

Figure 1. Production of inhibition zone by the antagonistic strains of *Cicer-Rhizobium* against the sensitive strain PR2005b (center: 2005b, from ↑ clockwise: PR2303, PR2042c, PR2015b and PR2109a). **a**, Growth; **b**, Halo zone seen after removal of growth.

observed. It was found that when the bottom layer of YEDXA contained 0.5% Qualigens and 1.5% Difco agar, and the top layer contained 0.5% of each of these two agars, the halo zone was most sharp

(Table 1, Figure 1). Both the layers of gel prepared in Sisco agar showed poorly visible halo zones, while with Qualigens, the halo zone was of moderate intensity. Similar type of results were observed when different media varying in composition, particularly in carbon sources were used. A well-like halo zone under the spot, which was clearer after removing the growth with cotton swab, was considered as positive antagonism.

The examination of 38 strains as test strains against 15 non-bacteriocin producing sensitive strains on YEDXA, made with a mixture of Qualigens and Difco agars, showed 23 strains producing clearly visible inhibitory halo zones. These 23 strains showed an inhibitory spectrum identical to that obtained with deferred antagonism procedure.

Based on this modified simple method, 8648 transconjugants could successfully be screened to identify three bacteriocin-defective mutants¹⁰.

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The hottest chilli variety in India

Chillies or cayenne, are one of India's major export commodities. An annual plant, chilli comes in a wide variety of shapes, sizes, colours and in different degrees of pungency. India is the only country rich in many varieties with different quality factors. The medium pungent *sannam* and the mildly pungent *mundu* chillies are internationally recognized as the finest in quality. Products are also available as powder and oleoresins. Indian chilli is exported to many countries, notably USA.

The hot flavour of chillies is due to the presence of a group of seven closely related compounds called capsaicinoids, but capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) and dihydrocapsaicin are responsible for approx. 90% of the pungency¹⁻³. Chilli hotness is measured in Scoville Heat Units (SHU)^{4,5} which is originally a subjective measure but today, chilli hotness is more frequently determined by HPLC (high performance liquid chromatography), whose results can be correlated to traditional Scoville ratings:

the conversion generally accepted is that 15 Scoville units is equal to 1 ppm capsaicin plus capsaicinoids. The hottest chilli, 'Red Savina' Habanero, has been tested at over 577,000 Scoville units. The extremely fiery Thai chillies barely reach 100,000; more common varieties like the *jalapeno* or the Italian *peperoncino* generally lie below 5000 Scoville units.

Chillies as a natural product have been a subject of study in pharmaceuticals, food industry and for law enforcement since the past few decades. Oleoresin

Table 1. Capsaicin and dihydrocapsaicin content and pungency in different varieties of Indian red chillies*

Capsicum type	Oleoresin (% w/w)	Capsaicin (% w/w)	Dihydrocapsaicin (% w/w)	Pungency (SHU)
Tezpur	15.0	4.28	1.42	855000*
Gwalior	12.5	0.47	0.23	112500
Patna	19.1	0.44	0.21	96000
Guntur	12.0	0.20	0.16	53250
Kashmir	11.0	0.18	0.09	40500

*Red Savina Habanero, a Mexican chilli reported to be hottest in the world has a pungency of 577,000 SHU.

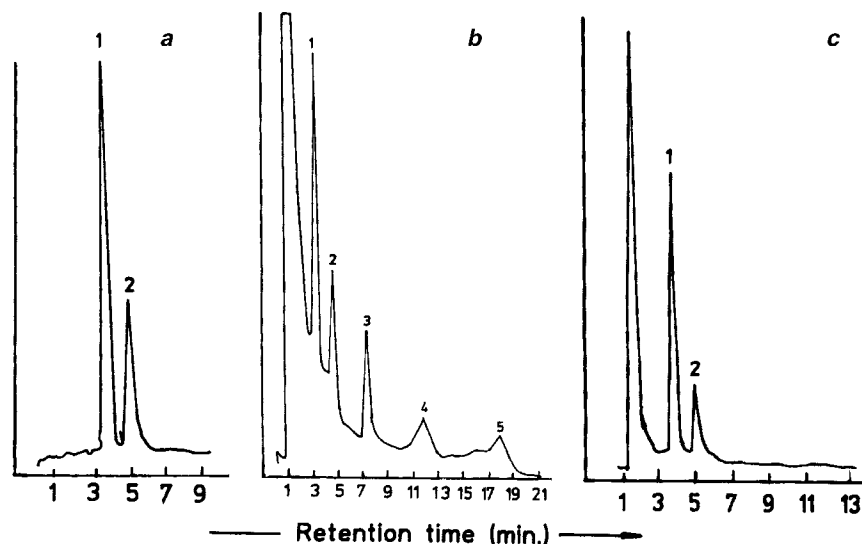


Figure 1. HPLC chromatogram of *a*, Standard capsaicin showing capsaicin (1) and dihydrocapsaicin (2); *b*, Patna oleoresin showing capsaicin (1) and dihydrocapsaicin (2). Peaks 3, 4 and 5 represent other naturally occurring compounds which could not be identified; *c*, Tezpur oleoresin showing capsaicin (1) and dihydrocapsaicin (2).

capsicum (OC) is an extract of the cayenne pepper. OC is less potentially lethal than its synthetic counterparts, ortho-chlorobenzalmalononitrile (CS) and chloroacetophenone (CN). It is environment-friendly and much safer than CS or CN. In most cases, OC is dispersed by the use of aerosols but use of OC powders is growing and it is predicted to dominate the market in the coming years as the mainstay of riot control agents.

The objective of our work was to find out a chilli which contains maximum capsaicin and dihydrocapsaicin, so that both can be extracted from it to be used as OC or as powder after isolation. We have analysed various varieties of chillies from different states of India. The chillies

have been assigned their local names because the botanical identification of these chillies could not be ascertained, except for the Tezpur chilli. The chillies were dried and then extracted with acetone which was found to be the best solvent, the extract was concentrated and the resulting concentrate was analysed by HPLC using Shimadzu Model LC6A liquid chromatograph, Polygosil C-18 column, MeOH-water (60 : 40 v/v), 10% acetonitrile and 1% citric acid as mobile phase and a variable wavelength UV-VIS detector set at 201 nm. From the above analysis, we calculated the amount of capsaicin and dihydrocapsaicin in ppm. Table 1 shows the capsaicin and dihydrocapsaicin content in chilli varieties and

decreasing order of pungency in SHU. We found that the Tezpur variety (*Capsicum frutescens* var. *Nagahari*) of Indian chilli contains maximum capsaicin and dihydrocapsaicin contributing to a pungency of 855,000 SHU, which seems to be the hottest chilli known so far. This chilli evokes a biting sensation. Interestingly, it contains only capsaicin and dihydrocapsaicin among the capsaicinoids (Figure 1) which contribute maximum to the pungency of chillies, perhaps this is the reason for its high Scoville heat value. The effect of seasonal variation on the amount of capsaicin and dihydrocapsaicin was also observed, as reported by Yahia and Padilla⁶. The above results have also been confirmed by the isolation of capsaicinoids from Tezpur and Patna varieties. Thus, we have identified the hottest chilli variety in India.

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