The Effects of Synthetic Iron Chelators and Flavonoids on Siderophore-producing *P. fluorescens*

Kamila Zakowicz

Big Picture

- Siderophore producing bacteria
- Iron chelation therapy to induce hypoferramia

Background

- Iron is essential
- 25% of world population is affected by iron abnormalities [1]
- 4 main iron abnormalities
  - Iron deficiency
  - Iron overload
  - Chronic disease induced anemia
  - Free radical pathology

Free Radical Pathogenicity

- Free radicals are highly reactive molecules with an unpaired electron that are essential for biological processes
- can cause cell damage when they bind to important cell structures
- much of the cell degeneration and cell aging in certain chronic diseases, such as kidney disease and Alzheimer's, is attributed to free radical oxidative damage [2]
- play a role in numerous infectious diseases
  - Body induces hypoferremia in pathogen (iron deficiency)

Hypoferramia

- To combat the free radicals in infectious diseases, the body uses two different pathways to induce hypoferremia (anaemia) in the infectious disease: the hepcidin-dependent pathway and an hepcidin-independent pathway
- hepcidin-dependent:
  - body will increase its production of hepcidin, a metabolic iron-regulating mechanism, to reduce iron plasma and induce hypoferremia of the infectious disease

Hypoferremia

- Hepcidin independent:
  - iron chelation may be used in order to to correct an iron abnormality caused by an infectious disease

Why *P. fluorescens*?

- Nonpathogenic but produces siderophores called pyoverdine
- Same siderophores as *P. areuginosa*

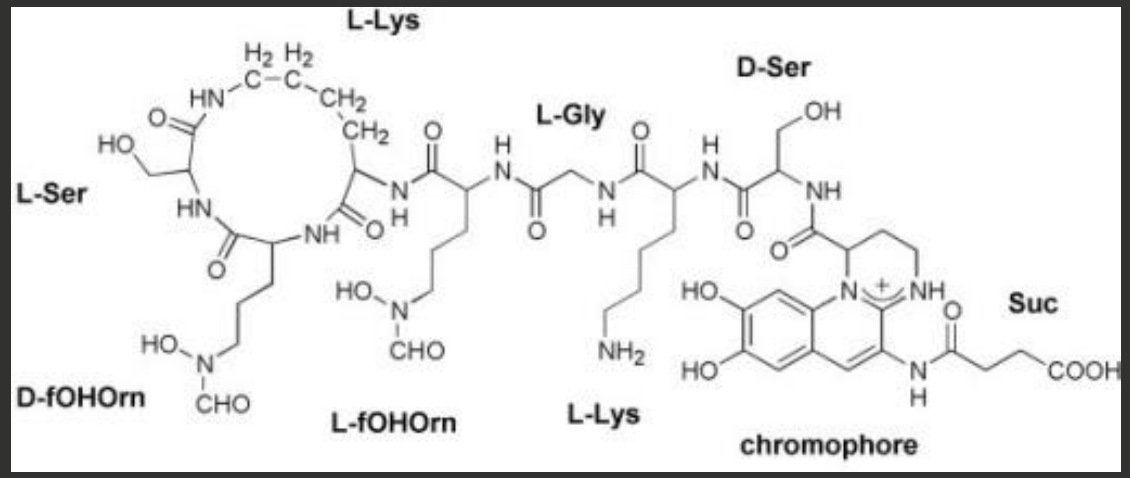


Fig. 1: P. fluorescens (Bio Control Agents. (n.d.). Retrieved April 03, 2017, from https://www.indiamart.com/rocky-imports-exports/bio-control-agents.html)

Siderophores

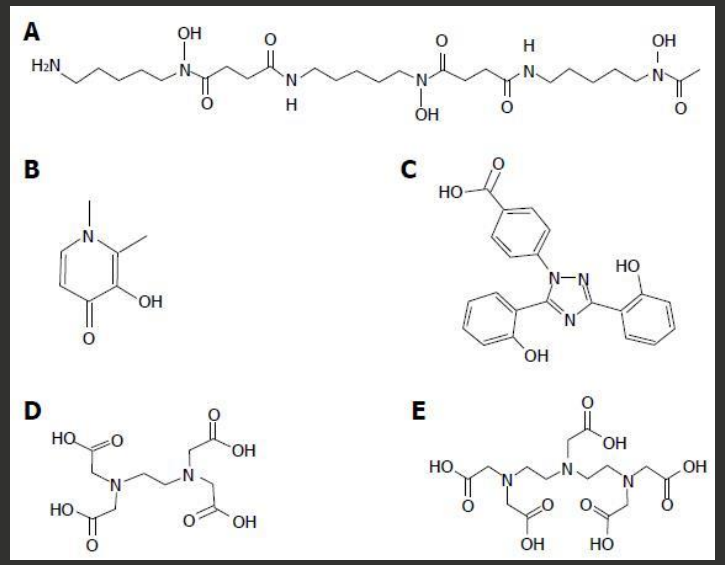
- Produced only by certain bacteria
- Mechanisms that help facilitate iron uptake in iron deficient conditions

