



Cocaine degradation by *Pseudomonas stutzeri*: an alternative approach for the final disposal of a dangerous solid waste

C.A. Díaz, E. Restrepo, W. F. Garzón and J.C. Higuita

Research Group in Chemical, Catalytic and Biotechnological Processes









Overview

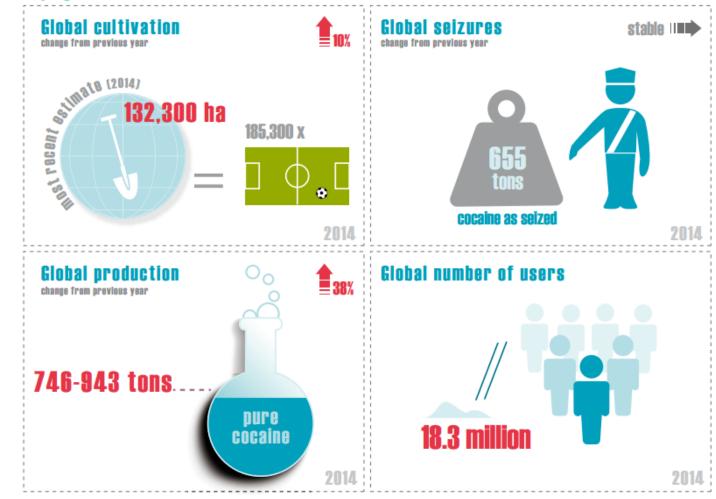
- Introduction
- Methods
- Results and discussion
- Conclusions
- References



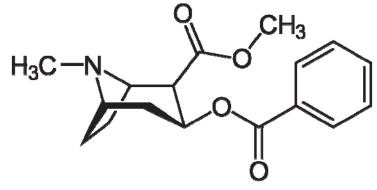
Introduction

COCAINE

Key figures

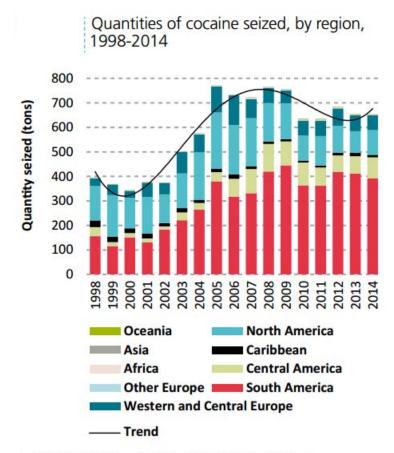






Note: Cocaine seizures are mostly of cocaine hydrochloride (of varying purity), but also include other cocaine products (paste, base and "crack").

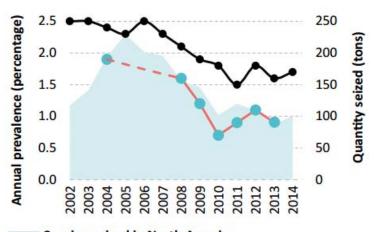
Introduction



Source: Responses to the annual report questionnaire.

Note: The seized forms of cocaine included cocaine hydrochloride, coca paste and base and "crack" cocaine, and the quantities seized were not adjusted for purity.

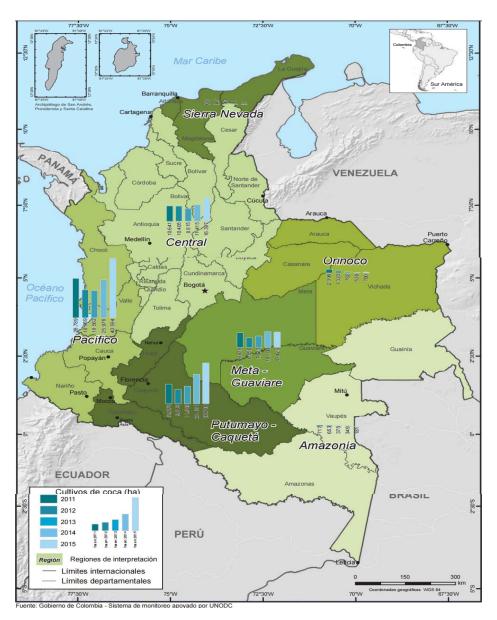
Quantities of cocaine seized in North America and prevalence of past-year cocaine use in Canada and the United States, 2002-2014



