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Enhanced production of thermostable laccases from a native strain of *Pycnoporus sanguineus* using central composite design

Key words: Enhanced laccase production, Screening media, Inducers, Tomato juice medium, Central composite design, Soybean oil, Copper sulfate

- The search for enzymes capable of substituting conventional chemical methods in the synthesis of commercial products and the transformation of highly toxic compounds into non-toxic species has received considerable attention.

***P. sanguineus* CS43**

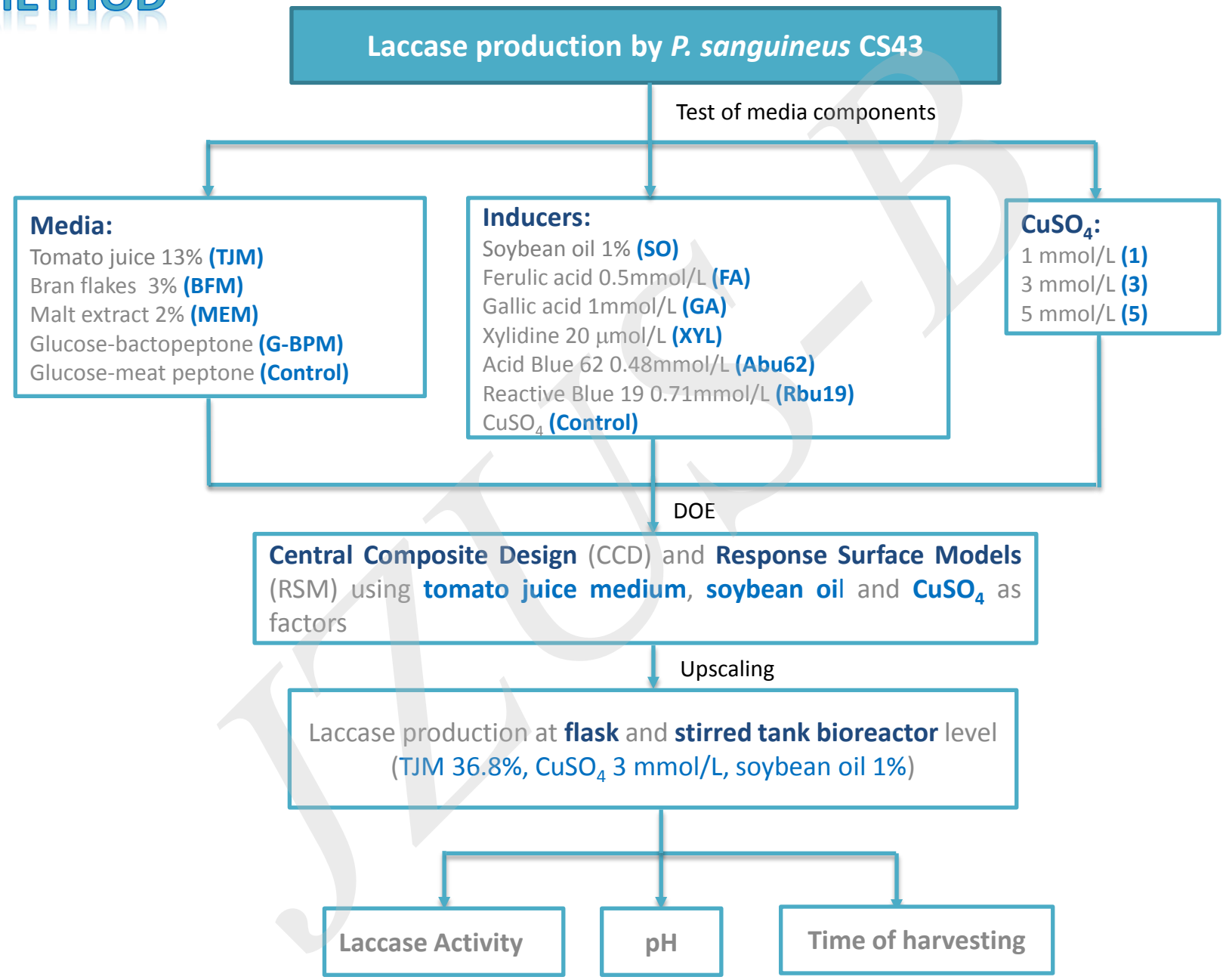
- Laccases (EC 1.10.3.2) are an example of enzymes that catalyze the oxidation of a wide range of arylamines or phenolic compounds, either natural or xenobiotic.

