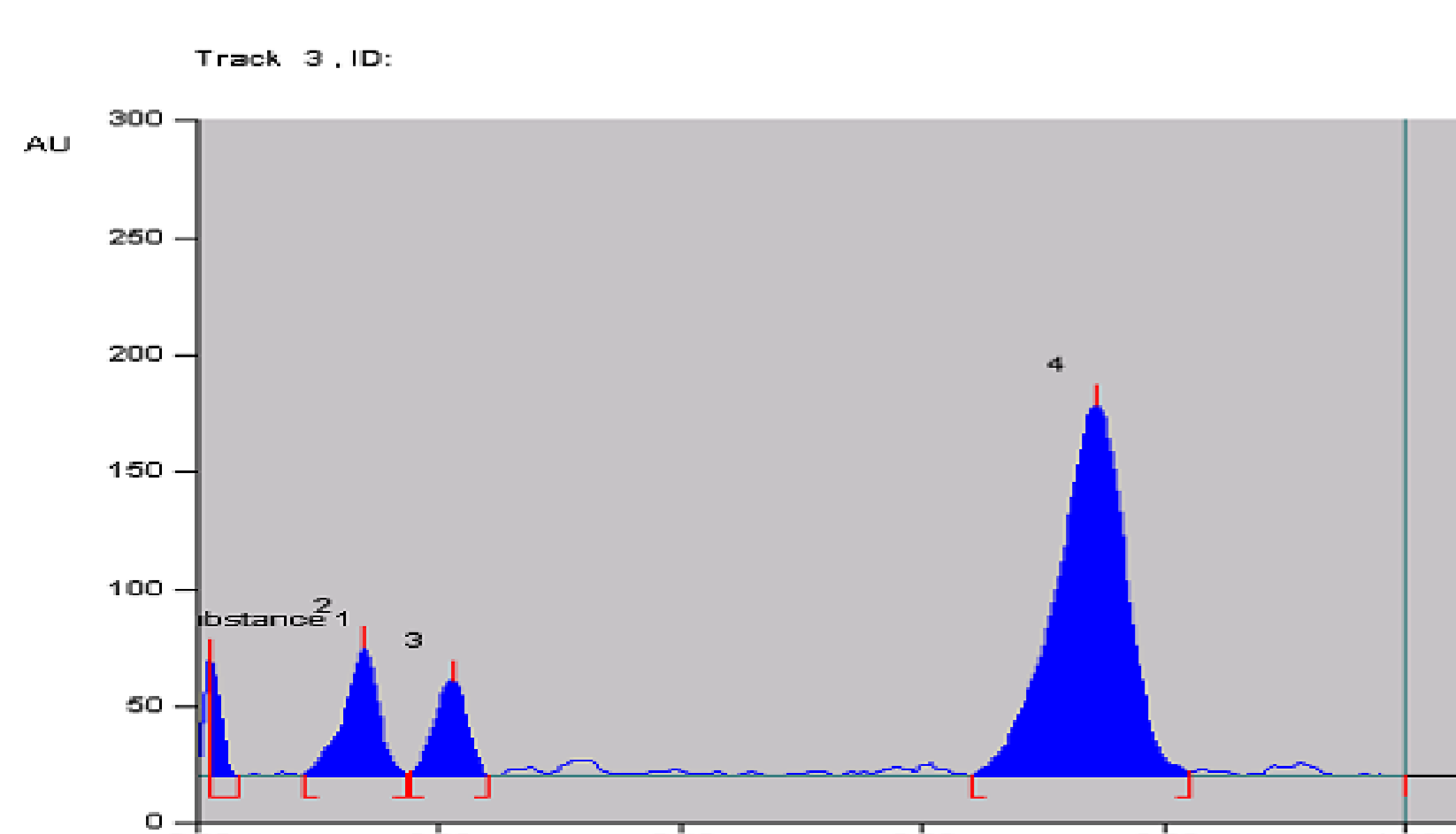
Sr. NO	Organism	CODE	Substrate
1	<i>Candida tropicalis</i>	JSN-3	7-hydroxy-4-methylcoumarin
2	<i>Gliocladium roseum</i> (NCIM 1037)	JSN-4	3-acetyl coumarin
3	<i>Streptomyces griseus</i>	JSN-7	7-methoxy coumarin
4	<i>Streptomyces griseus</i>	JSN-8	7-ethoxy-4-methylcoumarin
5	<i>Streptomyces griseus</i>	JSN-9	7-methoxy-4methylcoumarin

