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## CHARACTERISING PET DEGRADING IDEONELLA SAKAIENSIS & ENGINEERING OF PETase THROUGH MUTAGENESIS

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# CHARACTERISING PET DEGRADING IDEONELLA SAKAIENSIS & ENGINEERING OF PETase THROUGH MUTAGENESIS



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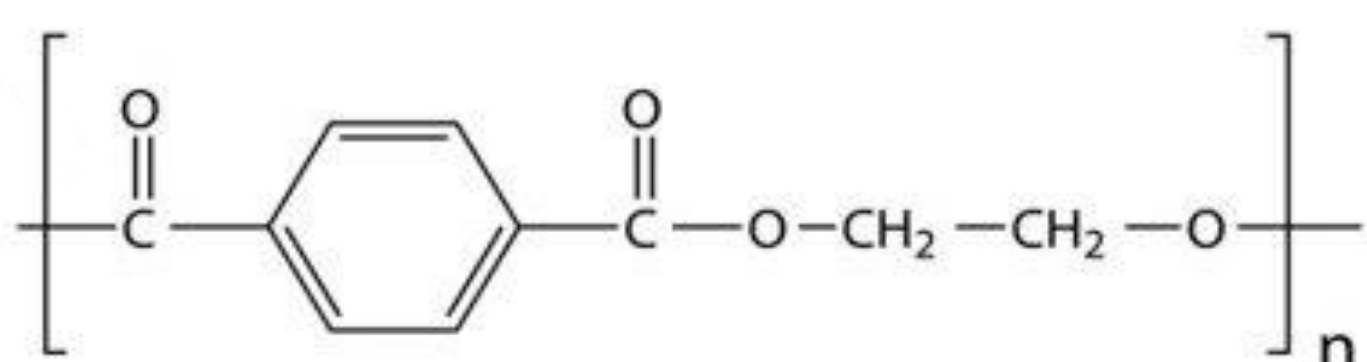
## INTRODUCTION

8300 million tonnes of plastic produced since early 20th century. Plastic pollution leads to toxic substances being released into our oceans, land and even air. Methods such as recycling and depolymerisation whilst excellent will only ever result in more plastic.

- 33% used once, and thrown away. Only 8% recycled (Wang et al., 2015).
  - 8 million tonnes p/y released into oceans
- Degrade into microplastics
- Enter the food chain and are on the rise
  - Unexamined health impacts

## PET (POLYETHYLENE TEREPHTHALATE)

- Crystalline plastic
- Drinks bottles, fleece, carpets
- Low melting temp
- Easy reformation
- Only 7% recycled into new products
- Made from ethylene monomers and terephthalic acid



## BIODEGRADATION

- Plastic degrading microorganisms exist in abundance (see table), the process is just too slow
- Biodegradation removes plastic from cycle and can turn plastic waste into a resource
- and result in profitable products (product engineering)

Plastic	Microorganism	Enzyme	Source	Reference
Polyethylene	<i>Brevibacterium borinquense</i>		Bacterial	[51][14]
	<i>Rhodococcus ruber</i>		Bacterial	[36][57][14]
	<i>Penicillium simplicissimum JK</i>		Bacterial	[58][14]
Polyethylene adipate (PEA)	<i>Ideonella sakaiensis</i>	lipase	Bacterial	[6]
	<i>Pseudomonas putida</i>	peroxidase	Bacterial	[6]
Polyhydroxyalkanoate (PHA)	<i>Penicillium, Rhizopus arizus</i>	lipase	Bacterial	[6]
	<i>Pseudomonas stutzeri</i>	Serine hydrolase	Bacterial	[6]
Polyterephthalate	<i>Ideonella sakaiensis</i>	Hydrolase	Bacterial	[6]
	<i>penicillium microspora</i>	Hydrolase	Fungal	[59]
Polyurethane	<i>Comamonas acidovorans FB-37</i>	urease	Bacterial	[40][11][14]
	<i>Penicillium microspora</i>	Hydrolase	Fungal	[59]
	<i>Phanerochaete chrysosporium</i>	Manganese peroxidase	Fungal	[59]
Polyvinyl chloride	<i>Pseudomonas chlorophila</i>		Bacterial	[14]
	<i>Curvularia zovae</i>		Fungal	[42][14]
	<i>Fusarium solani</i>		Fungal	[14]
	<i>Clostridium sp.</i>		Fungal	[14]
	<i>Pseudomonas putida AJ</i>		Bacterial	[14]
Polycaprolactone (PCL)	<i>Chryseobacterium J2</i>		Bacterial	[14]
	<i>Pseudomonas fluorescens B-22</i>		Bacterial	[43][14]
	<i>Aspergillus niger</i>		Bacterial	[14]
Polybutylene succinate (PBS)	<i>Aspergillus niger</i>	Glycosidase	Fungal	[6]
	<i>Aspergillus niger</i>		Fungal	[14]
	<i>Fusarium</i>		Fungal	[14]
	<i>Rhizopus delemar</i>		Bacterial	[14]
Polyethylene succinate (PES)	<i>Penicillium, Rhizopus arizus</i>	Lipase	Bacterial	[6]
	<i>Aspergillus oryzae</i>	cutinase	Fungal	[6]
	<i>Penicillium, Rhizopus arizus</i>	Lipase	Bacterial	[6]

