

Supplementary file

Production, optimization and characterization of esterase isolated from a new endophytic *Trichoderma afroharzianum* strain AUMC 16433 and its applications in dye decolorization

Yehia A.-G. Mahmoud¹, Nisrin S. Alamin², Tarek M. Mohamed², Nesma A. El-Zawawy¹ and Maha M. Salem^{2*}.

¹ Botany and Microbiology Department, Faculty of Science, Tanta University, 31257, Tanta, Egypt.

² Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, 31257, Tanta, Egypt.

Emails:

yehia.mahmoud@science.tanta.edu.eg

Nisreen_181425_pg@science.tanta.edu.eg

Tarek.ali@science.tanta.edu.eg

nesma.elzawawi@science.tanta.edu.eg

maha_salem@science.tanta.edu.eg

*Correspondence and proof:

Dr. Maha M. Salem (Ph.D.)

Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, 31257, Tanta, Egypt.

Email: maha_salem@science.tanta.edu.eg

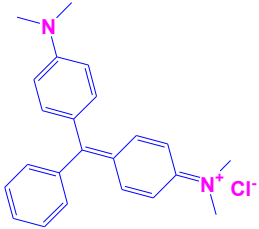
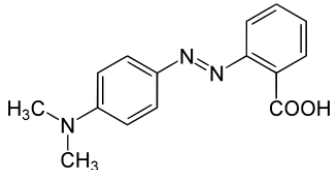
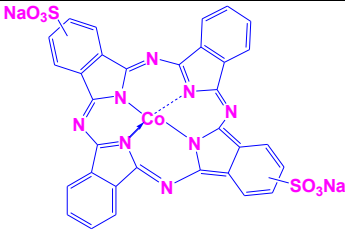
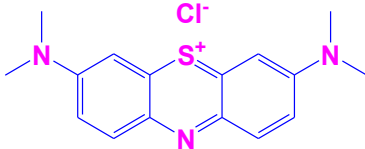
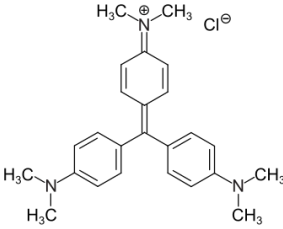
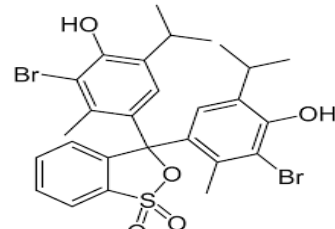
Table S1. Experimental variables for Plackett-Burman at different levels.

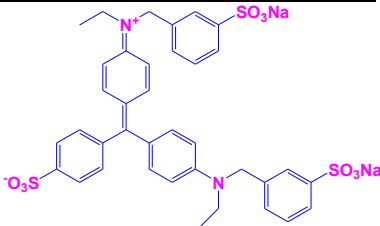
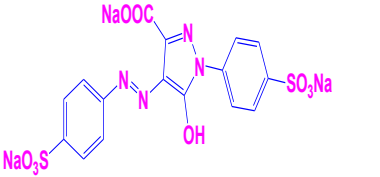
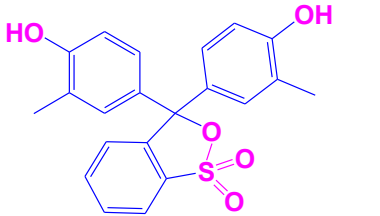
Factors	Variables	Units	Experimental values	
			Low level (-1)	High level (+1)
A	Cotton seed oil	mL	3	5
B	Peptone	Gram	1	2
C	Maltose	Gram	0.5	1.5
D	pH	-	7	9
E	Temperature	°C	28	40
F	Inoculum size	-	0.5	1.5

Table S2. Experimental variables for central composite design at different levels.

Factors	Variables	Units	Experimental values		
			Low level (-1)	Intermediate level (0)	High level (+1)
A	Cotton seed oil	mL	3	4	5
B	Peptone	Gram	1	1.5	2
C	Maltose	Gram	0.5	1	1.5

Table S3. Synthetic dyes were used in this study to estimate the ability of esterase for dye decolorization.