HPTLC: Figure 1 shows, HPTLC plate developed in Bnz:MeOH(9:1) loaded with crude test extracts, substrate and standards, scanned at 366nm.

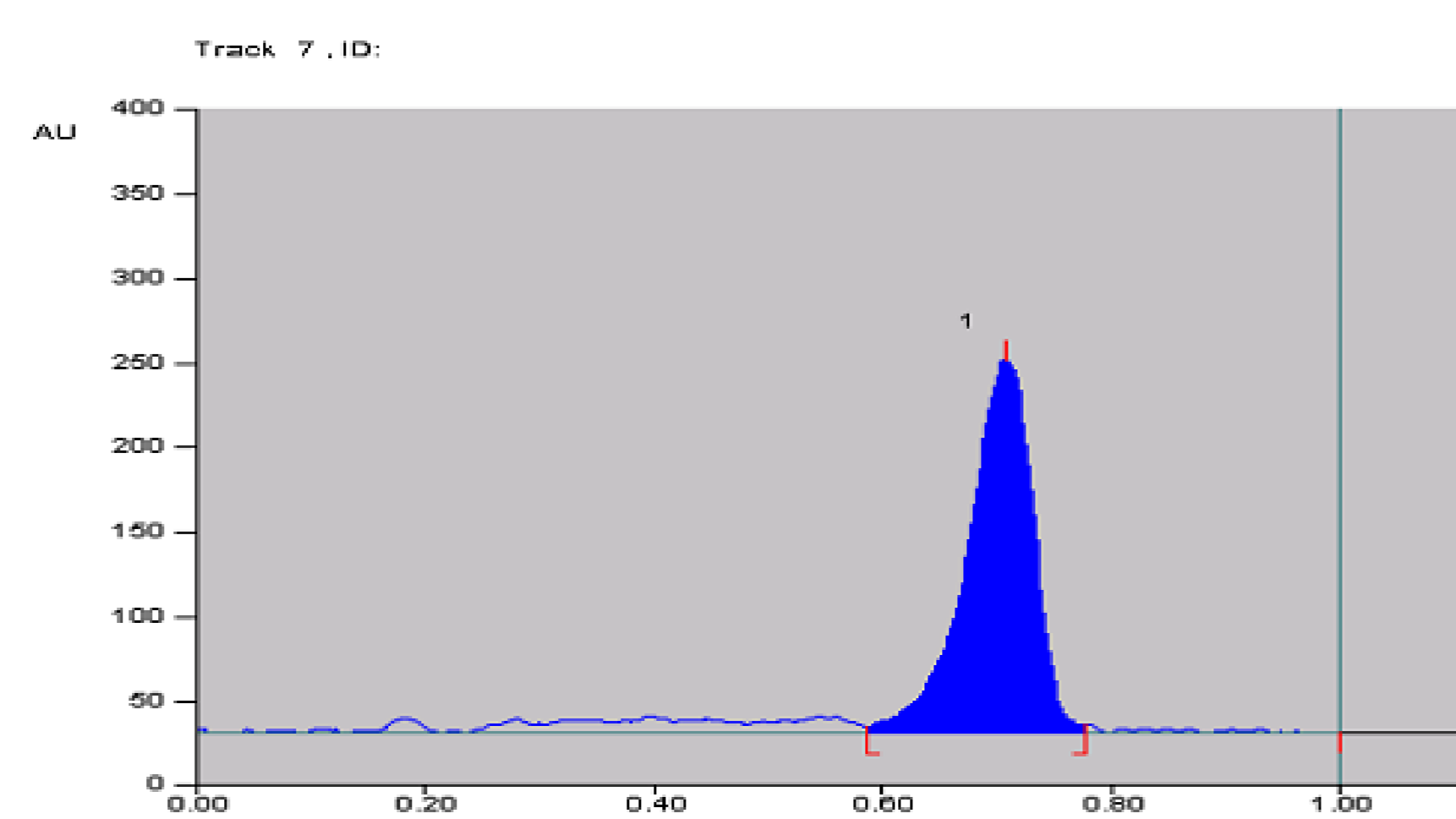
- ❖ Extract JSN-8 showed 4.24% conversion of test compound 7-ethoxy-4methylcoumarin to 7-hydroxy-4-methylcoumarin, whereas extract JSN-3 showed 5.52% conversion of 7-hydroxy-4-methylcoumarin to 7-methoxy 4-methylcoumarin.