## IDEONELLA SAKAIENSIS

Most effective plastic (PET) degrading organism. (Oda group, Kyoto Institute of Technology, 2016)

Gram negative, aerobic, rod-shaped, non-spore forming, single flagellated



Image of *Ideonella sakaiensis* producing tendrils which attach to substrate. (Yoshida et al., 2016)

## PROJECT AIM

Characterise the bacterial strain *Ideonella sakaiensis* for the engineering of its plastic degrading PETase enzyme to increase its catalytic activity using genetic engineering techniques such as directed mutagenesis.

## MATERIALS AND METHODS

### CHARACTERISING I. SAKAIENSIS

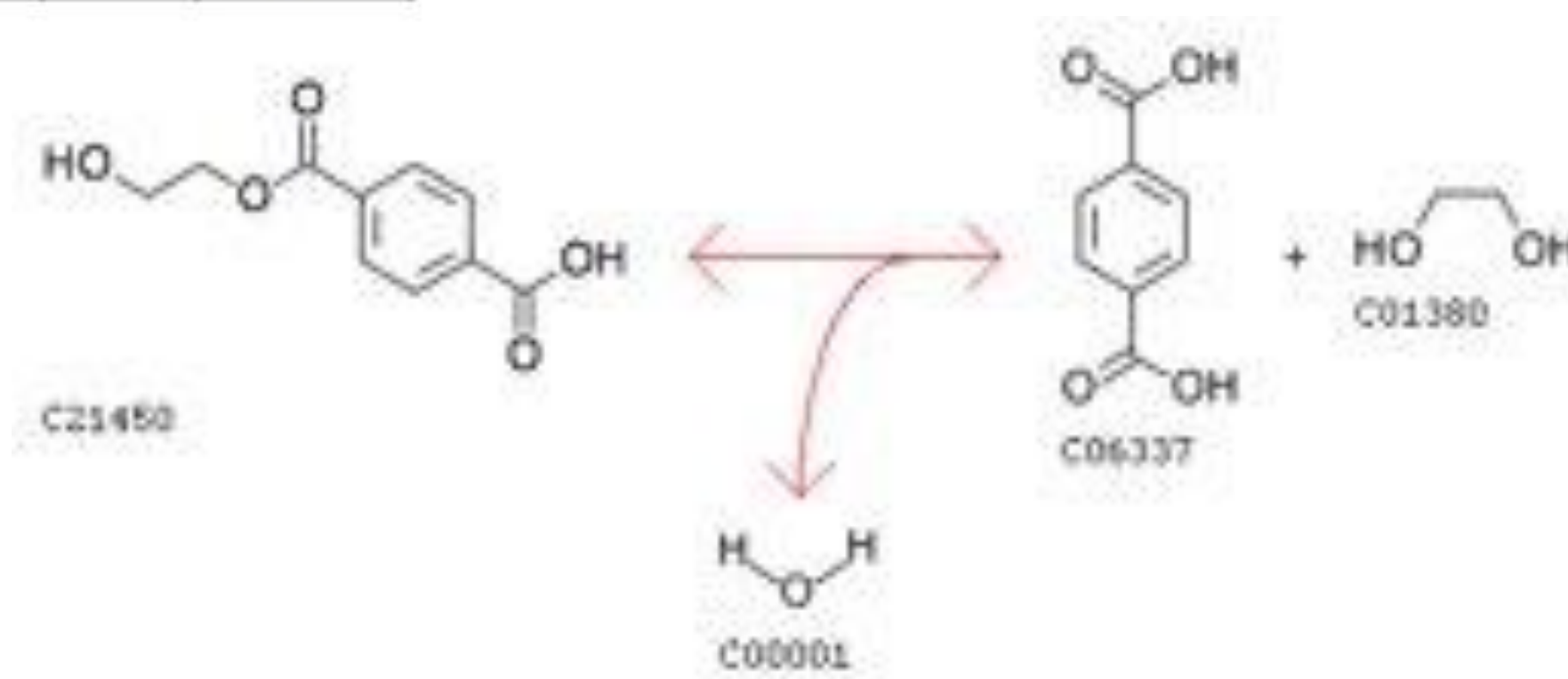
Preference to grow on solid medium with PET or PE plastic as attachment of tendrils to substrate is require to begin degradation:

