LIPASES & CHICKEN FEATHERS Matthew Barcus Lei Lab

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WHAT IS LIPASE?



hydrolyzes lipids (fats)



works at water/lipid interface (think oil and water)

LIPASE STRUCTURE

- Active site includes serine, aspartic acid (sometimes glutamate), and histidine
 - serine acts as the nucleophile
- many lipases have a 'lid' that slides out of the way when the hydrophobic interaction occurs between the substrate and the enzyme

LIPASE CATALYTIC MECHANISM



P. Reis et al. / Advances in Colloid and Interface Science 147-148 (2009) 237-250

LIPASE REGIOSELECTIVITY

 lipase favors different ester bonds on the glycerol's backbone of triglyceride



LIPASE USES

- detergents
- digestion
- biofuels
- enantioselectivity





LIPASE IN DETERGENTS

- Removes fat containing stains:
 - frying fats
 - butter
 - human sebum
 - sauces and oils
 - some cosmetics



LIPASE IN DIGESTION

- · lipase helps break down fats to be used or stored in the body
- one growing market is for lipase inhibitors that prevent the body from utilizing the lipids from a high fat diet.



LIPASE IN BIOFUELS



- Participates in the transesterification step
 - replacing the organic group of an ester with the organic group of an alcohol
- Lipase insensitive to FFA, an advantage over other methods

LIPASE FOR ENANTIOSELECTIVITY

- non-steroid anti-inflammatory drugs
- penicillins
- herbicides
- surfactants
- scents





CHICKEN FEATHERS



CHICKEN FEATHERS

• 24 billion chickens will be slaughtered this year



- This will leave behind 8.5 billion pounds of feather waste for us to deal with
- accumulates in landfills



CHICKEN FEATHER RECYCLING



- bioplastics
- animal feed
- cosmetics



CHICKEN FEATHER HYDROLYSIS

- chemicals (Na₂S, NaOH)
- fermentation with keratinolytic bacteria
- enzymatically





THE FEATHER STRUCTURE

- Crude Protein 85%
 - majority is ß-keratin
- fat 7.8%
- Ash 2%



- Calcium 0.4%
- Phosphorus 0.2%



CHICKEN FEATHERS DEFENSE

- preen gland
- epidermal excreta





Now it's your turn to work with feathers!





LIPASE & CHICKEN FEATHERS

LIPASE AND CHICKEN FEATHERS

- lipase can remove protective lipids
- allows for greater access for keratinase to penetrate the keratin
- possible synergistic relationship between lipase and keratinase for feather hydrolysis

THE APPROACH

- clone lipase genes
- express enzyme
- isolate lipids from feathers



- screen for lipase with activity against feather lipids
- combine lipase with feather degrading enzymes and determine if activity is enhanced

BIOINFORMATIC BACKGROUND

- what is the function of the gene?
- is there a signal peptide?
- how stable is this protein?



AMPLIFY GENE USING PCR



TA CLONING



DOUBLE DIGEST



YEAST EXPRESSION

- Why did we choose Pichia pastoris?
 - high yield
 - some groups struggled with E. coli expression systems using similar genes
 - inducible expression
 - does not require constant selection pressure

TRANSFORM INTO YEAST



- Homologous recombination into yeast genome
 - stable transformation

EXPRESSION

- induce growth with medium containing glycerol
- transfer to a medium with methanol
- feed cultures methanol every 24 hrs
- after ~100 hours, harvest protein



4-NITROPHENYL PALMITATE LIPASE ASSAY

ЭΗ

+

FFA



lipase

EXTRACTING LIPIDS FROM FEATHERS

- etroleum
- Soxhlet apparatus



THE FUTURE

CHARACTERIZE FEATHER LIPIDS

- Gas chromatography
- Thin-layer chromatography



Homo sapiens Non-human primates Saturated paraffins Cholesterol esters Squalene Cholesterol esters Cholesterol esters Wax monoesters Wax diesters Wax monoesters Oxidation end products Oxidation end products Triglycerides Triglycerides Free fatty acids Free fatty acids Diglycerides cholesterol Diglycerides cholesterol Diglycerides monoglycerides Diglycerides monoglycerides Polar lipids Polar lipids Gorilla Pongo Macaca mulatta Man Pan Hylobates

MEASURE IMPACT OF LIPASE ON FEATHER HYDROLYSIS

- Look at Free Fatty acid release
- lipase inhibitor effects on Streptomyces feather hydrolysis
- identify feather lipids which are hydrolyzed using TLC

CHECK LIPASE FOR FURTHER POTENTIAL

- nutritional improvement
- detergents
- enantioselectivity
- biofuels





CONCLUSION

WHAT DID WE LEARN?

- lipase is a powerful enzyme with a multitude of uses
- feather waste has the potential to be of high economic value
- lipase may help enhance feather hydrolysis

