











**Figure 4:** Dendrogram constructed with Dice similarity coefficient of GelCompar II on the basis of ARDRA patterns of selected carob endophytic bacteria (IEC and IRC), symbiotic bacteria

RCM (Missbah et al. 1996) and reference strains.

The clustering analysis of combined *MspI*, *Hinfl*, *HhaI* and *TaqI* restriction patterns showed a wide diversity among endophytic strains isolated from roots and epicotyls (Fig. 4). The result showed six main clusters at a mean Dice similarity coefficient of 50%.

Clusters 2, 4 and 6 contain only endophytes and RCM strains. Cluster 6 was formed by a large number of endophytic strains. In fact, this cluster was divided at 54% of similarity to two groups: the first group, formed mainly by IRC stains, one RCM strain and two IEC strains which were revealed identical by REP analysis, and the second group formed only by IRC strains which show more than 96% of similarity. Cluster 1 contains two strains IEC10 and IRC54 which has 53% of similarity with *Ag. Rhizogenes*. Cluster 3 is formed by rhizobial reference strains of *Mesorhizobium* and *Sinorhizobium*. Cluster 5 is divided at 54% of similarity in two groups: the first group which contain an epicotyls isolated strain: IEC11 and two strains nodulating carob: RCM9 and RCM11 that were tightly related to reference strains of the genus *Rhizobium*. The second group contain only endophytic strains originating from roots.

#### 4. Discussion

Biological test conducted in the presence of insoluble phosphate showed that associative bacteria isolated from roots (IRC) and epicotyls (IEC) of the carob tree (*Ceratonia siliqua* L.) exhibited a similar manner the ability to solubilize phosphate di-calcium *in vitro*. These endophytic bacteria are Gram negative and according Rashith et al. [34], Gram-negative strains are the most efficient in the solubilization of phosphate across the production of organics acids and the phosphatases enzymes. Solubilization index (*SI*) obtained in our study is consistent with the results reported for various rhizobacteria. It is 2.48 for *Rhizobium leguminosarum* by *Vicia*, 1.41 *Mesorhizobium ceceri*, *M. mediterraneun* and *Sinorhizobium meliloti* [14, 15, 35], 2.15 for *Burkholderia* [18] and 4,1 for *Pseudomonas* sp. [14].

For antibiotic test, the strains of carob tree exhibited a multiple antibiotic resistance. They were highly sensitive to tetracyclin and kanamycin and mostly resistant to nalidixic acid and moderately to the different concentrations tested of ampicillin, chloramphenicol, erythromycin, Nalidixic acid, spectinomycin and streptomycin (Fig. 2). The same result was described for many rhizobacteria. Various strains belonging to the genus *Rhizobium* and *Burkholderia* exhibited a multiple antibiotic resistance [35, 36].

The technology based on DNA digestion by endonucleases results in pattern of bands, is called ARDRA and it offers the opportunity to review polymorphism in the analyzed DNA [37]. Selected endophytic bacteria and RCM strains compared by ARDRA analysis to each other and to thirteen reference strains were present in the all clusters, except cluster 3 (Fig. 4). These endophytes of *Ceratonia siliqua* plant were very heterogeneous and some of them were related to the rhizobial reference strains such as *Rhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Ag. Rhizogenes*. The rhizobia strains that usually known to form nodule organ with legumes plants are recently reported to be as well as natural associative bacteria colonized wide range of host plants non-legumes. Here, we cited some natural associative rhizobia: *Rhizobium leguminosarum* bv. *phaseoli*, *trifolii* and *viceae* [3, 35, 38], *Rhizobium* sp. [14, 39], *Bradyrhizobium japonicum* [40], photosynthetic bradyrhizobia [41], *Sinorhizobium meliloti* [40], *Azorhizobium caulinodans* [42, 43], *Burkholderia brasilensis* [44] and *Burkholderia cepacia* [35].

## 5. Conclusion

The similarity of physiological, cultural, biological and molecular data between endophytes associations (IRC and IEC) and symbiotic (RCM) isolates of the carob (*Ceratonia siliqua* L.) tree confirms that these soil bacteria have the same host plant. On the other hand, these rhizobacteria (PGPB) promote the growth and development of their host, contribute significantly to the favorable installation of carob on poor soils and it resist face various abiotic stresses such as salinity, toxic metals, antibiotic substances, strong drought, ...

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