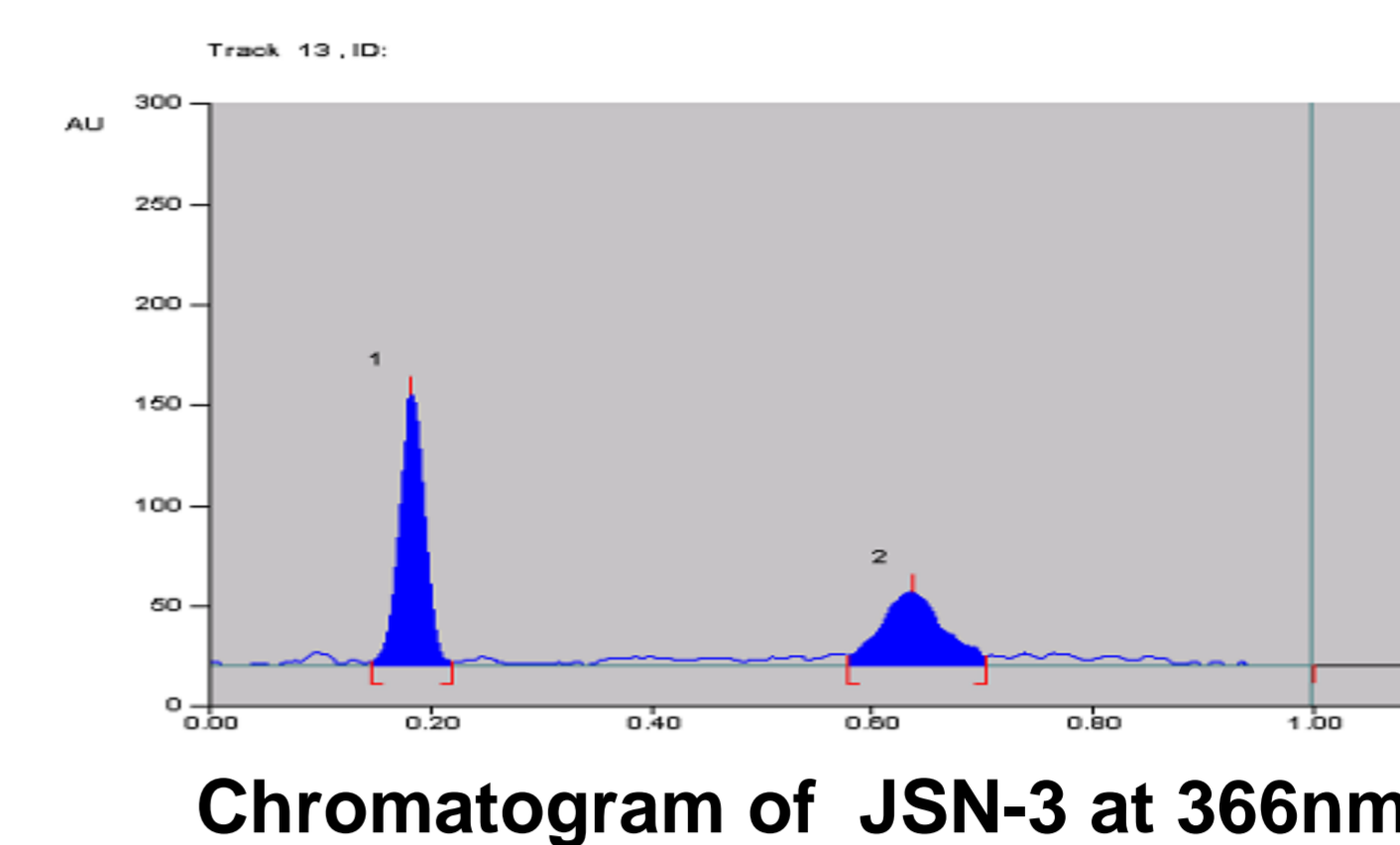
Figure 1



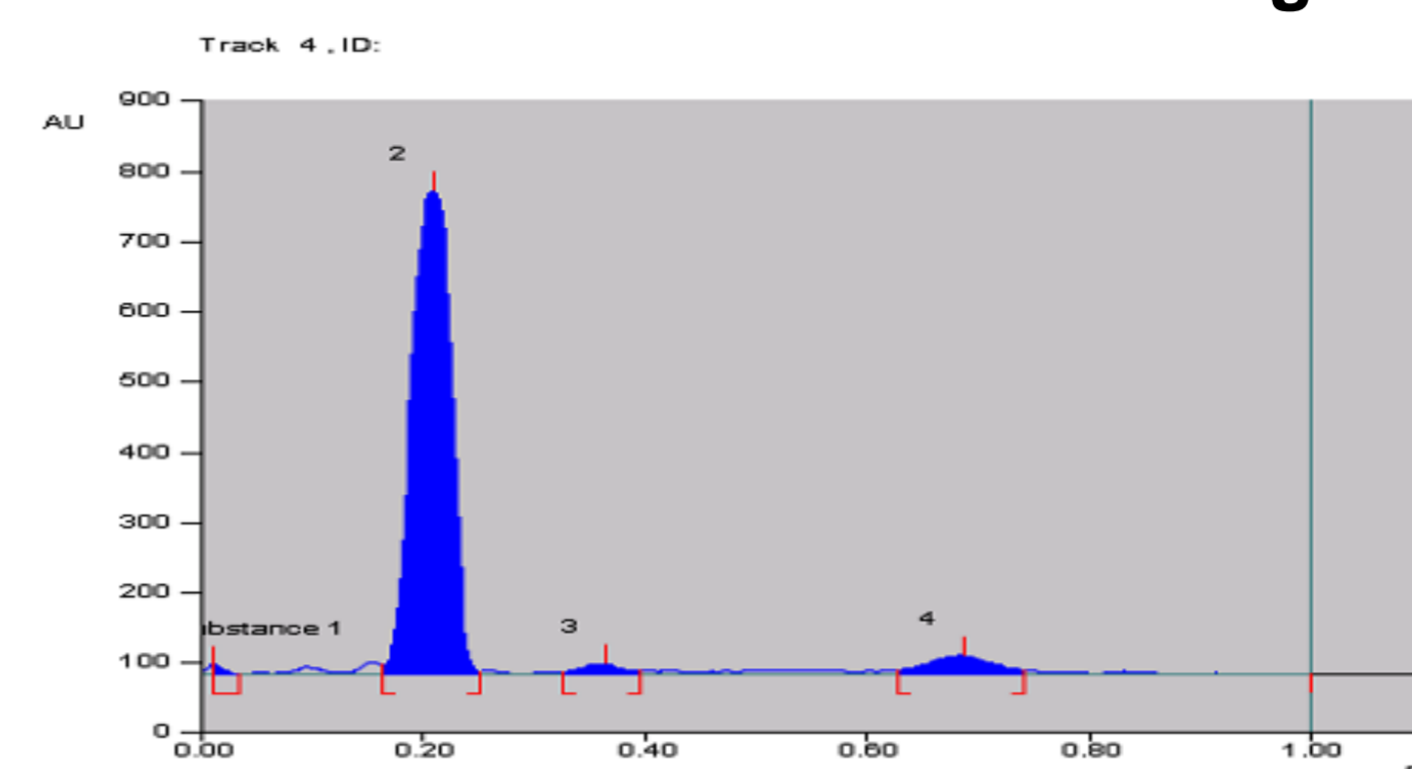