- Cocaine seized in North America
- Annual prevalence of cocaine use in the United States among the population aged 12 and older
- Annual prevalence of cocaine use in Canada among the population aged 15 and older

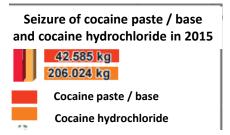
Source: Responses to the annual report questionnaire, the United States National Household Survey on Drug Use and Health and the Canadian Tobacco, Alcohol and Drugs Survey (CTADS) 2013.

Note: Data for 2002 have been used as baseline data, as the United States National Household Survey changed its methodology several times between 1998 and 2002.



United Nations Office On Drugs And Crime. (2016). Monitoring affected ladn by illicit crops 2105: United Nations publication.

Seizure figures in Colombia in a decade reached 2,000 tons

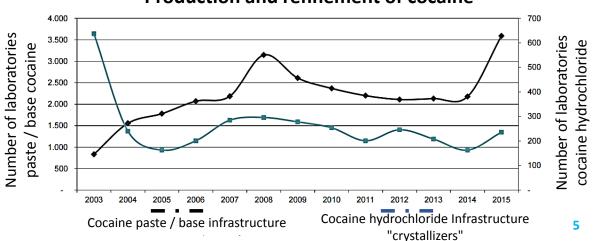


In 2016, Colombia seized 308 metric tons, 59 more than in all 2015

The Narcotics Division of the National Police of Colombia has already seized 130 tons in 2017

6,8 kg per hectare where four harvests are exceeded per year.

Production and refinement of cocaine



Objective

The aim of this research was to grow and adapt *Pseudomonas stutzeri* in a minimum medium using cocaine hydrochloride as the only carbon source as an alternative disposal method to reduce the contamination of this drug of abuse.