Dyes	Molecular Weight (g/mol)	chemical structure
Malachite green (ϵ_{max} = 617 nm)	364.91	
Methyl red (ϵ_{max} = 410 nm)	269.30	
Fast turquoise blue (ϵ_{max} = 550 nm)	780.17	
Methylene blue (ϵ_{max} = 665 nm)	319.85	
Crystal violet (ϵ_{max} = 590 nm)	407.99	
Bromothymol blue (ϵ_{max} = 460 nm)	624.4	

Light green SF yellowish ($\epsilon_{\text{max}} = >600$ at 631-634 nm in water)	749.893	
Tartarazine ($\epsilon_{\text{max}} = 435\text{-}500$ at 425-429 nm in water)	534.3	
Cresol red ($\epsilon_{\text{max}} = 442.01\text{-}590.16$ nm)	404.41	

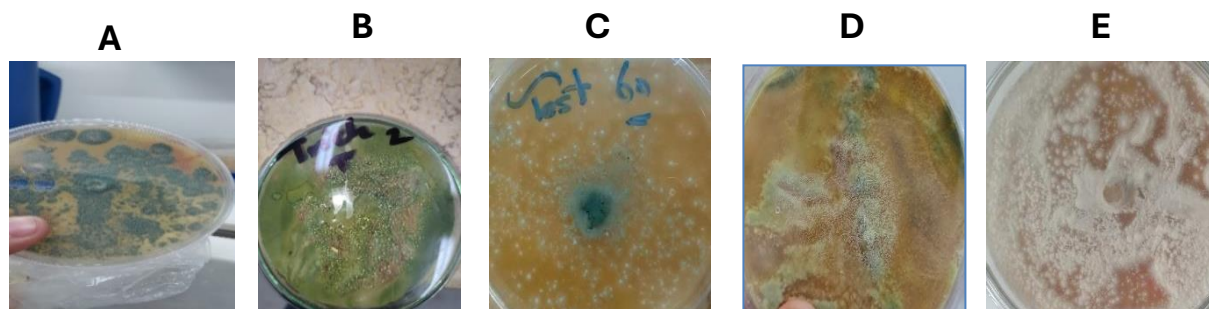


Figure S1. Fungal endophytes were isolated from *O. ficus-indica* and screened for esterase activity
A. EF-1, B. EF-2, C. EF-3, D. EF-4 and E. EF-5 strain were used in this study.

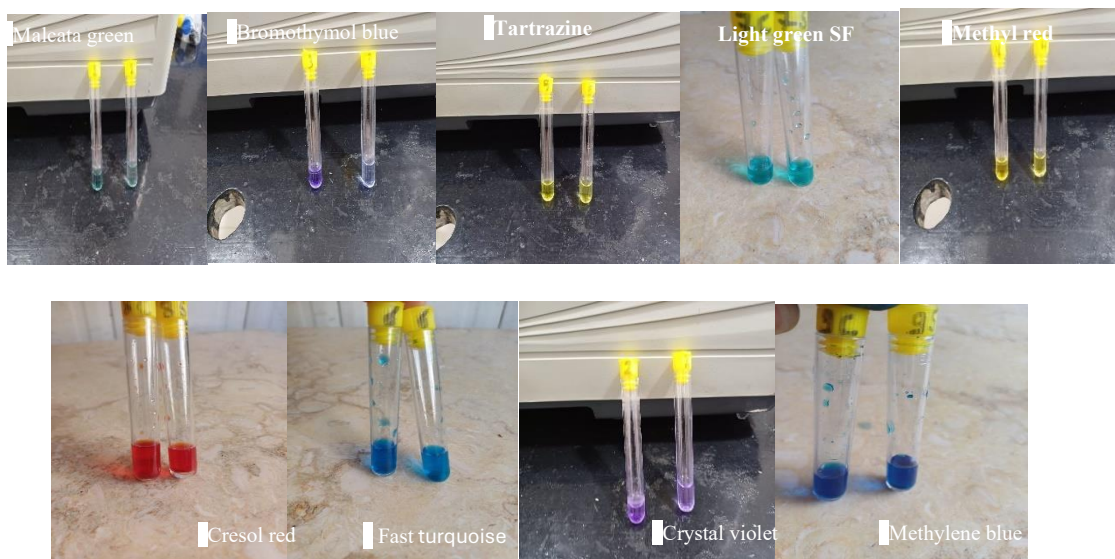


Figure S2. The image of the dye sample before and after 24 hours of treatment with purified esterase enzyme.