- There are multiple factors influencing laccase production. Laccase synthesis has been found to be highly dependent on cultivation conditions and growth medium composition.

- Reducing costs of laccase production by optimizing the fermentation medium and conditions remains one of the basic research objectives at the industrial level.

- In the present work, different media, novel inducers, and critical parameters (temperature, pH, and time of harvesting) were investigated in order to enhance the production of thermostable laccases by a native strain of *P. sanguineus*, CS43.

METHOD


Laccase production by *P. sanguineus* CS43
Test of media components
Media:
Tomato juice 13% **(TJM)**
Bran flakes 3% **(BFM)**
Malt extract 2% **(MEM)**
Glucose-bactopeptone **(G-BPM)**
Glucose-meat peptone **(Control)**
Inducers:
Soybean oil 1% **(SO)**
Ferulic acid 0.5mmol/L **(FA)**
Gallic acid 1mmol/L **(GA)**
Xylidine 20 μmol/L **(XYL)**
Acid Blue 62 0.48mmol/L **(Abu62)**
Reactive Blue 19 0.71mmol/L **(Rbu19)**
$CuSO_4$ **(Control)**
$CuSO_4$:
1 mmol/L **(1)**
3 mmol/L **(3)**
5 mmol/L **(5)**
DOE
Central Composite Design (CCD) and **Response Surface Models** (RSM) using **tomato juice medium**, **soybean oil** and **$CuSO_4$** as factors
Upscaling
Laccase production at **flask** and **stirred tank bioreactor** level (TJM 36.8%, $CuSO_4$ 3 mmol/L, soybean oil 1%)
Laccase Activity
pH
Time of harvesting

RESULTS AND CONLUSIONS

- The strains CS2 and CS43 of *P. sanguineus* had similar titers of laccase, but the half-life of CS43 laccases was 22% and 5% higher at 50 and 60°C respectively. The highest laccase activities (20000 IU/L) showed by the strain CS43 were detected using glucose-bactopeptone and tomato juice media. This latter was chosen for subsequent experiments due to its low cost. The best result for laccase induction was obtained with soybean oil (45000 IU/L) and in the testing of different $CuSO_4$ levels, the two highest activities were produced by $CuSO_4$ 5 and 3 mmol/L (11150 IU/L).

- The best condition in the CCD experiments achieved 52538 IU/L when tomato juice medium was at the highest level, whereas $CuSO_4$ and soybean oil were in the central level. A prediction equation was also generated, and the model fit for the observed vs. predicted effects on laccase activity showed a good correlation with the experimental data (r^2=0.94, P<0.001).

- Three-dimensional response surface plots indicated that increasing tomato juice medium concentration and selecting a lower level of CuSO4 were beneficial. An important interaction between $CuSO_4$ and soybean oil could observed maintaining tomato juice at medium level.

- The CS43 culture in tomato juice medium 36.8%, $CuSO_4$ 3 mmol/L and soybean oil 1% (A00) showed the higher laccase activity (77816 IU/L) at flask level, whereas in the scaling up to 10L stirred tank bioreactor the highest activity (143000 IU/L) was obtained after 13 days of growth, with harvesting on day 15 (85700 IU/L). The pH fluctuated from 3.7 to 6.0 and after day 16 increased concomitantly with the laccase activity.

- In conclusion, the production of thermostable laccases from *P. sanguineus* CS43 was successfully enhanced and scalable, where the cultivation time neccesary to reach the highest laccase activities was considerably reduced, from 28 to 13 days with a total productivity increment of up to 26 times. To the best of our knowledge, the levels of laccase activity achieved by CS43 in stirred tank bioreactor (143000 IU/L) are the highest reported in literature.