

ORIGINAL RESEARCH ARTICLE

**Comparative Performance of Rough (R) and Smooth (S) Colonies of *Acetobacter aceti* (CRF – 3) for Acetic Acid Production**

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**ABSTRACT**

The comparative performance of *Acetobacter aceti* CRF-3, rotten fruit isolate of cashew was developed to rough and smooth surface colonies of efficient strains (CRF-3 (R) and CRF-(S)) under static and shaking culture *in vitro* condition. The comparative evaluation of Roush and smooth strains of *Acetobacter aceti* CRF-3(R) and CRF-(S) following parameters viz., ethanol tolerance, acetic acid tolerance, thermal tolerance, pH tolerance, and log phase activities revealed the superior performance of the rough strain CRF-3 (R) then the smooth strain CRF-3 (S).

**Key words:** Acetic acid, Smooth colonies, Rough colonies and *Acetobacter aceti*.

**1. INTRODUCTION**

Acetic acid is an important chemical reagent and industrial chemical used in the production of polyethylene terephthalate mainly used in soft drink bottles; cellulose acetate, mainly for photographic film; and polyvinyl acetate for wood glue, as well as synthetic fibers. In households, diluted acid is often used in descaling agents. In the food industry, acetic acid is used under the food additive code E260, as an acidity regulator.

The global demand of acetic acid is around 6.5 million tonnes per year (Mt/a), of which approximately 1.5 Mt/a is met by recycling; the remainder is manufactured from petrochemical feedstocks or from biological sources as a key ingredient of vinegar.

Acetic acid is produced both synthetically and by batch fermentation. Today, the biological route accounts for only about 10% of world production, as many nations food purity laws stipulate that vinegar used in foods must be of biological origin. Biological approach for vinegar production deals with the action of acetic acid bacteria (AAB) on dilute solutions of ethyl alcohol derived from yeast fermentation. It is also produced from fermented cider, fruit juices or other fermented alcoholic solutions derived from barley malt, hydrolyzed cereals and starches.

In this context, production of vinegar has been gaining momentum from various low cost,

renewable agricultural waste materials<sup>[1]</sup> through biological process. However, the bioconversion processes are slow materials and the bacterial fermentation proceeds slowly over the course of weeks or months. The longer fermentation period allows for the accumulation of non-toxic slime composed of acetic acid bacteria and soluble cellulose known as the mother of vinegar emphasize the need for research in several areas including, 1. Use of improved mutant strains, 2. Yeast strain development from cheaper sources, 3. Use of cheaper source raw materials (renewable sources) and 4. Provide better nutrients for optimum cell growth<sup>[2]</sup> etc. in order to maximize the vinegar production through biological process.

**2. MATERIALS AND METHODS**

**Isolation of rough and smooth strains of *Acetobacter aceti* isolates**

The *Acetobacter aceti* strain was cultured in potato medium with agitation. The culture was then diluted and onto Potato agar, where almost all colonies were R - type, and R – type colony was isolated as the R – strain. To isolate the S – type of colony, repeated shaking cultures were performed several times by transferring the culture to fresh Potato medium every 24 hrs. The culture was then diluted and spread onto a plate from which an S – type of colonies were isolated as S – strain.

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### Comparison of characteristics of rough and smooth strains of *Acetobacter aceti* CRF – 3:

The characteristics of rough (R) and smooth (S) surfaced colonies of *Acetobacter aceti* (CRF – 3) were compared. The isolates were examined for their phenotypic characteristics by following the procedure of Asai *et al.* [3].

### Comparative performance of rough and smooth strains of *Acetobacter aceti* for acetic acid production

*Acetobacter aceti* CRF - 3 strains was cultured in potato medium with agitation. The culture was then diluted and spread onto Potato agar, all colonies were R - type and S - type colonies were isolated. S - Type colony repeated straining culture was performed several times by transferring the culture to fresh potato medium every 24 hrs. The culture was then diluted and spread onto a plate from which an S - type colony was isolated.

The comparative performance of R and S colonies of *Acetobacter aceti* CRF - 3 was evaluated for the parameters viz., different ethanol concentration, acetic acid concentration, temperature tolerance, different pH and time course as described earlier in the chapter (3.9.1, 3.9.2, 3.9.3, 3.9.4) respectively.

### Comparative performance of rough and smooth strain of *Acetobacter aceti* for acetic acid production at lag phase

*Acetobacter aceti* CRF - 3 strains was cultured in Potato medium with agitation for 24 hrs to harvest in lag phase cells. The culture was then diluted and spread on to potato agar, R - type colonies were isolated as the R - strain. To isolate the S - type colony, shaking culture was performed several times by transferring the culture to fresh potato medium every 24 hrs. The culture was then diluted and spread onto a plate from which an S - type colony was isolated.

## 3. RESULTS AND DISCUSSION

The comparative performance of rough (R) and smooth (S) colonies of *Acetobacter aceti* CRF – 3 for acetic acid production was investigated (Table 1). The comparison was carried out under static condition and shaking condition of Rough and smooth colonies of *Acetobacter aceti*. Rough colonies showed more acetic acid production when compared to smooth colonies. Maximum acetic acid production was noticed by the rough colony of *Acetobacter aceti* CRF – 3 (42.43 g/L)

in static condition. Acetic acid production was more in static culture than shaking culture.