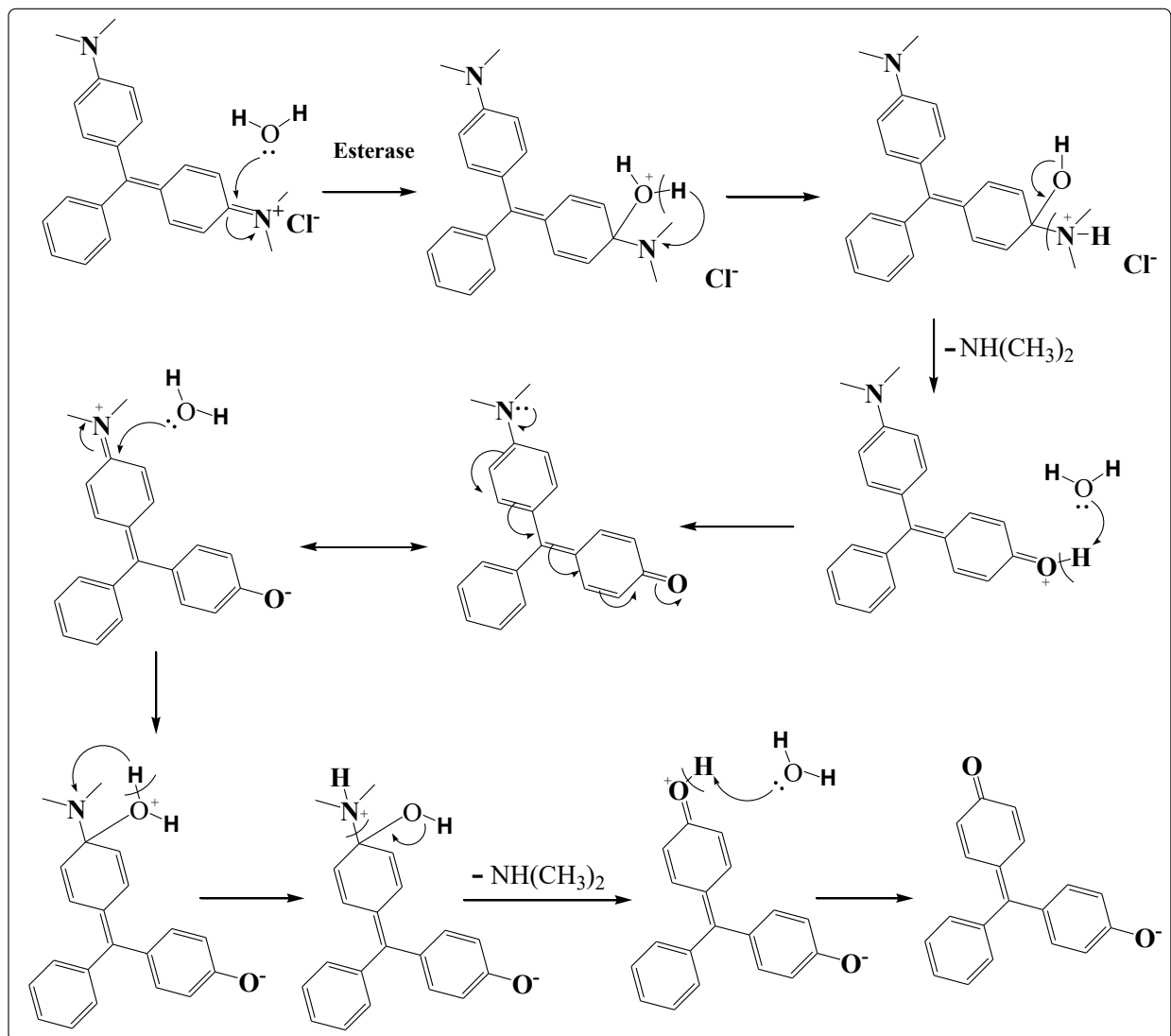


Figure S3: The suggested mechanism of malachite green dye decolorization by *T. afroharzianum* esterase.