Chromatogram of JSN-8 at 366nm



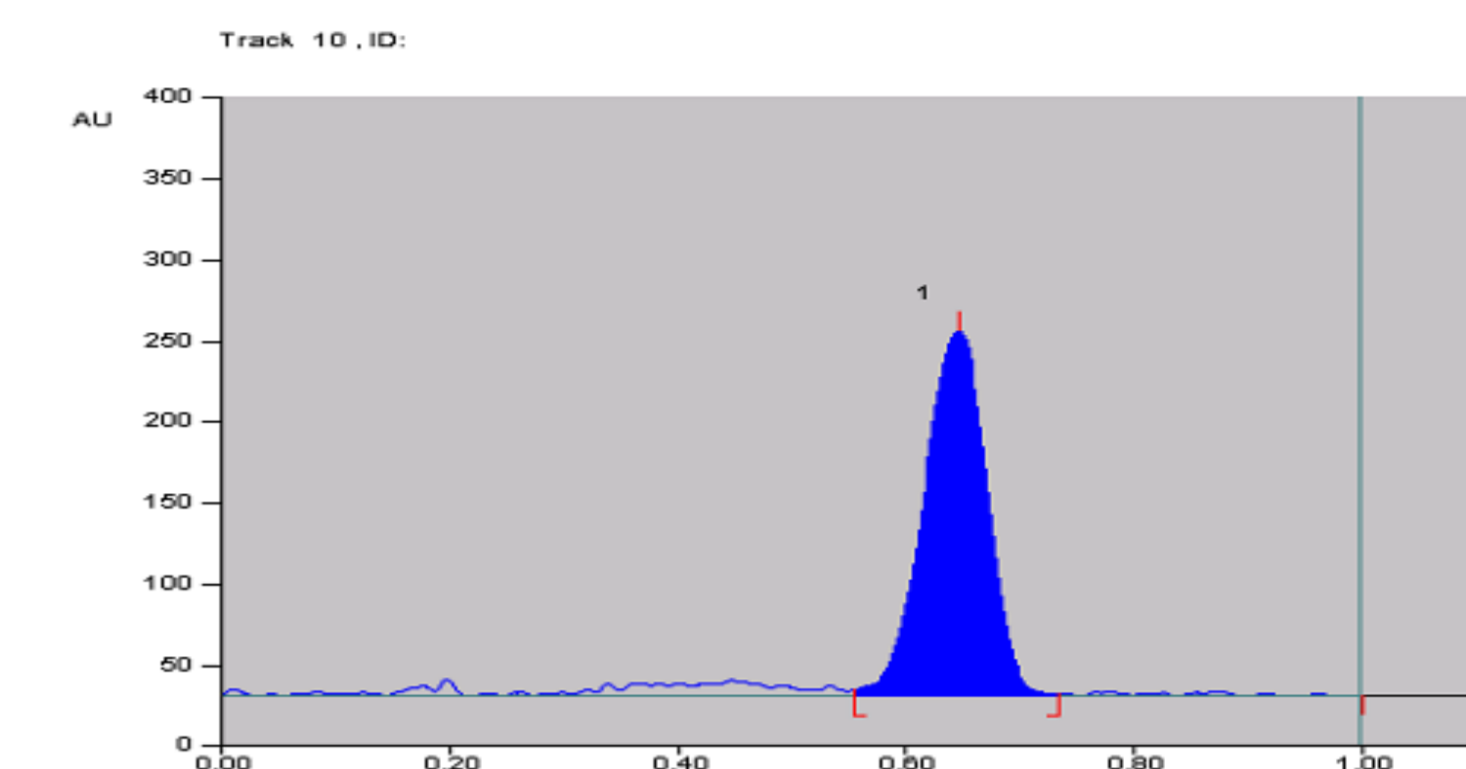
Chromatogram of 7-ethoxy-4-methylcoumarin at 366nm