Iron Chelators

- molecules that bond to iron and may transport it
- may be a key component in treatment of iron abnormalities
- Types of Chelators
  - natural and weaker e.g. ascorbic acid may help with minor iron deficiency and be helpful as an adjuvant to oral supplements of iron
  - Bacteria produced e.g. siderophores like pyoverdine
  - Phytochemicals with chelating properties e.g. quercetin and rutin
  - Synthetic iron chelators used in treating thalassemia (iron overload)

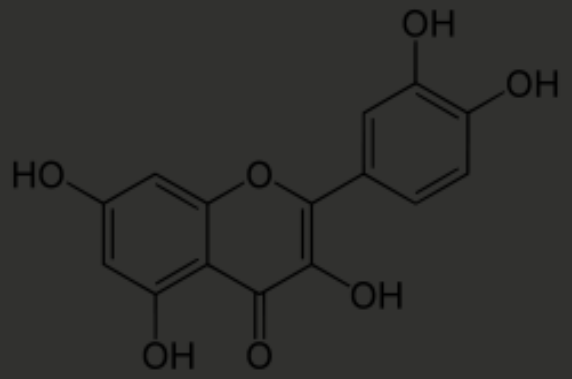
Synthetic Iron Chelators

- Five main: deferiprone (L1), deferoxamine (DFO), deferasirox (DFRA), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA)
- L1 was used because it is one of the safest and effective treatments for thalassemia and is not produced by bacteria (i.e. DFO)



Flavonoids

- one of the most common phytochemicals found in plants
- catechin = green tea
- quercetin = buckwheat, green tea, berries, etc.
- Metabolic, insecticide, antifungal properties
- Found to exhibit strong antioxidant properties towards iron [3]



Iron Chelation Therapy

Variables/Control

- The variables include the following: the concentration of iron in the media, the type of chelator added to treatments, and the concentration of chelator added to treatments.

Hypothesis

- The flavonoids, quercetin and catechin, and the iron-chelating drug, L1, are predicted to have inhibitory effects on the growth of P. fluorescens in both concentrations of iron media. Siderophore production is expected to positively correlate with bacterial growth

Materials

- 100 mg Deferiprone (L1) (UCLA)
- 1 g Catechin Hydrate (96 %, Sigma Aldrich)
- 1 g Quercetin (98%, Samsara Herbs)
- Slant culture P. fluorescens (Flinn Scientific, Inc)
- 1 g iron (iii) citrate (Flinn Scientific, Inc)
- 50 ml DMSO (Flinn Scientific, Inc, Catalog)
- M9 minimal media (Na_2HPO_4, NH_4Cl, KH_2PO_4, NaCl, $MgSO_4$, $CaCl_2$, 10% glucose)
- 2 and 3 mL microcentrifuge tubes
- Analytical balance
- Electronic Plate Reader (accuSkan FC)
- Spectrophotometer (V5000 Visible
- Spectrophotometer)
- Centrifuge (Eppendorf Centrifuge 5418

Methods

M9 Minimal Media Preparation

- M9 minimal media was prepared (Na_2HPO_4, NH_4Cl, KH_2PO_4, NaCl, $MgSo_4$, $CaCl_2$, 10% glucose)
- P. fluorescens running culture and a standard curve was made
- 400 µM and 4 µM iron media preparation
- a stock solution of 0.1 M iron (iii) citrate was serially diluted in the M9 minimal media to make 400 µM and 4 µM iron media

