

**ISOLATION AND OPTIMIZATION OF INDIGO- REDUCING
BACTERIA FROM FERMENTATION OF TARUM LEAVES**
(*Indigofera tinctoria* L.)

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ABSTRACT

Indigo dye is a type of natural dye which needs to be reduced in its application. Bacteria as a biological agent are able to reduce indigo, and it reduces the utilization of harmful chemical reducing agent, sodium dithionite. The aim of this research was to isolate and identify the genus of bacteria that are able to reduce indigo, to optimize the bacterial growth and its activity with variation of carbon sources, temperature and also pH value. Another aim was to know the correlation of bacterial cell growth and leuco product during the certain incubation by spectrophotometric.

The bacteria were isolated from the fermentation liquor of *I. tinctoria* (indigo plant). The fermentation liquor was inoculated on pepton- yeast extract broth medium (PYB) and (PYA) media (indigo 0,01%). The pure bacteria isolate is characterized and identified by the genus groups. The cultivation process and the measurement of reduction activity were performed in PYB medium (indigo 0.05%) for 72 hours under anaerobic conditions. The optical density (OD) values of bacterial growth and the leuco product were measured at 600 nm and 390 nm. The concentration of leuco product is known by matching the standard curve. The data were analyzed descriptively.

Its characters showed a rod-shaped bacteria, gram positive, catalase positive, motile, homofermentative, and able to produce the endospores. Three bacterial isolates, ST2, ST3 and IB1, showed the indigo-reducing activity under anoxic conditions, and identified phenotypically as group of genus *Bacillus*. Based on the concentrations of leuco indigo that reduced from indigo, the three isolates showed the characteristics of alkaliphilics and mesophilics bacteria, which optimally function at pH 10.0 and 25-37°C whereas *Bacillus* ST2 was 292,45 mg/L; 216,25 mg/L; and 239,45 mg/L, *Bacillus* ST3 was 260,45 mg/L; 222,7 mg/L; and 246,40 mg/L, also *Bacillus* IB1 was 261,10 mg/L; 202,85 mg/L; and 246,60 mg/L of leuco indigo. The “tajin” water is able to increase the cell growth and the indigo reducing activity. The highest growth rate of bacteria that was found at logarithmic phase was coincide with the highest capacity of bacteria in reducing indigo to leuco indigo. It occurred on the 6th hours to the 26th hours of cultivation time. In particular, the late-exponential phase was expected to be the best time for the indigo-reducing activity of bacteria.

Keywords: bacteria, indigo reduction, *Indigofera tinctoria*, leuco indigo, optimization.

ISOLASI DAN OPTIMASI AKTIVITAS BAKTERI PEREDUKSI INDIGO DARI FERMENTASI DAUN TANAMAN TARUM (*Indigofera tinctoria* L.)

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INTISARI

Senyawa indigo perlu direduksi menjadi *leuco indigo*. Penggunaan natrium ditionit sebagai agen pereduksi senyawa indigo bersifat iritan dan mencemari lingkungan, sehingga perlu dieksplorasi bakteri yang mampu mereduksi indigo secara aman dan efisien. Penelitian ini bertujuan mengisolasi dan mengidentifikasi isolat bakteri pereduksi indigo dari cairan fermentasi tanaman *I. tinctoria* secara fenotipik, optimasi pertumbuhan dan aktivitas reduksi bakteri dengan variasi sumber karbon, suhu dan pH serta mengetahui hubungan pertumbuhan sel bakteri dan produk *leuco indigo* selama waktu inkubasi tertentu dengan metode spektrofotometri.

Isolat bakteri diperoleh dari fermentasi daun indigo. Sampel cairan fermentasi diinokulasi pada media PYB dan PYA (indigo 0,01%). Isolat murni dikarakterisasi dan diidentifikasi genusnya. Kultivasi bakteri dan pengukuran aktivitas reduksi dilakukan dengan media PYB (indigo 0,05%) selama 72 jam dalam kondisi anaerob. Nilai OD pertumbuhan bakteri dan *leuco indigo* diukur pada panjang gelombang 600 nm dan 390 nm. Konsentrasi *leuco indigo* diketahui melalui pencocokan kurva standar. Data dianalisis secara deksriptif.

Isolat bakteri memiliki karakteristik bentuk sel batang, bersifat gram positif, katalase positif, motil, homofermentatif, menghasilkan endospora, dan mampu mereduksi senyawa indigo menjadi *leuco indigo* sehingga diidentifikasi sebagai kelompok genus *Bacillus*. Kecepatan tumbuh tertinggi bakteri dalam mereduksi senyawa indigo diperoleh pada fase logaritmik jam ke-6 hingga jam ke-26. Hubungan nilai OD dan produk *leuco indigo* bersifat linear yaitu semakin tinggi nilai OD maka semakin tinggi konsentrasi *leuco indigo*. Penambahan sumber karbon berupa air tajin, pH 10 kisaran basa atau tergolong alkalifilik dan mesofilik yakni suhu 25°C merupakan kondisi lingkungan optimal untuk pertumbuhan dan aktivitas reduksi indigo tiap strain. Konsentrasi *leuco indigo* yang dihasilkan yakni *Bacillus* ST2 sebesar 292,45 mg/L; 216,25 mg/L; dan 239,45 mg/L, *Bacillus* ST3 sebesar 260,45 mg/L; 222,7 mg/L; dan 246,40 mg/L, serta *Bacillus* IB1 sebesar 261,10 mg/L; 202,85 mg/L; 246,60 mg/L.

Kata kunci: bakteri, *Indigofera tinctoria*, *leuco indigo*, optimasi, reduksi indigo.