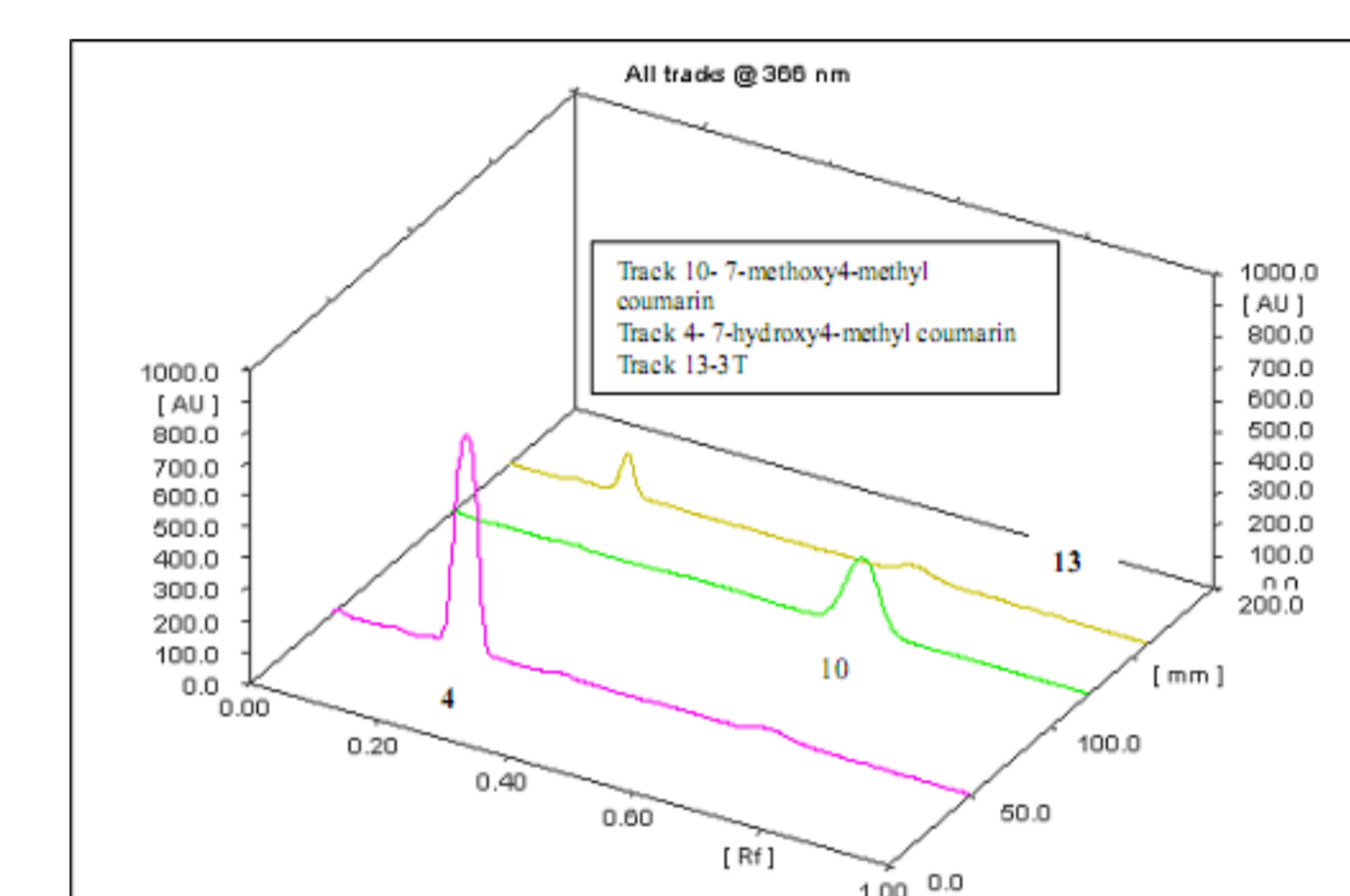