Vs



General Method

<u>Cryopreserved</u> <u>strains</u>



Incubator 32°C, 150 rpm



Inoculate each strain separately in salt medium



Spectrophotometry analysis

Bacterial growth kinetics and quantification by HPLC-MS

Reactivation of strains and adaptation to test substrate

Methods

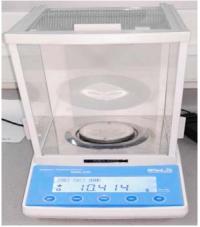








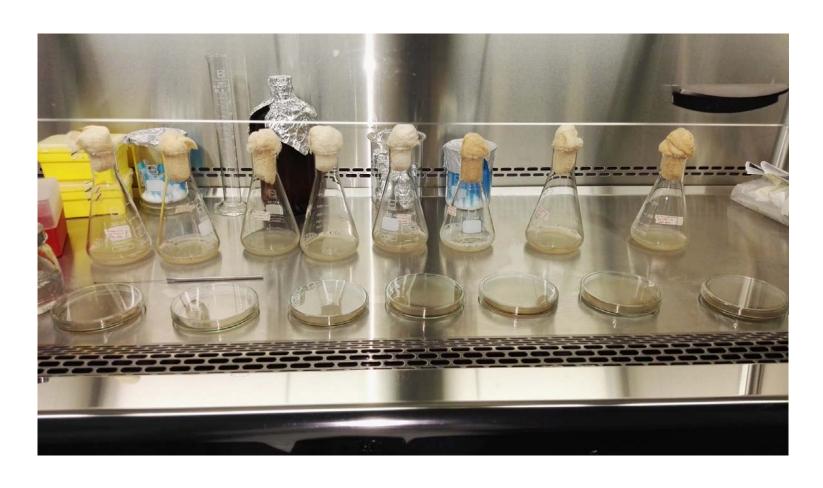








Methods

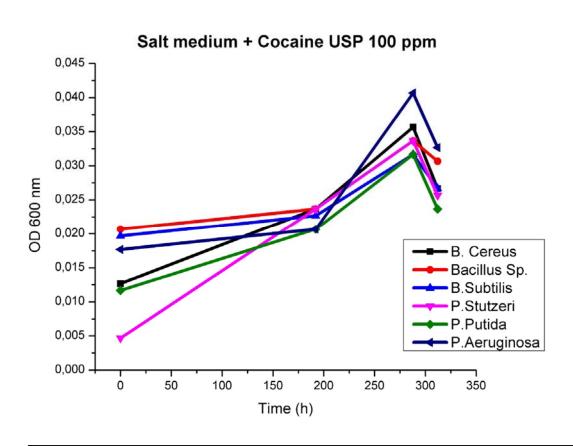


Cromatographic conditions	
Parameters	Value
Mobile Phase	Mixture of the dibasic sodium phosphate solution 0.05mol L-1 pH 8.0 and acetonitrile (70:30 v/v).
Mode	Gradient
Detector	UV 235nm
Column Flow	1,0 mL/min
Rt Cocaine	1,802 min
Work temperature	RT

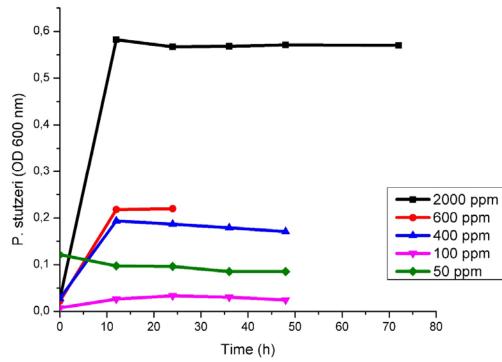


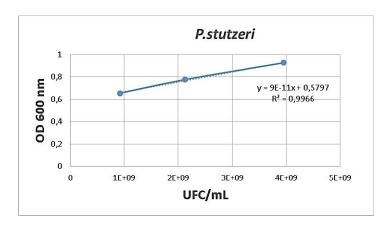
Results

Growth of Pseudomonas stutzeri at different cocaine concentrations

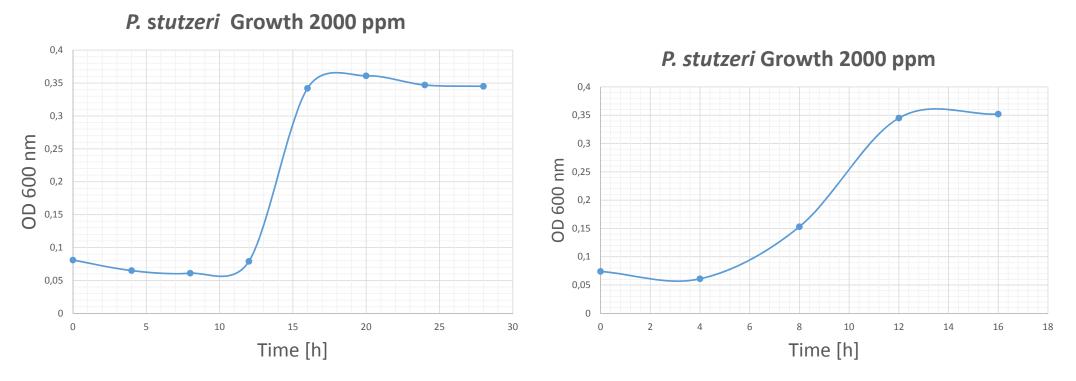


Several strains were evaluated and the one that showed the best adaptation was *P. stutzeri*.



