Characterisation and Optimisation of Carotenoid Production from a Marine Bacterial Isolate

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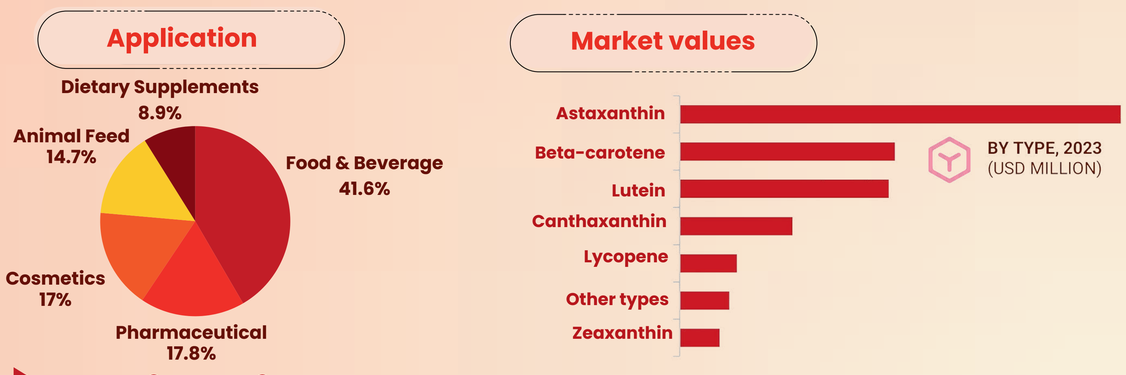
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Abstract

Many bacterial species from marine environments produce secondary metabolites and pigments of high economic value. This study aimed to characterise a marine bacterial isolate and determine optimum conditions for its growth and carotenoid production. A pigment-producing bacterial isolate, W11, was identified from seawater using 16S rRNA gene sequencing. Growth and pigment production were assessed under different pH levels (pH 6–12), incubation temperatures (30, 37, and 40°C), and agitation conditions (150 rpm and static culture). Pigments were extracted using various solvents and sonication methods and analysed using high-performance liquid chromatography (HPLC). Isolate W11 was identified as *Bacillus infantis*, and its optimal carotenoid production was achieved with culture grown in Trypticase Soy Yeast Extract Broth at pH 8 and 37°C with agitation at 150 rpm. The most effective carotenoid extraction was achieved using sonication in combination with 99.9% methanol. The total carotenoid obtained under these conditions was 2.13 mg/L. HPLC analysis confirmed the presence of β-carotene and other carotenoid compounds. Additional characterisation via LC-MS is required to identify the carotenoid profiles of this isolate.

Economic Importance of Carotenoids



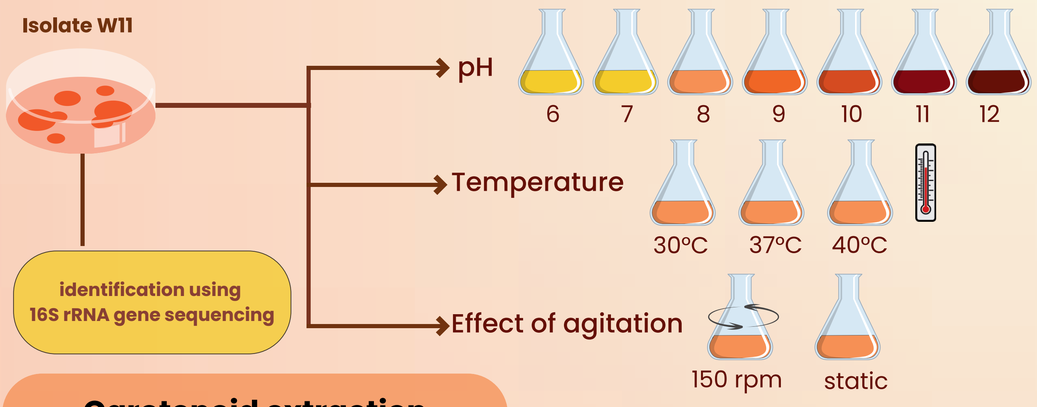
(MarketsandMarkets, 2024)

Objectives

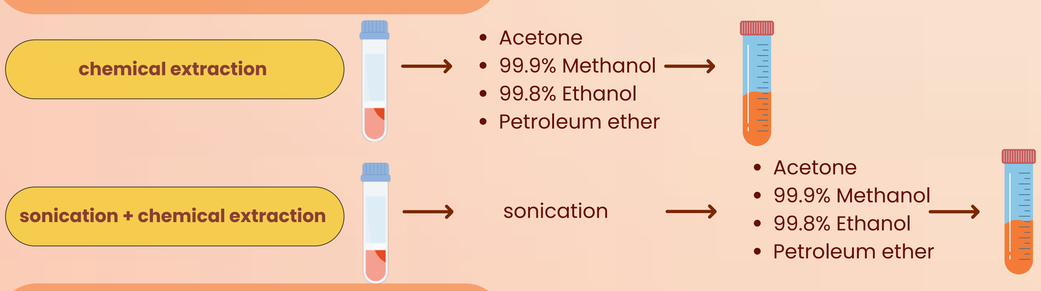
- To identify species of a marine carotenoid-producing bacterial isolate W11
- To optimise process parameters for carotenoid production by isolate W11
- To identify carotenoid type produced by the W11 isolate

