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Screening For Carotenoid-Producing Halophilic Bacteria Isolated From Saline Environments In Uzbekistan

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ABSTRACT

Uzbekistan's salt-affected ecosystems are plausible reservoirs of halophilic prokaryotes capable of producing carotenoid pigments. The Aral Sea basin, partly within Uzbekistan's Republic of Karakalpakstan, has markedly shrunk since the 1960s, exposing salt-rich sediments and creating harsh conditions that can enrich for stress-tolerant microbiota. In halophiles, carotenoids are associated with membrane stabilization, photoprotection, and mitigation of oxidative stress, including well-known C40 pigments (e.g., β-carotene, lycopene) and, in many haloarchaea, C50 bacterioruberin derivatives. This article presents an IMRAD-structured screening workflow suitable for Uzbekistan's saline matrices (brines, sediments, salt crusts, saline soils). The approach integrates graded-salinity cultivation, pigment-stability checks, solvent extraction, UV–Vis fingerprinting focused on carotenoid absorption in the 450–550 nm window, and 16S rRNA identification, with confirmatory chromatography reserved for top candidates. The proposed pipeline prioritizes isolates based on convergent evidence rather than colony color alone, supporting resource-efficient discovery and the establishment of a local culture collection for downstream characterization and process development.

KEYWORDS: Uzbekistan; halophiles; carotenoids; bacterioruberin; Aral Sea basin; screening; UV–Vis spectroscopy; 16S rRNA.

INTRODUCTION

Microbial carotenoids are increasingly valued because they provide both coloration and functional bioactivity and can be produced year-round by controlled fermentation. Halophilic microorganisms add a practical advantage to pigment bioprospecting: high salinity suppresses many non-halophilic contaminants, which can reduce operational complexity during early discovery and, in some cases, simplify cultivation management. Carotenoids share conjugated double bonds that generate characteristic visible-light absorption; this property makes spectrophotometry a convenient first-pass tool for distinguishing carotenoid-like pigments from other chromophores.

Uzbekistan is a rational target for halophile screening because it contains persistent salinity gradients, including the Aral Sea dry-bottom region characterized by exposed salt-laden sediments and severe environmental pressure. Independent studies of the Large Aral Sea report diverse extremophilic bacterial and archaeal groups, supporting the premise that regional saline sites can host pigment-producing halophiles and related extremophiles. The methodological challenge is to translate visible colony pigmentation into evidence-based prioritization, so that a small number of the most promising isolates proceed to confirmatory chemistry and taxonomy.

The screening workflow targets brines, wet sediments, salt crusts, and salt-affected soils typical of Uzbekistan's saline environments. Samples are collected aseptically and transported without dilution to minimize osmotic shock, then processed rapidly to preserve community structure. Cultivation is initiated on halophile-compatible media across graded NaCl to capture a broad salt-response spectrum, accommodating slight (about 1–3% NaCl), moderate (3–15% NaCl), and extreme (>15% NaCl) halophiles in parallel. This graded design reduces "blind spots" that occur when a single salinity level is used and supports recovery of both bacterial halophiles and haloarchaea.

Isolation proceeds by repeated streaking at the salinity supporting best growth. Pigmented colonies (yellow, orange, pink, red) are treated as putative carotenoid producers and are subjected to pigment-stability checks: re-growth near the salt optimum is used to confirm that coloration persists rather than appearing only as a transient stress response. Growth dependence on elevated NaCl is recorded to distinguish true halophiles from salt-tolerant background flora.

Pigment extraction is performed on broth cultures grown at near-optimal salinity. Biomass is harvested by centrifugation and extracted with acetone–methanol (7:3, v/v) until cells are visibly decolorized, consistent with established halophile carotenoid practice. Clarified extracts are scanned by UV–Vis spectrophotometry across the visible region. Because carotenoids absorb strongly in the blue–green window (approximately 450–550 nm), extracts are classified as carotenoid-like when they display characteristic maxima and band shapes within this range. UV–Vis is used as a prioritization tool, not as final identification.

Taxonomic placement is obtained via 16S rRNA gene sequencing for UV–Vis-positive isolates, enabling separation of bacterial and archaeal candidates. High-priority strains are advanced to confirmatory profiling (TLC and LC–MS/HPLC-DAD), since red halophiles can contain pigment mixtures; LC–MS, for example, has resolved bacterioruberin together with lycopene and β-carotene in a red haloarchaeal isolate, illustrating why confirmation beyond UV–Vis is necessary.

The principal outcome of the workflow is a prioritized shortlist of candidate carotenoid producers supported by convergent evidence: salt-requiring growth, stable pigmentation upon subculture, and carotenoid-like UV–Vis fingerprints. Two pigment–salinity patterns are especially informative for ranking. Orange–yellow isolates that grow under moderate salinity frequently yield UV–Vis profiles consistent with C40 carotenoids typical of many bacterial pigment systems. Deep red or pink isolates that require very high NaCl commonly show broader and longer-wavelength banding compatible with bacterioruberin-dominated systems characteristic of many haloarchaea. The combined use of salt-dependence, pigment stability, and spectral behavior reduces reliance on colony color alone and helps focus confirmatory analytics on strains most likely to deliver meaningful pigment yields and interpretable carotenoid profiles.

The screening logic is ecologically grounded. Salt-affected environments impose chronic osmotic stress and, in exposed basins such as the Aral Sea dry-bottom region, high irradiance and oxidative pressure are also relevant; these conditions plausibly favor microorganisms that invest in protective metabolites. Genomic and biochemical studies emphasize that carotenoids in extreme halophiles can function as membrane stabilizers and photoprotectants and that C50 bacterioruberin pigments can contribute to UV protection and reactive oxygen species

scavenging under hypersaline stress. This supports targeting visible pigmentation as an initial signal, but only when it is paired with objective measurements and stability checks.

UV–Vis fingerprinting is central because it directly interrogates the carotenoid chromophore and is accessible to most laboratories. At the same time, UV–Vis spectra are not uniquely diagnostic: mixtures, solvent effects, and geometric isomerization can shift maxima and alter band shapes. For that reason, chromatographic confirmation remains essential before claiming a specific carotenoid composition or comparing yield across strains. The staged design is therefore deliberately resource-efficient: broad isolation and UV–Vis triage can be performed locally, while LC–MS/HPLC capacity is reserved for the most evidence-supported candidates.

From a bioprocess perspective, halophiles may offer advantages for later scale-up, including reduced contamination pressure at high salinity. Additionally, some extreme halophiles can lyse when salt is lowered, which can simplify pigment release during extraction. However, high-salt cultivation introduces constraints (corrosion risk, saline waste handling), which reinforces the value of documenting "process-friendly" traits early in screening alongside pigment phenotype and spectral stability.

A staged screening workflow can accelerate discovery of carotenoid-producing halophilic bacteria and archaea from Uzbekistan's saline environments by combining graded-salinity cultivation, pigment-stability checks, UV–Vis fingerprinting, and 16S rRNA identification, with confirmatory chromatography reserved for top candidates. Grounded in the ecological reality of salt-affected Uzbek landscapes, including the Aral Sea basin, the approach supports building a local culture collection and selecting strains with credible potential for downstream pigment characterization and process development.

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