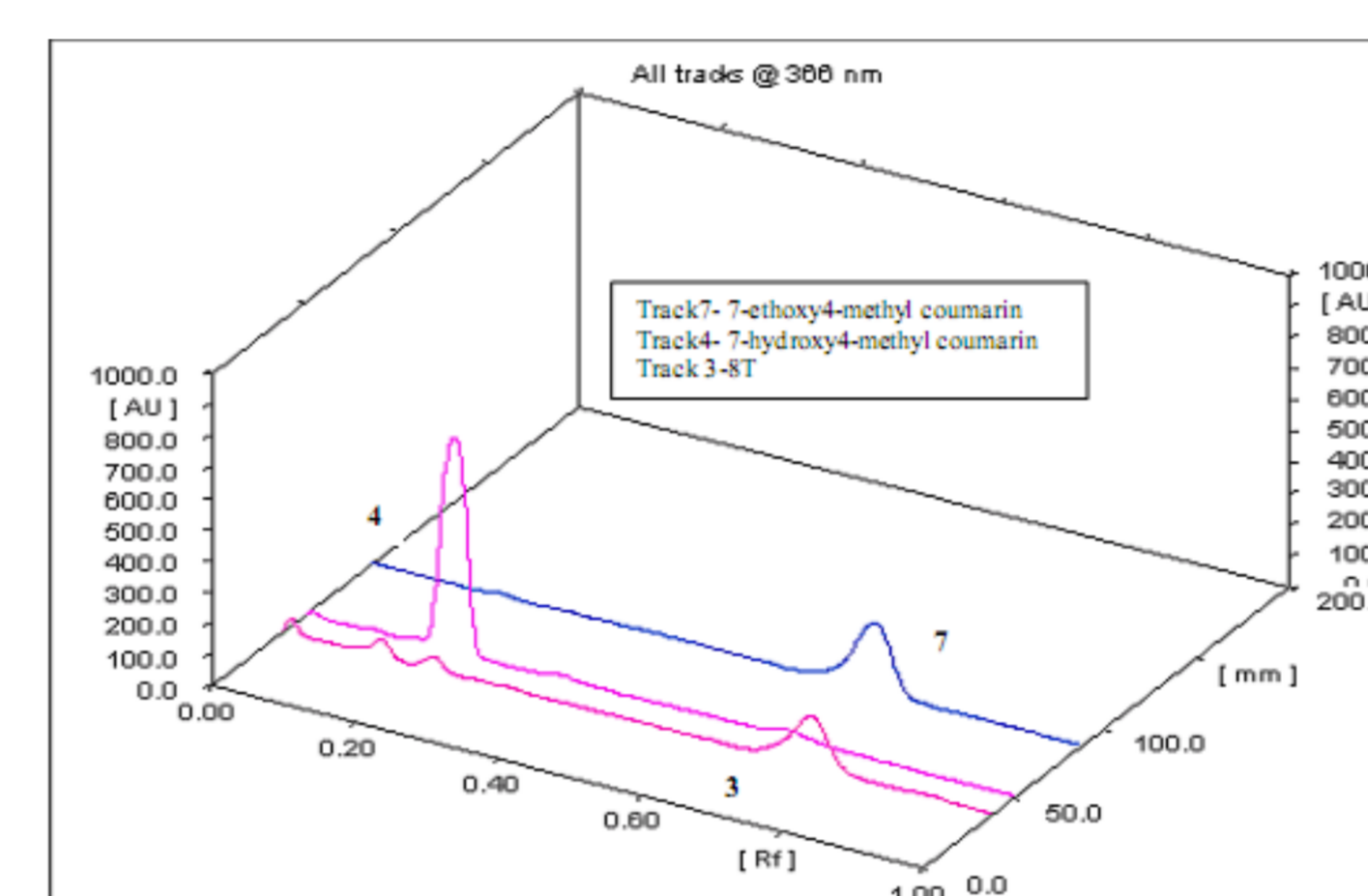
Chromatogram of JSN-3 at 366nm