Materials and Methods

Optimisation



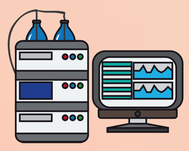
Carotenoid extraction



TLC / HPLC Analysis



Mobile phase:
→Chloroform (99%)
→ Dichloromethane : Ethyl acetate 99:1 (v/v)



Instrument	SpectraSYSTEM ™ UV2000 HPLC
Column	YMC Carotenoid C30 (5 µm, 250 x 4.6 mm)
Conditions	Mobile phase A (MeOH: MTBE: H_2O 81:15:4) and B (MeOH: MTBE 10:90); flow rate 1 mL/min; detection at 480 nm

Results

Identification of Bacterial Isolate

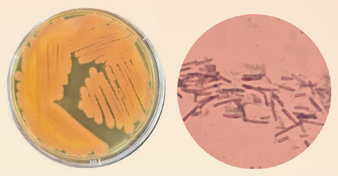


Figure 1 Colonies and cell morphology of pigmented bacterial isolate W11

Table 1 16S rRNA gene sequencing results of bacterial isolate W11

Closest relative	Query Cover	Percentage of identity	Accession NO.
Bacillus infantis NRRL B-14911, complete genome	100%	100.00%	CP006643.1
Bacillus infantis strain AR001 chromosome	100%	100.00%	CP160000.1
Bacillus infantis strain OT1 16S ribosomal RNA gene, partial sequence	100%	100.00%	OR691661.1

Optimisation of culturing conditions

(significant differences at $p<0.05$)

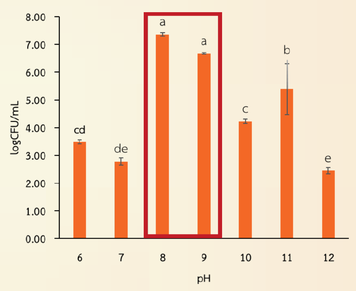


Figure 2 Effect of pH on growth of isolate W11

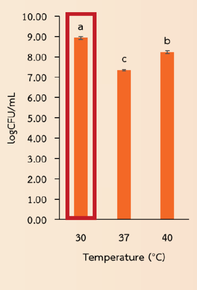


Figure 3 Effect of temperature on growth of isolate W11

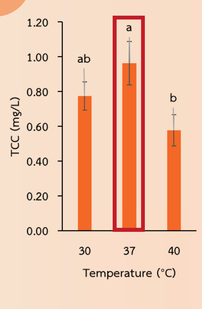


Figure 4 Effect of temperature on carotenoid production by isolate W11

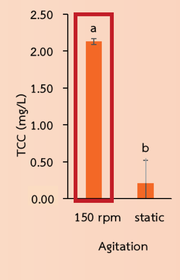


Figure 5 Effect of agitation on carotenoid production

Efficiency of Extraction Methods

(significant differences at $p<0.05$)

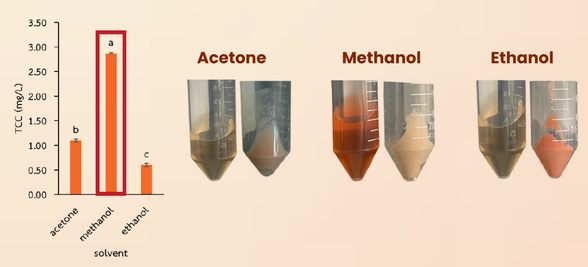


Figure 6 Comparison of carotenoid extraction efficiency using different solvents

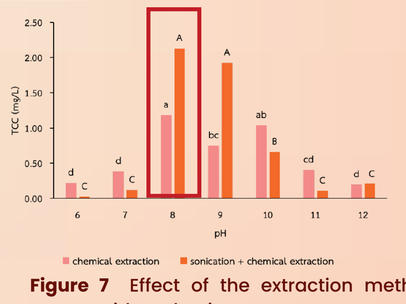


Figure 7 Effect of the extraction methods on total carotenoid production

TLC and HPLC Analysis of Carotenoid



Figure 8 TLC analysis showing a yellow band of β-carotene standard with a band from W11 sample



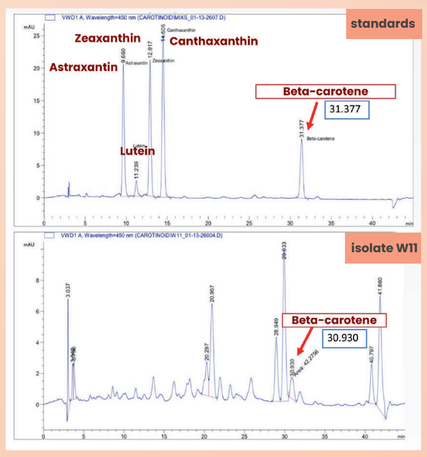


Figure 9 HPLC chromatograms of carotenoid standards and sample

Acknowledgements

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Conclusions

- The bacterial isolate W11 a marine carotenoid producer was identified as *Bacillus infantis*.
- Optimal carotenoid production were observed in TSYEB pH8 at 37°C with agitation at 150 rpm. Sonication combined with 99.9% methanol resulted in highest carotenoid recovery.
- β-carotene was identified as the major carotenoid compound produced by isolate W11 .