Examples of *I. Sakaiensis* Colonies; round Clear and small

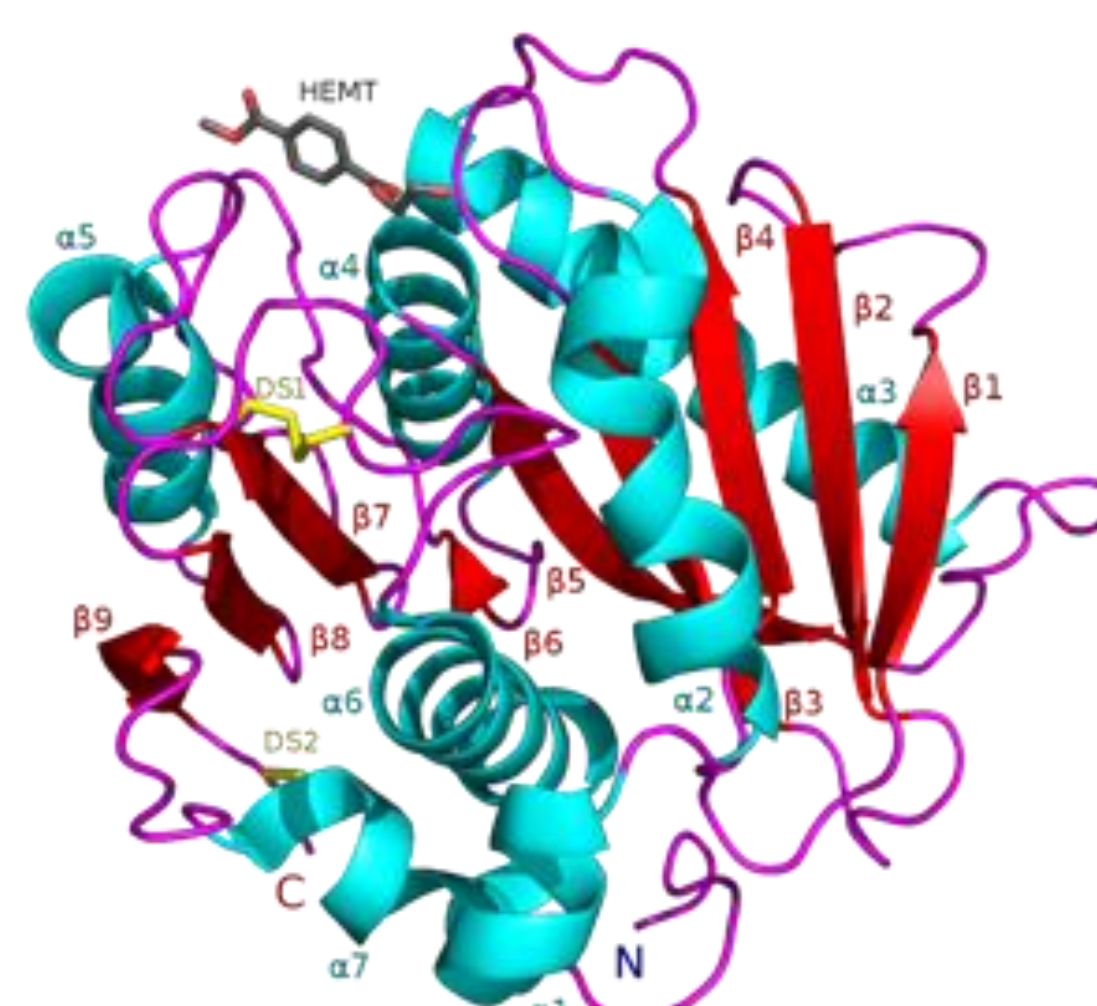


## ENZYME

- Serine hydrolase, charge relay system
- Coupled with MHETase which breakdown the products further in ethylene glycol and terephthalic acid
- PETase bottleneck of enzyme cascade



Degradation of PET into Ethylene glycol and terephthalic acid by PETase