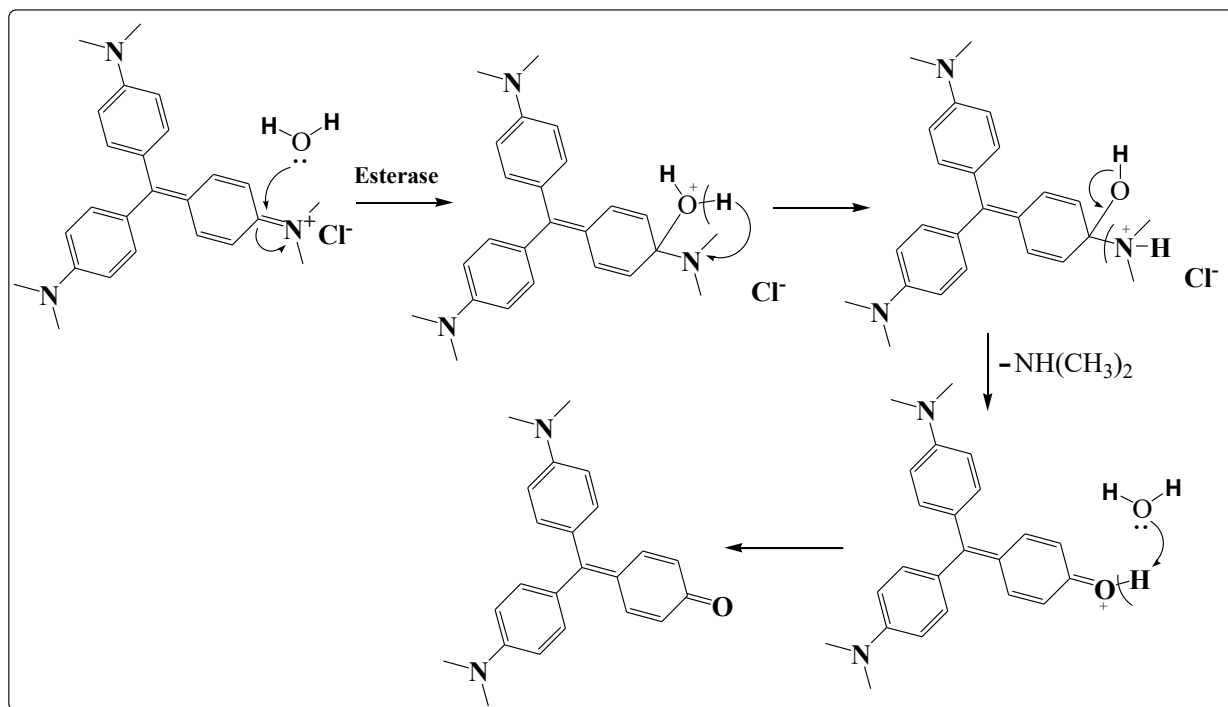


Figure S4: The suggested mechanism of crystal violet dye decolorization by *T. afroharzianum* esterase.

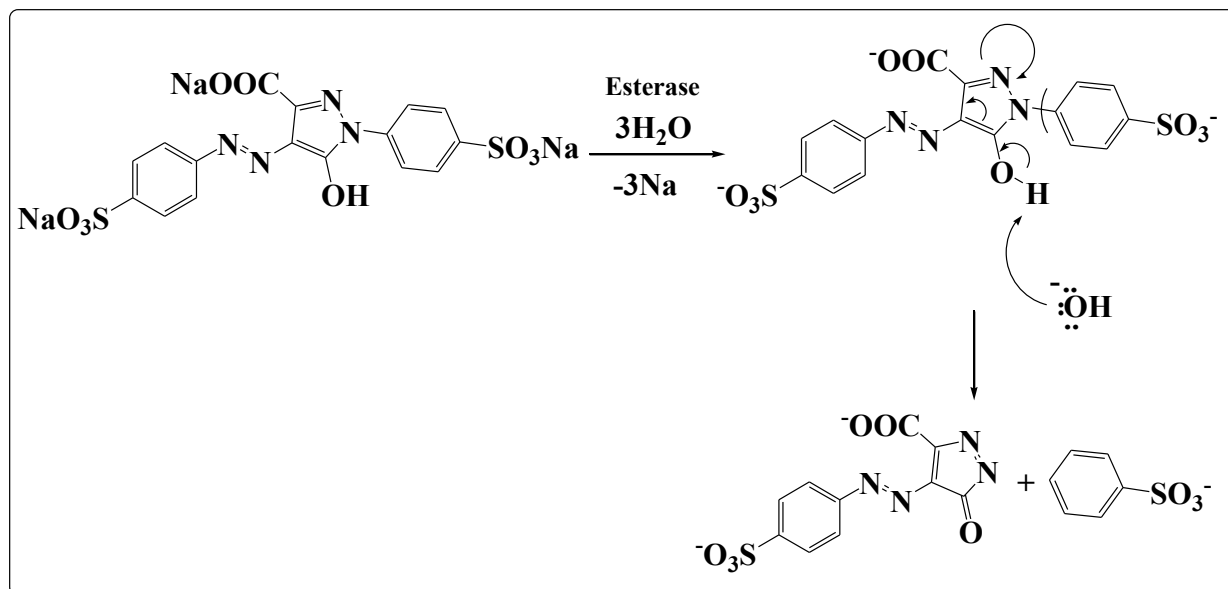


Figure S5: The suggested mechanism of tartrazine dye decolorization by *T. afroharzianum* esterase.