Chelator Preparation

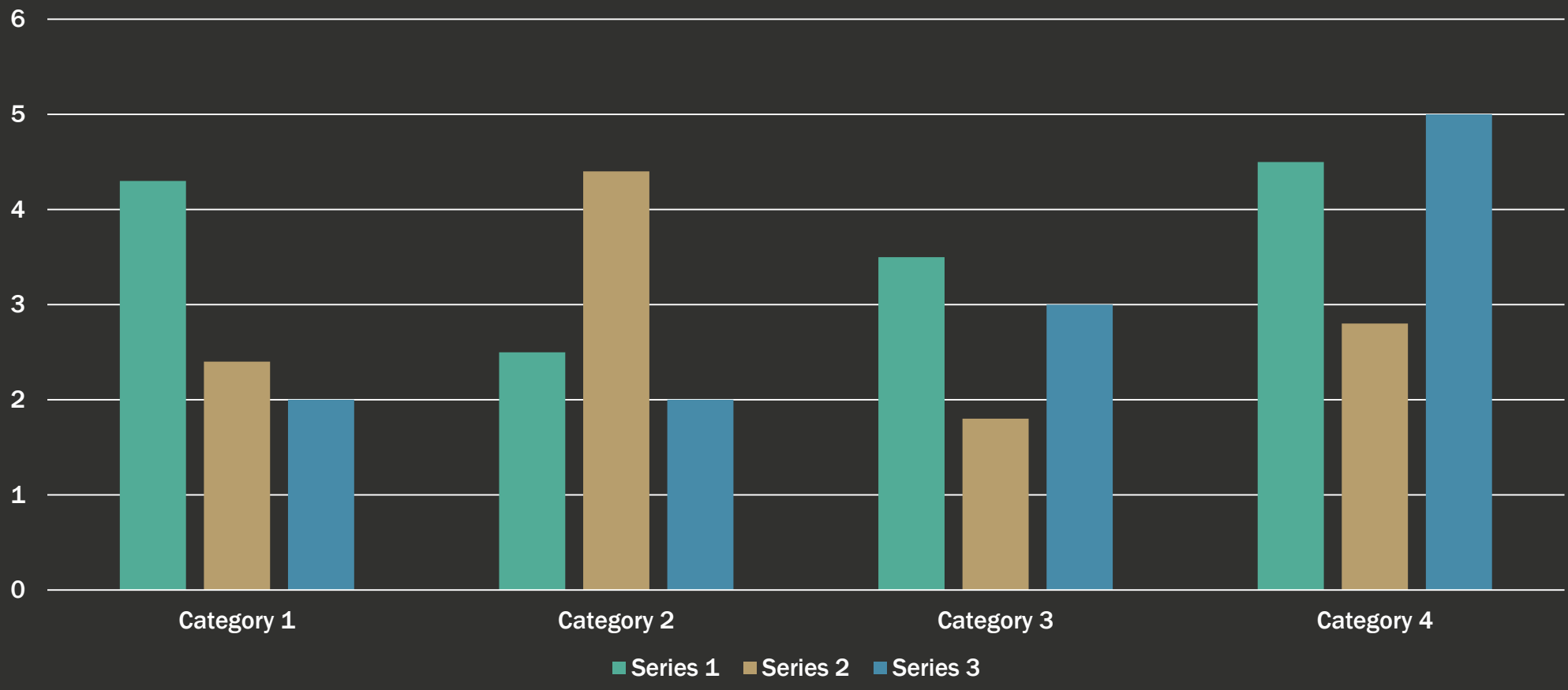
- 1 mL of each desired flavonoid concentration (0.2 mM, 0.5mM, 1 mM, 1.5 mM) was dissolved in 100% DMSO at 10 x concentration and was added to treatments at 1 x concentration
- 1 mL of each L1 concentration (0.2 mM, 0.5 mM. 1 mM, 1.5 mM) was dissolved in warm water at 10x concentration, and added to treatments at 1 x concentration

Methods

Optical Density Analysis

- the OD of each sample was taken using a spectrophotometer and/or a plate reader at ≈600 nm after 24 hours and compared to the growth curve in order to determine growth inhibition
- the samples were centrifuged and the OD of the supernatant was taken at 405 nm using the plate reader to determine pyoverdine presence

Title and content layout with chart



Two content layout with table

- First bullet point here
- Second bullet point here
- Third bullet point here

	Group 1	Group 2
Class 1	82	95
Class 2	76	88
Class 3	84	90

Two content layout with SmartArt

- First bullet point here
- Second bullet point here
- Third bullet point here