3D structure of PETase enzyme, (Han et al., 2017)

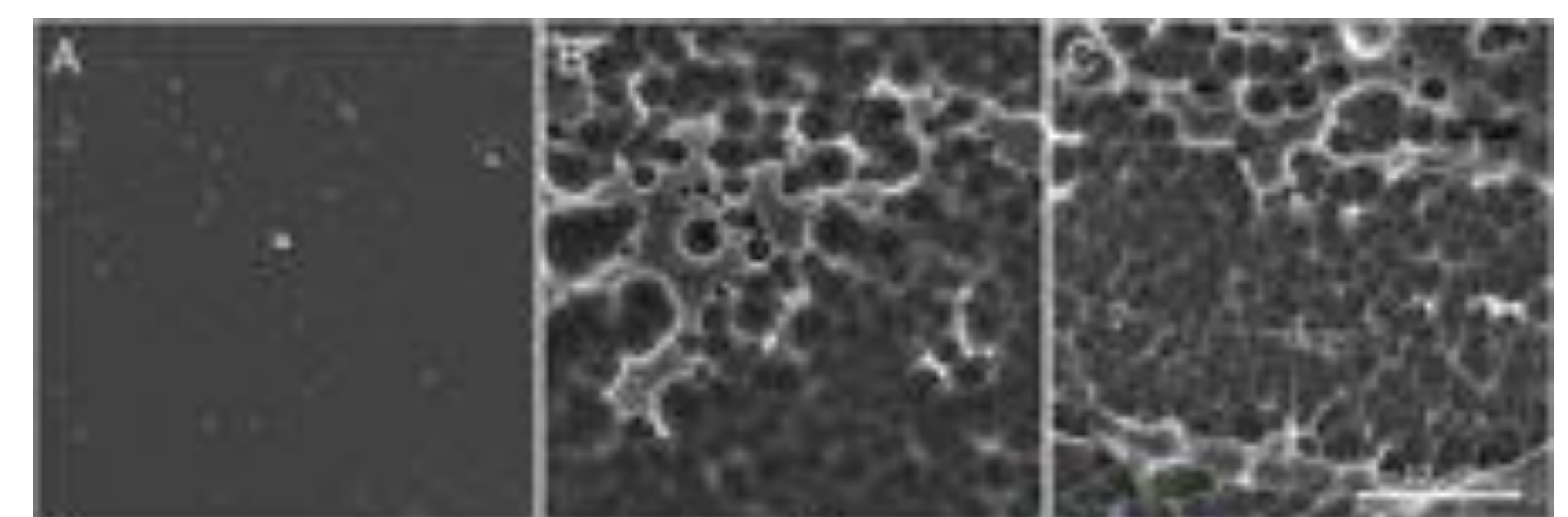
### DIRECTED MUTAGENESIS

Modifications to enzyme active site improve binding of substrate:

#### S238F & W159H mutation:

- Serine to phenalalynine (S238F) = more aromatic interactions & stable 'docking' for PET
- Wild-type PET stabilised by W185 and W159 interactions only.