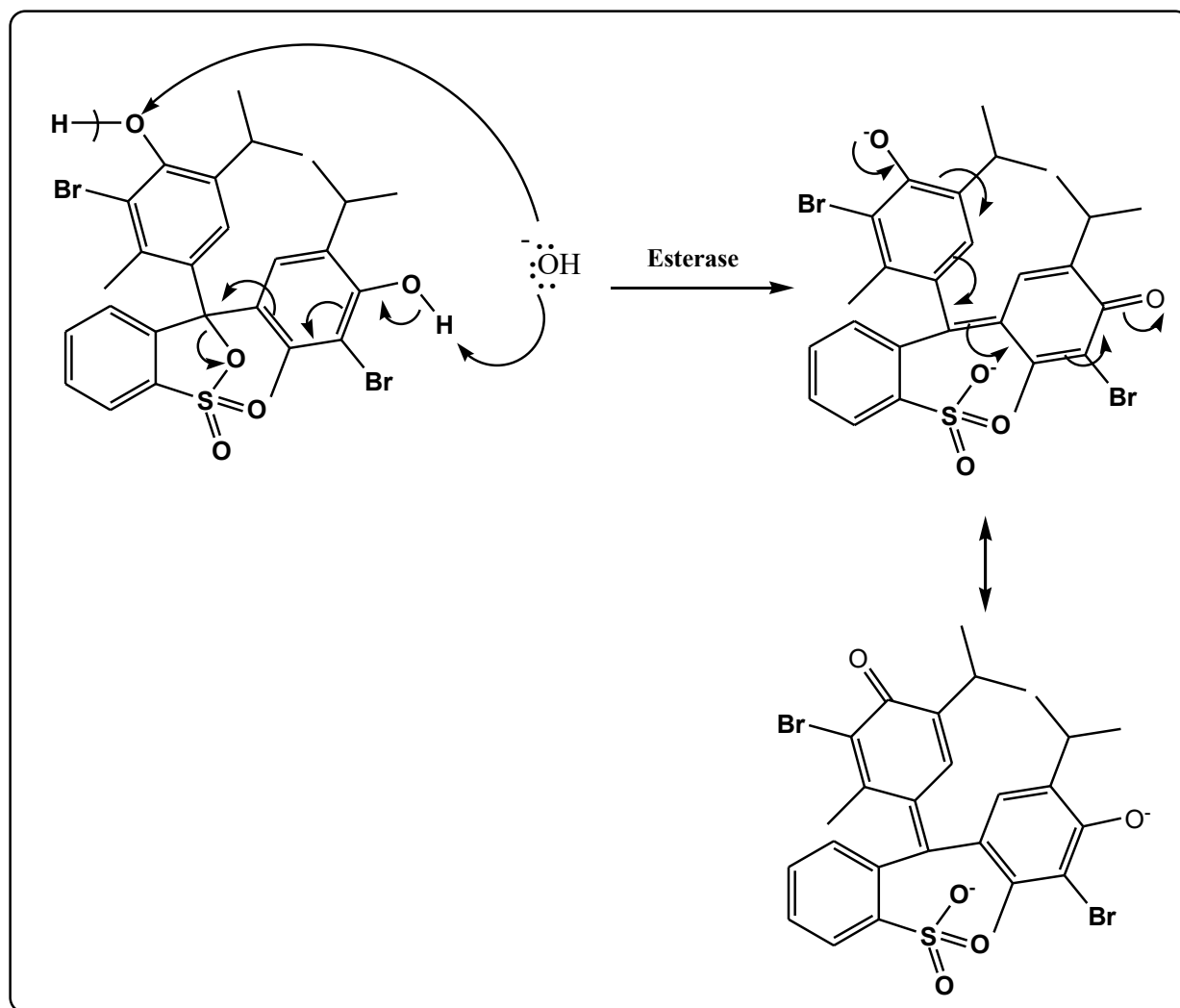


Figure S6: The suggested mechanism of bromothymol blue dye decolorization by *T. afroharzianum* esterase.