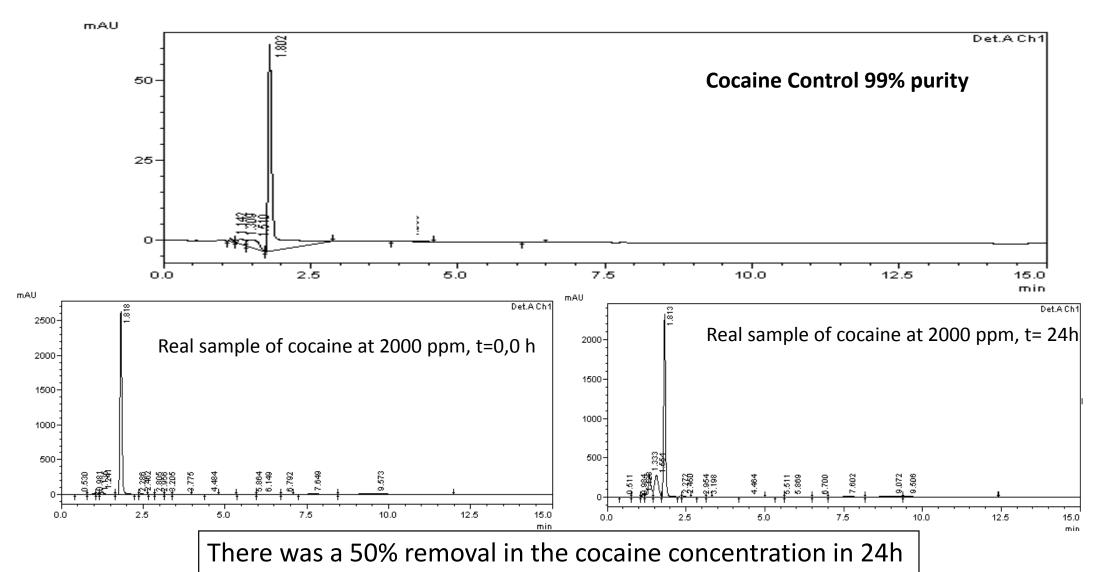


Growth kinetics



The first growth was between 12 to 16 h, while succesive growths reduced the time growth between 4 to 12 h.

Chromatographic results of cocaine consumption by *Pseudomonas stutzeri*



Conclusions

- There was a good adaptation of *P. stutzeri* to the cocaine substrate as the only carbon source in a saline medium.
- The major adaptation was at 2000 ppm.
- The removal of cocaine, so far, was 50%.
- The adaptation process of *P. stutzeri* to use cocaine as carbon source, might help as an alternative destruction method of this substance.
- Further studies must be conducted in order to adapt microorganisms to higher concentrations of cocaine and also to obtain better removal values.

References

- Ahmad, A., Leete, E.. (1970). Biosynthesis of the tropine moiety of hyoscyamine from δ -N-methylornithine. *Phytochemistry*, 9(11), 2345-2347.
- Alves, M. M. (2010). Portugal Patente nº WO/2007/058557
- Barton, A., Sagers, R., Pitt, W. (1996). Measurement of bacterial growth rates on polymers. Journal of Biomedical Materials Research, 32, 271-278.
- Baranyi, J., Roberts, T. A. (1994). Mathematics of predictive food microbiology. *International Journal of Food Microbiology*, (26), 199-218.
- Bartholomew, B. A, Smith .M. J. (1993). The isolation and identification of 6-hydroxycyclohepta-1,4-dione as a novel intermediate in the bacterial degradation of atropine. *Biochemical Journal*, (1), 115-118.
- Kosieradzka, K., Le Faouder, P., Molinié, R., Gentil, E., Lebreton, J., Robins, R., (2014). Tropane alkaloid metabolism by Pseudomonas AT3 cell cultures: Interchange between the nortropine and norpseudotropine catabolic pathways. Phytochemistry Letters 10, 60-68.
- Kloss, M., Rosen, G., Rauckman, E., (1983). N-demethylation of cocaine to norcocaine. Mol. Pharmacol. 23, 482–485.

Research Group in Chemical, Catalytic and Biotechnological Processes, Laboratory Group of Biological and Molecular Sciences



Research Group of the International Center for Strategic Studies Against Drug Trafficking - Colombia