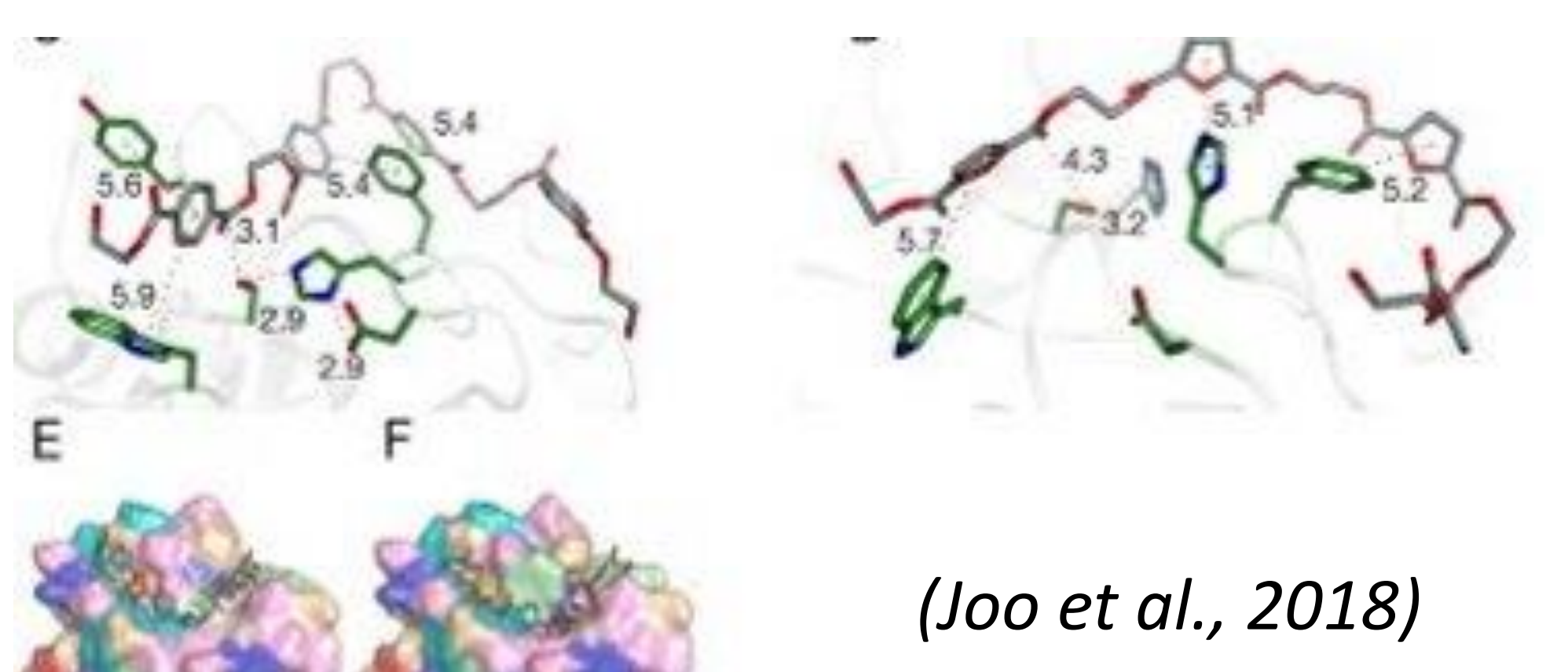
Double mutant provides four aromatic interactions to stabilise the PET



Degradation of PET film by WT *I. sakaiensis* (left) and double mutant (far right) (Austin et al., 2018)

#### Arg280 mutation:

- Residue in the binding site (Arg280) = polar and protruding region
- Substituting Arg280 for Alanine, = longer, unhindered, substrate binding.



(Joo et al., 2018)

## FUTURE PERSPECTIVES

- Faster and more broad spectrum plastic biodegradation
- Make plastic into a resource by engineering products
- Bio-sensors: Ocean microplastic problem
- Demobilization onto filters
- Demobilization onto underwater structures (artificial reefs)
- Synthetic consortia

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