The comparative performance of rough (R) and (S) smooth colonies of *Acetobacter aceti* CRF – 3 for acetic acid production at 5% ethanol concentration was determined (Table 2). The comparison was carried out under static condition and shaking condition. Rough colonies of *Acetobacter aceti* showed more acetic acid production when compared to smooth colonies. Maximum acetic acid production was recorded by the rough colony of *Acetobacter aceti* CRF – 3 (43.19 g/L) at 5% ethanol concentration in static condition, acetic acid production was more in static culture when compared to shaking culture.

The comparative performance of rough and smooth colonies of *Acetobacter aceti* (CRF – 3) for acetic acid production at 4% acetic acid concentration was evaluated and the results were presented in (Table 3). The comparison was carried out under static condition and shaking condition. Rough colonies of *Acetobacter aceti* showed more acetic acid production when compared to smooth colonies. Maximum acetic acid production was observed by the rough colony of *Acetobacter aceti* CRF – 3 (40.56 g/L) at 4% acetic acid concentration in static condition. Acetic acid production was more in static culture when compared to shaking culture.

The comparative performance of rough and smooth colonies of *Acetobacter aceti* CRF – 3 for acetic acid production at 37°C was studied and the results were showed in (Table 4). The comparison was carried out under static condition and shaking condition. Rough colonies of *Acetobacter aceti* showed more acetic acid production when compared to smooth colonies. Maximum acetic acid production was recorded by the rough colony of *Acetobacter aceti* CRF – 3 (41.53 g/L) at 37°C in static condition. Acetic acid production was more in static culture when compared to shaking culture.

The comparative performance of rough (R) and smooth (S) colonies of *Acetobacter aceti* CRF – 3 for acetic acid production in lag phase was tested and the results are furnished in (Table 5). The comparison was carried out under static condition and shaking condition. Rough colonies of *Acetobacter aceti* showed more acetic acid production when compared to smooth colonies. Maximum acetic acid production was noticed by the rough colony of *Acetobacter aceti* CRF – 3

(13.00 g/L) in lag phase under static condition. Acetic acid production was more in static culture when compared to shaking culture.

The comparative evaluation of rough (R) smooth (S) strain of *Acetobacter aceti* CRF – 3 at lag phase was studied. The studies on the acetic acid productivity of rough and smooth strains of *Acetobacter aceti* CRF - 3 revealed that the rough strain of CRF - 3 revealed maximum acetic acid production in lag phase when compared to the smooth strain. Lee *et al.* [4] developed thermotolerant *Acetobacter aceti* strain accumulated acetic acid under lag period. Saeki *et al.* [5] formed that lag phase for producing acetic acid in *Acetobacter aceti* was very long. In the present study also, the rough (R) strain of CRF-3 *Acetobacter aceti* showed higher acetic acid in static condition when compare to the smooth strain and the result of the present study are in conformity with the earlier findings.

**Table 1: Comparative performance of rough (R) and smooth (S) strains of *Acetobacter aceti* (CRF – 3) for acetic acid production**

Isolate	Acetic acid production (g/L)	
	Static culture	Shaking culture
CRF – 3 (R) Rough colony	42.43 ± 0.06	38.92 ± 0.07
CRF – 3 (S) Smooth colony	36.13 ± 0.05	39.52 ± 0.05

**Table 2: Comparative performance of rough (R) and smooth (S) strains of *Acetobacter aceti* (CRF – 3) for acetic acid production at 4% ethanol concentration**

Isolate	Acetic acid production (g/L) at 5% ethanol concentration	
	Static culture	Shaking culture
CRF – 3 (R) Rough colony	43.19 ± 0.02	39.91 ± 0.03
CRF – 3 (S) Smooth colony	40.21 ± 0.01	41.32 ± 0.04

**Table 3: Comparative performance of rough (R) and smooth (S) strains of *Acetobacter aceti* (CRF – 3) for acetic acid production at 4% acetic acid concentration**

Isolate	Acetic acid production (g/L) at 4% acetic acid concentration	
	Static culture	Shaking culture
CRF – 3 (R) Rough colony	40.56 ± 0.05	37.23 ± 0.06
CRF – 3 (S) Smooth colony	37.42 ± 0.04	39.17 ± 0.07

**Table 4: Comparative performance of rough (R) and smooth (S) strains of *Acetobacter aceti* (CRF – 3) for acetic acid production at 37°C**

Isolate <sup>†</sup>	Acetic acid production (g/L) at 37°C	
	Static culture	Shaking culture
CRF – 3 (R) Rough colony	41.53	38.23
CRF – 3 (S) Smooth colony	36.13	39.09

**Table 5: Comparative performance of rough (R) and smooth (S) strains of *Acetobacter aceti* (CRF – 3) for acetic acid production at lag phase**

Isolate	Acetic acid production (g/L) in lag phase	
	Static culture	Shaking culture
CRF – 3 (R) Rough colony	13.00 ± 0.04	10.00 ± 0.03
CRF – 3 (S) Smooth colony	9.00 ± 0.03	11.00 ± 0.04

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