Chromatogram of 7-hydroxy-4-methylcoumarin at 366nm

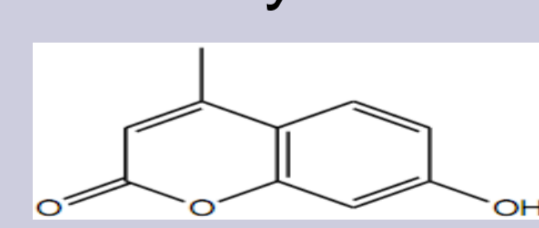
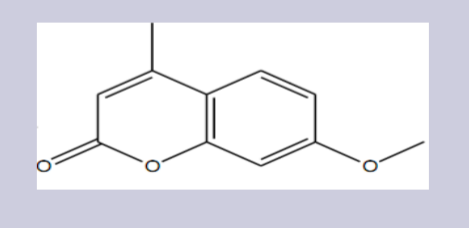
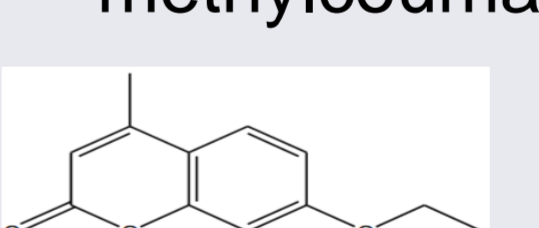
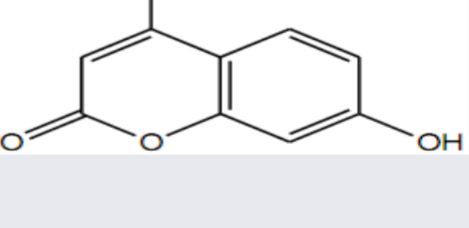


Chromatogram of 7-methoxy-4-methylcoumarin at 366nm



Results and Discussions

Out of the nine biotransformation experiments performed on different substrates using specific organism, the following transformations have been expected to occur successfully:

Sr. NO	Organism	CODE	Substrate	Transformed product
1	<i>Candida tropicalis</i>	JSN-3	7-hydroxy-4-methylcoumarin 	7-methoxy-4methylcoumarin  (5.52% yield)
2	<i>Streptomyces griseus</i>	JSN-8	7-ethoxy-4-methylcoumarin 	7-hydroxy-4-methylcoumarin  (4.24% yield)

Conclusion

- ❖ HPTLC was performed for crude test extracts such as JSN-3, JSN-4, JSN-8, JSN9 and JSN-7 using solvent system Benzene: Methanol (9:1). The crude test extracts showed the presence of both starting substrate and the final product.
- ❖ Mass spectra and HPTLC data analysis, supported the occurrence of two biotransformation, such as, conversion of 7-ethoxy-4-methylcoumarin to crude product of 7-hydroxy-4-methylcoumarin in 4.24% yield by *Streptomyces griseus* and the conversion of 7-hydroxy-4-methylcoumarin to crude product of 7-methoxy 4methylcoumarin in 5.52% yield by *Candida tropicalis*.

References

- 1Lawrence Philip Wackett, C. Douglas Hershberger. Biocatalysis and biodegradation microbial transformation of organic compounds. Marcel Dekker, New York, NY 9. 1995; 22: 24.
2. James E Leresche, Hans-Peter Meyer. Chemocatalysis and biocatalysis (biotransformation): Some thoughts of a chemist and of a biotechnologist. Organic Process Research & Development 2006; 10(3):572-580.
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